ISSCR23

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THE GLOBAL STEM CELL EVENT

POSTER ABSTRACT GUIDE

CO-SPONSORED BY:

BlueRock THERAPEUTICS

ISSCR ANNUAL MEETING 2023

14–17 JUNE 2023
BOSTON, USA + VIRTUAL

POSTER SESSIONS

All Times in EDT

Posters 101 – 1140 available in-person and virtually. Presentation times listed below. Posters will be continuously displayed during Exhibit Hall hours Wednesday through Friday.

POSTER SET-UP & TAKE DOWN HOURS

Set-up: Wednesday, 14 June, 2:30 PM – 5:45 PM

Take down: Friday, 16 June, 2:45 PM – 3:00 PM

Posters 2001 – 2140 available in Virtual Poster Theater only. No assigned presentation time; presenters available through online messaging.

WEDNESDAY, 14 JUNE 2023

POSTER SESSION 1

6:00 PM - 7:30 PM

TRACK: ETHICS, POLICY AND STANDARDS (EPS) – all posters # 101-110
TRACK: CELLULAR IDENTITY (CI) – all posters # 181-380
TRACK: MODELING DEVELOPMENT AND DISEASE (MDD) – partial track # 381-848; refer to session listings

ODD NUMBERED POSTERS
6:00 PM - 6:45 PM

EVEN NUMBERED POSTERS
6:45 PM - 7:30 PM

THURSDAY, 15 JUNE 2023

POSTER SESSION 2

10:00 AM -11:30 AM

TRACK: TISSUE STEM CELLS AND REGENERATION (TSC) - All Posters #967 - 1140

ODD NUMBERED POSTERS
10:00 AM – 10:45 AM

EVEN NUMBERED POSTERS
10:45 AM – 11:30 AM

POSTER SESSION 3

1:30 PM -3:00 PM

TRACK: MODELING DEVELOPMENT AND DISEASE (MDD) - partial track # 381-848; refer to session listings

ODD NUMBERED POSTERS
1:30 PM – 2:15 PM

EVEN NUMBERED POSTERS
2:15 PM – 3:00 PM

FRIDAY, 16 JUNE 2023

POSTER SESSION 4

9:30 AM -11:00 AM

TRACK: NEW TECHNOLOGIES (NT) - all posters #849 - 966

ODD NUMBERED POSTERS
9:30 AM – 10:15 AM

EVEN NUMBERED POSTERS
10:15 AM – 11:00 AM

POSTER SESSION 5

1:15 PM -2:45 PM

TRACK: CLINICAL APPLICATIONS (CA) – all posters # 111-180

ODD NUMBERED POSTERS
1:15 PM – 2:00 PM

EVEN NUMBERED POSTERS
2:00 PM – 2:45 PM

VIRTUAL POSTERS

POSTER ABSTRACTS

WEDNESDAY, 14 JUNE 2023

TRACK: ETHICS, POLICY, AND STANDARDS (EPS)

Session 1: Odd

6:00 PM - 6:45 PM

TOPIC: NO TISSUE SPECIFICITY

101

THE TECHNOLOGY, OPPORTUNITIES and CHALLENGES BEHIND AN ETHICAL FRAMEWORK FOR SYNTHETIC BIOLOGICAL INTELLIGENCE

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The use of pluripotent stem cells to produce neural systems capable of basic goal-directed intelligence has recently been described by our group and termed Synthetic Biological Intelligence (SBI). Stem cell-derived in vitro neurons were embodied in a simulated game-world and showed statistically significant performance in a simplified version of the game 'Pong'. The scalability and versatility of applying pluripotent stem cells as a key building block of this technology provides a crucial enabling foundation, however it also creates potential ethical, semantic, and philosophical questions. Here we shall share recent updates from our group in the production and capabilities of these systems as an approach to consider these questions. Moreover, we outline details of SBI as an emerging technology by breaking this down into advancements in 1) stem cell technology and synthetic biology; 2) enhanced hardware and software applications; 3) neurocomputational theories and informatic analyses. From this we are able to propose some foreseeable applications and ethical considerations that may arise. In particular, we focus on the uses of pluripotent stem cell technologies to create highly specified and reproducible neural substrates in the short-, medium-, and long-term. In short, these applications, which vary greatly in likely timelines to realization, can broadly be subdivided into the use of SBI technologies for pre-clinical applications or for computational and intelligent processes. As part of this, we present considerations of how existing metrics may or may not appropriately capture key phenomena that are currently proposed to reflect potentially morally valuable states and touch on the broader debate of the appropriateness of using human derived neural tissue in vitro. Finally, we propose a pathway for promoting constructive dialogue and adopting an ethical approach that balances potential utility with foreseeable risks of harm and the uncertainty inherent to novel technologies.

Keywords: in vitro neural systems, synthetic biology, intelligence

103

AUTOLOGOUS CELL-BASED THERAPIES: THE PATIENT PERSPECTIVE

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Autologous cell-based therapies (ACBTs) are treatments where the patient's own cells are sampled, manipulated, and administered. ACBTs are mostly commonly used in the treatment of osteoarthritis and sports injuries, but they have also been used in cosmetic procedures.This study aims to highlight the patient journey from the time patients first learn about ACBTs, their experiences of receiving ACBTs, and the effects the ACBTs have had on their lives since receiving the treatment. This will enable us to better understand the health and financial context of patients receiving ACBTs in the current regulatory context. Participants will be patients who have received or are currently receiving treatments involving ACBTs. We aim to recruit a minimum of 200 participants through public ads and posts on social media platforms, such as Facebook and Twitter. Data collection will be conducted through the administration of an online survey via Qualtrics. Once data collection has been completed, the survey data will be cleaned by a member of our research team. The data will be analysed and verified by two coders who will identify themes emerging from the data using NVivo. We will use a grounded theory approach to explore patterns in the knowledge, perceptions, and experiences of patients who have received ACBTs in medical procedures and how these patterns may relate to legal, ethical, and regulatory issues associated with the clinical and regulatory implementation of ACBTs. The findings may then be used to inform the design of Health Canada's regulatory processes for ACBTs.

Funding Source: Stem Cell Network

Keywords: autologous cell-based therapies, patient perspectives, clinical trial regulation

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MATCHING DYNAMIC SCIENCE WITH CONSENT MODELS: TOWARDS PARTICIPATORY STEM CELL RESEARCH

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Stem cell research leverages thousands of diverse biosamples and pluripotent cell lines obtained, distributed and transformed over time, across the world, under heterogenous regulatory requirements. This phenomenon necessitates complex, iterative, provenance determinations as well as compatibility assessments between intended uses with scope of informed consent and legal-ethical regimens. Thereby, the documentation of ethical data as e.g. sample provenance is as heterogeneous as the legal-ethical frameworks existing worldwide. With the stem cell field advancing towards clinical translation and more sophisticated – if not controversial - research continuing been carried out (e.g., pluripotent stem cell-based models of human development, organoids, chimeric human-animal models, etc.), it is imperative to revisit the adequacy of traditional approaches to informed consent. After all, it is the breadth and scope of the informed consent which ultimately justifies the use and control of donated biosamples for stem cell derivation and of downstream uses. In the context of rapidly evolving science, participatory research platforms have been heralded as effective tools to promote longitudinal engagement and to support interactive participant decision-making. Building on existing governance strategies providing ongoing support for broad consent frameworks, and against the background conventional (one-time) consent approaches, this presentation will explore the benefits and challenges of implementing dynamic consent models for stem cell research including data aspects.

Funding Source: This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No. 726320

Keywords: Ethics, policy, regulatory, participatory research,, pluripotent stem cells, consent,

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THE INVISIBLE RESOURCE: MOST AUSTRALIAN STEM CELL LINES ARE NOT FORMALLY REGISTERED

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Governance of human pluripotent stem cells (hPSCs) in Australia has largely been driven by the recommendations from the 2005 Lockhart review. Although one of its recommendations was the establishment of embryonic stem cell (hESC) banks, no such resource has been implemented. Many of the legislative requirements governing hESC do not apply to newer technologies such as induced pluripotent stem cells. Nevertheless, some oversight of which hPSC lines have been generated in Australia would be valuable. While stem cell banks do exist internationally, a more common practise is the development of a stem cell registry. These are online catalogues of lines that promote research visibility and cell line provenance. Two examples of international hPSC registries are hPSCReg and SKIP. In this project, we reviewed Australian stem cell laboratories and their registered lines in hPSCreg and SKIP. We also conducted semi-structured interviews among Australian-based stem cell researchers and manufacturers to understand experiences and attitudes towards stem cell registries in the Australian context. The number of Australian hPSC lines registered internationally is comparatively low compared to what exists, and many Australian lines are only partially registered. Interviews reveal a general agreement with the value of registries, but lack of familiarity with the registries themselves. One of overarching themes from stakeholder interviews was the difficulty complying with registries designed for hPSC research. Any locally designed solution must serve local needs whilst allowing data exchange with global registries. We will also evaluate whether a local registry or bank is desirable.

Keywords: Stem Cell Registry, Governance, hPSC lines

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OPENNESS IN STEM CELL RESEARCH: PRACTICES, POSSIBILITIES, POLICIES

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On a global scale, open science is increasingly being offered as an important conduit for conducting scientific research, including within the stem cell sciences. Prominent international organizations like UNESCO and the International Science Council have

put forward recommendations, convened working groups, and produced papers that affirm the importance of broader access to scientific research and findings in order to facilitate more efficient, robust and legitimate research outcomes. To these ends, initiatives that prioritize openness and sharing have been developed within stem cell research (e.g. Stem Cell Commons) and in related fields (e.g. Global Alliance for Genomics and Health). Nevertheless, developing and implementing open science policies in particular national contexts requires careful consideration of specific research, institutional, and regulatory landscapes. This paper examines the prospects for cultivating openness in stem cell research in two ways. Firstly, it offers findings from an interdisciplinary research project investigating the practices, priorities, and possibilities of 'openness' with respect to stem cell research based in Australia. Drawing on interviews with stem cell researchers, this paper shares insights into understandings and motivations of openness, how it is enacted in acquiring and sharing research materials and forming collaborations, and the factors that both encourage and restrict possibilities for openness. Secondly, it compares these findings with similar existing initiatives in stem cell research and related fields in order to identify lessons for developing effective open science policies. Through drawing upon several case studies, this paper aims to provide insights that could inform the design of effective policies that encourage and consolidate open science practices in stem cell research.

Funding Source: National Health and Medical Research Council (NHMRC), Medical Research Future Fund (MRFF), Stem Cell Therapies Mission [Australia]

Keywords: Open science, Sharing, Collaboration

TRACK: CELLULAR IDENTITY (CI)

Session 1: Odd

6:00 PM - 6:45 PM

TOPIC: CARDIAC

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CHEMICAL HPSC CARDIAC PROGENITOR'S STABILIZATION IN VITRO, REFLECTS STAGE-SPECIFIC HEART DEVELOPMENT IN VIVO

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Cardiac progenitor cells (CPCs) have gained recent attention in developmental biology, due to their potential application in regenerative therapies. Owing to their transient nature, the maintenance and investigation of CPCs remains challenging, in both native hearts in vivo and upon human pluripotent stem cells (hPSCs) differentiation, in vitro. We show that chemical modulation of p300 (Histone Acetyl Transferase) activity by IQ-1 and WNT pathway activity by CHIR99021, synergistically enable the controlled and reversible block of cardiac differentiation progression. Stabilized Cardiac Progenitors (SCPs) resulting from this process are characterized by ISL1(pos)/KI-67(pos)/NKX2-5(neg) expression. In presence of the small molecule inhibitors, SCPs maintain a quiescent state. Upon compound removal, NKX2-5 upregulation hallmarks the cell-autonomous recoupling to the cardiomyogenic program and resumption of proliferation. Directed differentiation into endothelial and smooth muscle cells confirms SCPs full developmental potential, expected for bona fide cardiac progenitors. By combining global gene expression arrays and scRNAseq, we show that SCPs possess a distinct posterior Second Heart Field (pSHF) identity hallmarked by NR2F2 expression; additionally, epicardial and endocardial subpopulations were identified. These observations are validated by a significant transcriptional overlap with E8.25-E9.75 cardiac regions in mouse heart development, underscoring that our human in vitro model reflects relevant native cell populations. In agreement with our in vitro results, we show that IQ-1 arrests Isl1(pos) progenitors at the venous pole in zebrafish heart development in vivo, which are considered to resemble the mammalian posterior SHF progenitors. We also investigated mechanisms controlling SCPs' biology, revealing that Retinoic Acid stimulates SCPs proliferation and phenotypic homogeneity but ultimately disrupting their cardiomyogenic potential, whilst BMP signalling is pivotal for cells' autonomous recoupling to cardiomyogenesis. Due to its chemically defined and reversible nature, our approach provides an unprecedented opportunity to dissect key mechanisms in cardiac progenitor cell specification and investigations on their cellular and molecular properties.

Funding Source: German Research Foundation ZW64/4-1, ZW 64/4-2, KFO311 / ZW64/7-1 German Ministry for Education and Science 13N14086, 01EK1601A, 13XP5092B, 031L0249 European Union H2020 TECHNOBEAT 66724 Horizon Europe project HEAL 101056712

Keywords: Human Pluripotent Stem Cells differentiation, Multipotent Human Cardiac Progenitors', Histone acetyl transferases and WNT pathway

TOPIC: CARDIAC

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SINGLE CELL TRANSCRIPTOME OF HUMAN EMBRYONIC STEM CELL-DERIVED NODAL LIKE PACEMAKER CELLS REVEALED NOVEL REGULATORS MANIPULATING THE DEVELOPMENT OF CARDIAC CONDUCTION SYSTEM

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The cardiac conduction system contains specialized cells and nodes that control the heartbeat, which include sinoatrial node, atrioventricular node, bundle of His and purkinje fibers. The sinoatrial node (SAN), located in the right atrium, serves as the primary site for initiation of the normal heartbeat. The human SAN is poorly understood due to limited tissue access and unstable differentiation method in vitro. We developed an efficient and stable strategy to generate human embryonic stem cell-derived SAN-like cells (hESC-SAN) using our lab-made SHOX2-EGFP; MYH6:mcherry knock-in hESC reporter line. The derived hESC-SAN cells co-express the well-known bona-fide nodal cell markers with SHOX2 and exhibit the nodal-like electrophysiological characteristics. We performed single cell RNA sequencing (scRNA-seq) and detected SHOX2 positive cells co-expressing the marker genes. After sorting out the pure pacemaker cells using the two fluorescent reporter, we identified distinct 'SAN-head' and 'SAN-tail' subpopulations similar to the vivo SAN from scRNA-seq. Moreover, the correlation analysis between SHOX2 and other genes expressed in SAN cluster demonstrated that the genes that show high correlation with SHOX2. To discover the biological functions of the SHOX2-correlated genes, we performed perturb-seq using hESC-derived SAN progenitors to determine the key genes that regulates SAN differentiation. One lead hit encodes bone morphogenetic protein 4 (bmp4) BMP4 treatment at SAN progenitor enhance the directed differentiation toward SAN cells. We further performed the cellular identities and electrophysiological analysis to characterize the identified SAN cells. Together, we identified a key factor controlling SAN differentiation and developed an efficient strategy to derive functional SAN cells, which can be used for disease modeling and drug evaluation.

Keywords: sinoatrial node, perturb seq, conduction system

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P21 DEFICIENCY ENHANCES TISSUE REPAIR AND THE REACTIVATION OF EPICARDIAL REGENERATION

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Reactivating the human epicardium upon cardiac injury is one of the most exciting yet challenging goals in using stem cells for cardiac tissue regeneration. During development, the human epicardium undergoes a shift from a proliferative state marked by the expression of WT1 to a more mature but quiescent state after birth, implicating a temporal cell cycle withdrawal. Although the epicardium is not entirely a mitotic-refractory tissue, the mechanism leading to entering and retaining a cell quiescence is unknown. Interventions that ease the cell cycle checkpoints' transitory restrictions could help reactivate the regeneration in the epicardium in the postnatal stages. Studies on p21, an essential regulator of the G1 checkpoint, have demonstrated that its transient or permanent deficiency can enhance regeneration context-dependently. However, the mechanistic insights of the phenotype and its clinical application have yet to be elucidated. Here, we generated highly regenerative epicardial tissue using epicardial cells from human induced pluripotent stem cells (iPSCs) through the ectopic downregulation of CDKN1A, the gene encoding for the protein p21. Downregulation of CDKN1A improved epicardial re-epithelization, proliferation, and migration, an essential step in epicardial regeneration in vivo to differentiate into epicardial-derived cells (EPDCs). p21 deficiency led to the reactivation of WT1. Experiments performed in p21 -/- in vivo also demonstrated epicardial susceptibility to enhance their regenerative properties, suggesting that p21 could be a key regulator in the reactivation of the epicardium during adulthood. Our thorough evaluation of the safety of epicardial tissues derived from hiPSC cells downregulating p21 highlights their advanced degree of applicability to be used as a tool to understand the epicardial reactivation in cardiac regeneration, with high expectations to be used in cell therapy and clinical applications.

Keywords: epicardial regeneration, p21 deficiency, induced pluripotent stem cell

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MULTIOMIC ANALYSIS OF NADPH OXIDASE 4 HEART METABOLIC REPROGRAMMING: IMPLICATION FOR CARDIOGENESIS

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NADPH oxidases (NOXs) are enzymes that primarily function by generating reactive oxygen species, which are important regulators of heart physiology and post-insult response. While NOX-driven redox signalling has been well recognised in pathophysiological myocardial remodelling, Nox4 has attracted significant interest by protecting the heart from dysfunction and failure. Nox4 expression is increased during pathology, physiological stress and acute exercise, acting as a driver of adaptive stress responses. Nox4 is also capable of driving cardiomyocyte differentiation. However, the mechanisms of Nox4-mediated metabolic and transcriptomic remodelling remain elusive. Previously, it was shown that Nox4-driven cardiogenesis in pluripotent cells depends on c-Jun activation. Nox4 also induces reprogramming of heart intermediary metabolism, shifting cardiac energy substrate utilization from glucose to fatty acid oxidation, partially depending on ATF4. Because these mechanistic effects of Nox4 overexpression appear to rely on hydrogen peroxide-responsive transcription factors, we performed multi-omic analyses on the transcriptomes and epigenomes of wild-type and cardiomyocyte-specific Nox4-transgenic hearts. We found that Nox4 induced profound epigenetic changes such as gain of accessible chromatin at gene loci associated with cardiac conduction, energy metabolism and transport of inorganic ions and amino acids. These changes are positively associated with altered gene expression. In silico deconvolution of transcriptomes revealed an increase in macrophage population in Nox4-overexpressing hearts without an increase in inflammatory markers. Sarcomeric proteins were downregulated with Nox4 upregulation. Finally, integrated analysis connected these changes to diverted activities of hydrogen peroxide-responsive transcription factors. While NRF2 and ATF4 activity increased, the repressed activity of AP-1 and CREB was predicted, partly attributed to the loss of accessible cAMP-responsive elements. A deeper understanding of the interplay between Nox4-driven redox signalling and heart intermediary metabolism in human cardiac models is expected to unveil mechanisms of the adaptive cardiomyogenic programme, inspiring metabolism-targeted regenerative heart therapies.

Funding Source: Wellcome Trust British Heart Foundation

Keywords: NADPH oxidases, Cardiogenesis, Multiomics

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IMMUNOGENICITY OF INDUCED PLURIPOTENT STEM CELL (IPSC)-DERIVED SMOOTH MUSCLE CELLS RESULTS FROM A REDUCTION IN THE EXPRESSION OF INDOLEAMINE 2,3 DIOXYGENASE (IDO-1)

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Smooth muscle cells (SMCs) are a vital component of large blood vessels and SMC dysfunction is associated with many cardiovascular disease states. Despite progress in treatment strategies, including the development of tissue engineered vascular grafts (TEVGs), their successful application is limited by challenges including sourcing functional cells in high enough volumes for therapeutic use. The use of allogeneic iPSCs, differentiated to a cell type such as SMCs, is therefore an attractive alternative for the treatment of many cardiovascular diseases. However, before widescale therapeutic application of iPSC-derived SMCs, key aspects of these cells need to be characterised, including their propensity to trigger an immune reaction. Vascular SMCs are innately immunomodulatory, due to a lack of co-stimulatory molecule expression and interferon-γ-inducible expression of indoleamine 2,3 dioxygenase (IDO-1), whereas there is conflicting evidence for the iPSC-derived SMC immune profile. In this study, we investigate the functional immunogenicity of iPSC-SMCs compared to naturally derived vascular SMCs (v-SMC). We show that iPSC-SMCs trigger T effector memory (TEM) cell proliferation in both CD4 TEM (6.8-8.6%) and CD8 TEM (14.3-15.4%) in contrast to that of v-SMCs, which triggered minimal proliferation of both CD4 TEM (0.4-0.5%) and CD8 TEM (2.0-3.0%). However, expression of TEM activation-related antigens, HLA-ABC, CD86, LFA-3, and OX40-L, were comparable between the two cell types. Arterial v-SMC can establish immunoprivilege through IDO-1 activity, which is responsible for inhibiting T cell proliferation and promoting an anti-inflammatory phenotype. Therefore, we investigated the expression of IDO-1 in two independently engineered iPSC-SMC cell lines (N8 and Y6). Our data show that the expression and functionality of IDO-1 was significantly reduced in both iPSC-SMC cell lines compared to v-SMC, and that unlike v-SMC, neither iPSC-SMC line could modulate the immune response in a co-culture with CD3/CD28 activated PBMC. These results indicate that the impaired ability of iPSC-SMC to modulate the immune response through IDO-1 expression contributes to their immunogenicity and highlights the importance of immune phenotyping for therapeutic applications of iPSC-derivatives.

Funding Source: 1 Therapeutic Reference Materials, Medicines and Healthcare products Regulatory Agency (MHRA), South Mimms, United Kingdom. 2 Department of Immunobiology, Yale School of Medicine, New Haven, Connecticut, United States

Keywords: Induced Pluripotent Stem Cells (iPSCs), Immunogenicity, Vascular Smooth Muscle Cells (vSMCs)

TOPIC: EPITHELIAL INCLUDES SKIN GUT LUNG

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OPTIMAL WNT AGONIST FOR HIGH LGR5+ INTESTINAL ORGANOID GROWTH AND FUNCTIONAL DIFFERENTIATED ORGANOID INDUCTION

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Wnt has been widely used as an essential factor in human intestinal organoids. Although recombinant Wnt3a is commonly utilized as Wnt source in organoid culture, a single Wnt3a protein is water insoluble and is not stable in culture medium. Recently, recombinant Afamin/Wnt3a and Wnt Surrogate-Fc Fusion Protein were developed. These Wnt agonists are stable as a medium supplement and support long-term organoid proliferation. However, the information about differences of the characteristic among organoids cultured with each Wnt is limited. We compared three different Wnts (Recombinant Afamin/Wnt3a, Wnt Surrogate, CHIR99021). Gene expression analysis showed that LGR5 stem cell gene expression of organoids cultured with Afamin/Wnt3a was higher than that of the other Wnts and increased two-five-fold compared to human small intestinal tissue. This organoid could be passaged for long-term as high LGR5+ stem cell enrichment organoids. In contrast, low LGR5+ intestinal organoids could be cultured in low concentrations of Afamin/Wnt3a. The expression of transporters and cytochrome P450-related genes in these organoids was maintained as much as human small intestinal tissue. CYP3A4 activities of these cells also resembled that of human intestinal epithelial cells. Afamin/Wnt3a could support enriching LGR5+ stem cell. Furthermore, low LGR5+ intestinal organoids cultured in afamin/Wnt3a could be induced to functional enterocytes. It is indicated that intestinal organoid cultured with Afamin/Wnt3a could be a great tool for investigating and analyzing of stem cell and drug metabolism research.

Keywords: INTESTINAL ORGANOID, LGR5, CYP3A4

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FATE SPECIFICATION OF RARE CFTR HIGH EXPRESSER CELLS IN THE INTESTINE

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Rare cell types have been traditionally difficult to study due to their low abundance and lack of markers to facilitate isolation and enrichment. We lack foundational knowledge about how rare cell types contribute to tissue function and how their unique functional roles are established through differentiation from progenitors. Here, we investigate a rare and distinct population of intestinal epithelial cells found in rats and humans, CFTR High Expressers (CHEs), which are characterized by the high expression of CFTR (cystic fibrosis transmembrane conductance regulator). However, the physiological function and cell fate lineage origin of CHE cells have yet to be established. In order to understand the function of CHE cells in intestinal physiology, we must first determine how CHE cells fit into the cell fate specification hierarchy in the intestine. Here, we elucidate the fate specification of CHE cells in the small intestine using single-cell RNAseq and a novel rat intestinal organoid model. Our scRNAseq analysis has revealed a single CHE-specific transcription factor, Meis1, which may promote a unique CHE cell fate. Additionally, CHE cells express Prox1, similar to tuft cells and enteroendocrine cells. Our data support a model in which CHE cells, tuft cells, and enteroendocrine cells arise from a common progenitor. We demonstrate that CHE cell fate specification likely arises along the secretory lineage of intestinal epithelial cells in a shared trajectory with the poorly understood population of rare chemosensory tuft cells. Yet, paradoxically for the secretory lineage, which has been thought to remain Notch “off”, CHEs specification requires active Notch signaling. This suggests that Notch signaling is re-activated along the secretory lineage to specify CHE cells. Incorporating CHE cells into a revised map of intestinal cell fate specification will shift our knowledge of how intestinal secondary fates are determined and shed insight into diverse secretory progenitor populations.

Funding Source: Charles H. Hood Foundation; Yale School of Medicine; Yale Training Program in Genetics

Keywords: cell fate, intestine, Notch signaling

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MATERNAL 27-HYDROXYLASE ACTIVITY IS ESSENTIAL FOR FETAL LUNG DEVELOPMENT

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During pregnancy, the maternal body degrades toxic metabolites on behalf of the developing fetuses, since an accumulation of such factors could critically interrupt the proliferation and differentiation of tissue stem cells. However, what types of factors contribute to fetal-maternal crosstalk is still unclear. Here, we demonstrate that maternal 27-hydroxylase (Cyp27a1) activity is essential for fetal organ formation. Cyp27a1 is involved in various metabolic reactions, particularly in bile acid synthesis pathways. We discovered that depletion of Cyp27a1 in the mother resulted in a low pregnant rate and a smaller litter size. Survived newborn showed abnormalities in many organs, and all newborn mice died after birth due to respiratory distress syndrome (RDS) regardless of the newborn’s genotype. Histological analysis observed that alveolar structures were poorly developed in the lung of newborn mice delivered from Cyp27a1-/- mothers (mKO fetuses). Immunostainings and single-cell RNA-sequencing (scRNA-seq) revealed that E18.5 mKO embryos lack alveolar epithelial (AE) cells. scRNA-seq of E14.5 embryos found rather increased transcription levels of AE differentiation-related genes, e.g., Sftpc, and many ribosomal genes. GO analysis indicated that mKO fetuses have ribosomal defect signatures such as Diamond-Blackfan Anemia. Cyp27a1-/- mice are known for reduced bile acid pool and highly elevated levels of bile acid precursor, oxysterols. Notably, injection of one of the accumulating oxysterols, 7α-hydroxycholesterol (7α-HC), into wild-type pregnant mice led to the birth of newborn mice with RDS symptoms. Consistently, the addition of 7α-HC to the ex vivo whole organ culture of embryonic lung significantly reduced alveolar branching. An advanced proteomics technolo-

gy discovered that 7α-HC selectively binds to and destabilizes a protein Fau, which regulates the assembly of ribosomal proteins. In fact, both knockdown of Fau using shRNA and the addition of 7α-HC to the culture of murine lung cancer cell line (LLC) resulted in poor polysome formation, lower protein synthesis, and slow cycling. Our findings propose that the 27-hydroxylase activity is essential for fetal organ formation and maturation. It presumably correlates with some types of recurrent pregnancy loss and fetal growth retardation.

Funding Source: This work was supported by JSPS KAKENHI Grant Number JP20K23380 and AMED under Grant Number JP21gm6310030.

Keywords: Fetal-maternal crosstalk, Bile acid metabolism, Fetal growth retardation

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IMPOSING GEOMETRIC CONFINEMENT ON ANTERIOR FOREGUT ENDODERM CELLS TO UNDERSTAND PRIMITIVE GUT TUBE DEVELOPMENT

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The anterior foregut endoderm (AFE) is derived from the primitive gut tube (PGT) and eventually gives rise to the epithelia of many visceral organs such as the thyroid, thymus, trachea, esophagus, and lungs. Groups have developed various protocols to achieve the progenitor cell types of these organs in order to develop organoids. However, hPSC-derived thyroid, thymus, and trachea organoids are yet to be seen. These progenitor cell types have been difficult to achieve, perhaps due to their anterior positioning and requiring a complex signaling hierarchy. Here we employ micropatterns as a method to geometrically confine AFE cells. This promotes a microenvironment for self-organization and establishes internal signaling gradients amongst the cells. By introducing combinations of morphogens at different timepoints, we have developed a way to better understand the developmental pathways of progenitor cell types that arise from the AFE.

Keywords: Definitive endoderm, Micropattern, Signaling hierarchy

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DEFINING THE KEY ATTRIBUTES OF CANCER STEM CELLS IN HIGH-GRADE SEROUS OVARIAN CANCER

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Ovarian cancer is the 7th most common cancer in women globally. High-grade serous ovarian cancer (HGSOC) is a fatal gynecological cancer with a more than 80% recurrence rate for advanced cases within 24 months after treatment. Understanding the cause of cancer recurrence and optimizing therapy is highly vital. Cancer stem cells (CSCs) are a small subpopulation within the tumor that contribute to tumorigenesis, metastasis, chemo- and radio resistance, and pro-tumor immune phenotype. The present study utilized primary tumor, ascites, and chemo-resistant cells to examine stemness, epithelial-mesenchymal transition (EMT), and pro-tumor immunity. We hypothesize that in metastatic cells the proportion of CSCs will be higher, as observed by gene expression, mesenchymal traits, and stemness reporter expression. Our data indicate HGSOC ascites (metastatic) cells expressed higher levels of stemness-related genes, POU5F1 and SOX2, and an immune checkpoint inhibitor gene, PD-L1, than primary tumor cells. We observed EMT transcription factor genes Zeb1, SNAIL, SLUG, and TWIST were expressed at higher levels in primary tumor cells, while Zeb2 was higher in ascites cells. All mentioned genes were expressed at the highest levels in cisplatin-resistant cells. Furthermore, we incorporated a SORE6-GFP reporter to identify the cell population expressing Sox2/Oct4 and a 3' UTR-ZEB1-GFP reporter to detect the cell population with mesenchymal traits. To understand the role of CSCs in radiation, we transduced cancer cells with the reporters and then exposed them to 0, 1, 2, 4, and 8Gy of 250 MeV proton and 6 MeV photon beams. We observed that cisplatin-resistant cells had the highest resistance to radiation. We conclude that metastatic HGSOC cells exhibit higher levels of stemness, mesenchymal phenotype, and pro-tumor immunity genes. Studies to determine the role of these attributes in resistance to chemotherapy and radiation are ongoing.

Funding Source: California Institute for Regenerative Medicine

Keywords: Cancer Stem Cells, High-Grade Serous Ovarian Cancer, Epithelial-Mesenchymal Transition

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PRELIMINARY CHARACTERIZATION OF IPSCS DERIVED FROM THE BLACK-CAPPED CAPUCHIN MONKEY

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The plasticity of pluripotent stem cells, reflected in the capacity to originate every cell type of an organism, is one of the reasons they are abundantly investigated to this date. Additionally, the prospect of producing those cells from differentiated tissues, gained with the generation of the so-called “induced pluripotent stem cells (iPSCs)” opened countless possibilities for basic research, regenerative medicine, and cell therapy. Here, we aimed on the generation of iPSCs from the black-capped capuchin (Sapajus apella), a relevant non-human new world primate model. Capuchins are not only known for the usual physiological and anatomical similarities between human and non-human primates, but also for their high cognitive capacity, highlighting their relevance as a study models, especially for neurodevelopment, however, iPSCs from these models are still lacking and, to our knowledge, are unprecedented for the black-capped capuchin. In this study, fibroblasts isolated from vitrified skin biopsies were induced to pluripotency trough lentiviral reprogramming, using an excisable polycistronic vector containing human Oct4, Sox2, Klf4 and c-Myc factors. Colonies were first observed around 18 days after induction. Clonal colonies were first maintained in a feeder-dependent system, and later transferred onto a matrix (geltrex) based culture system, where cell lines were expanded and were partially characterized. Reprogrammed cells were positive for alkaline phosphatase detection, as well as Oct4 and Sox2, detected through immunostaining. Colonies were also able to generate embryoid bodies and further analysis aiming on molecular and morphological characterization are ongoing. We anticipate that the data generated in this study will contribute to the understanding of cellular reprogramming, pluripotency mechanisms and commitment, and in the future, can be used for studies in regard to differences and similarities between human and non-human primate models and also to generate specific cell lines.

Funding Source: FAPESP 2019/20527-0; FAPESP 2015/26818-5

Keywords: capuchin monkey, non-human primate, cellular reprogramming

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SINGLE-CELL ANALYSIS OF BIDIRECTIONAL REPROGRAMMING BETWEEN PLURIPOTENT EPIBLAST AND EXTRA-EMBRYONIC ENDODERM STATES REVEALS DIFFERENTIAL PLASTICITIES

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Two distinct cell fates, pluripotent epiblast (EPI) and primitive (extra-embryonic) endoderm (PrE), arise from common progenitor cells, the inner cell mass (ICM), in the mammalian embryo. To study how these sister identities are forged, we leveraged embryonic (ES) and eXtraembryonic ENdoderm (XEN) stem cells – in vitro counterparts of the EPI and PrE, respectively. Bidirectional reprogramming between ES and XEN coupled with single-cell RNA and ATAC-seq analyses uncovered distinct rates, efficiencies and trajectories of state conversions, identifying drivers and roadblocks of reciprocal conversions. While GATA4-mediated ES-to-iXEN conversion was rapid and nearly deterministic, OCT4, KLF4 and SOX2-induced XEN-to-iPS reprogramming progresses with low efficiency and slow kinetics. A dominant PrE transcriptional program, safeguarded by Gata4, and globally elevated chromatin accessibility of the EPI underscored the differential plasticities of the two states. Mapping in vitro trajectories to the embryo revealed reprogramming in either direction tracked along, and toggled between, EPI and PrE in vivo states without transitioning through the ICM, suggesting bistability of these states. In conclusion, our studies clearly demonstrate the differential plasticity

between ES and XEN cells and offer insights into the molecular basis of such differences.

Keywords: early blastocyst, reprogramming, single cell, epiblast, extra-embryonic endoderm, single-cell RNA-seq and scATAC-seq

TOPIC: GERMLINE AND EARLY EMBRYO

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HUMAN NAIVE PLURIPOTENT STEM CELLS ARE FUNCTIONAL BLASTOCYST-LIKE FORMING CELLS

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Self-organized blastocyst-like structures (blastoids) from human naive pluripotent stem cells (hnPSCs) provide an alternative and robust platform to investigate the pre- and post-implantation development of human early embryos in vitro. Various multi-step approaches have been established to generated human blastoids via inducing cell fate with specific factors at different stages. hnPSCs are postulated as being in a developmental continuum progressing towards embryo germ layer differentiation. However, the functional identity of hnPSCs is not well understood as they fail to respond to developmental cues. Here we report that robust self-renewing hnPSCs give rise to blastoids spontaneously, rapidly (within 3-4 days), and efficiently (>50%). In the poster we will communicate the mechanism underlying this process. Importantly, spontaneous blastoids model early stage human blastocysts in terms of structure, size and transcriptome characteristics. Further in vitro culture of spontaneous blastoids reaches a bipolar egg cylinder-like stage, morphologically and transcriptionally simulating human embryos at implantation. In conclusion, our study defines hnPSCs as functional blastocyst-like forming cells. This not only changes our conceptual understanding of hnPSCs but also sets up a new platform to explore early human embryo development in vitro.

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Keywords: HUMAN NAIVE PLURIPOTENT STEM CELLS, BLASTOIDS, IN VITRO CULTURE

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MODULATION OF HDAC ACTIVITY DIRECTLY REPROGRAMMES EMBRYONIC STEM CELL TO TROPHOBLAST STEM CELL

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Human naïve embryonic stem cells (ESC) show unrestricted potential to differentiate to trophoblast lineage. However, the epigenetic barrier between ESC and TSC in mouse is still largely unknow. Here, we show that mouse ESCs treated with sodium butyrate (NaB), an HDAC inhibitor can transdifferentiate into trophoblast stem cells (TSCs). Interestingly, although NaB treatment greatly increase the 2C-like cell population, this ESC to TSC transition is a direct reprogramming event that does not require transition through a 2C-like state. Mechanistically, butyrate inhibits Class I histone deacetylases activities in LSD1-HDAC1/2 corepressor complex, increasing acetylation levels in the regulatory regions and the mRNA expression level of abundant of TSC specific genes. Importantly, butyrate treated cells acquire the capacity to generate blastocyst-like structures that can develop beyond the implantation stage in vitro and induce decidualization in vivo. These results uncover how epigenetics restrict the trophectoderm fate in mouse ESCs.

Keywords: Transdifferentiation, Trophoblast stem cells, HDAC

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A NEW REGULATORS PARTICIPATES IN PRIMORDIAL GERM CELLS SPECIFICATION

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Germ cells are the carriers to pass genetic information between generations. Primordial germ cells (PGCs) are the first germ cell population established during development and are the common precursor for both oocytes and spermatogonia. It is essential to understand how PGCs acquire germ cell specific program and segregate from somatic fate at the embryonic stage. Although a few key transcription factors and signaling molecules (i.e., BMP4 and WNT) have been identified to play crucial roles in this process, the broader gene-regulatory network that controls PGC ontogeny remains unknown – feasibility limited by the low numbers of nascent PGCs and inefficient approaches to identify and functionally evaluate candidate regulators at large scales. A recently developed platform enables the generation of a large quantity of PGC-like cells (PGCLCs) from pluripotent stem cells (PSCs), and thus greatly facilitates molecular studies of germ cell specification. In addition, genome-wide screenings with CRISPR-based gene editing technologies have led to many discoveries but yet to be widely used for studying germ cell development. By using these platforms, our initial genome-wide screenings have identified more than putative regulators which include known PGC specifiers. We confirmed that many of top putative activators were highly expressed in PGCs, and some upregulated PGCLC

formation form PSCs when their expressions were enforced. In summary, our data unveil a broader regulatory network that controls PGC specification from pluripotent state.

Funding Source: This work was supported by NSF CAREER award (IOS2042908) and MSU startup fund to Dr. Yuan Wang.

Keywords: Primordial Germ Cells, CRISPR-based Gene Editing Technologies, Putative Regulators

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YAP1 KNOCKOUT IMPAIRED HUMAN PRIMORDIAL GERM CELL INDUCTION FROM HUMAN EXPANDED POTENTIAL STEM CELLS

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The Hippo signaling pathway is critical for stem cell self-renewal and differentiation during embryogenesis. The expression of YAP1, one of the Hippo pathway genes, is initiated in the outer cells of human morula. YAP1 is an essential modulator for first-lineage segregation. Previous studies reported that Yap1 modulates differentiation of mouse primordial germ cells (PGC) in vitro. The germline competence is compromised in Yap1-null mouse epiblast stem cells due to the reduced response to Wnt pathway, a critical pathway for mouse epiblast- PGC transition. However, the role of YAP1 in human germ cell lineage development is not fully elucidated. To address this question, we first re-analyzed the published dataset of human fetal PGC development. Interestingly, expression of YAP1 was detected from mitotic to meiotic stages of human PGC. We then utilized our novel human expanded potential stem cells derived from human embryos (hEPSC-em) as an in-vitro model for studying the role of YAP1 in early PGC development. Following the published protocol, hEPSC-em were first differentiated into pre-mesendoderm (pre-ME) stage, followed by 6 days differentiation of PGC-like cell (PGCLC). Gene expression analyses revealed significant induction of markers of pre-ME (Emoes, Mixl1) and PGCLC (Sox17, Nanos3, Tfap2c) at their respective stages, showing the efficient induction of germ cell lineage from hEPSC-em. In addition, decreased Dnmt3b and enhanced Tet2 expression were found during PGCLC differentiation, which was concordant with the reported global DNA demethylation. YAP1-knockout hEPSC-em generated by CRISPR/Cas9 approach was further used to confirm the role of YAP1 in PGC development. Our results showed that YAP1 depletion led to retention of markers of pre-ME but reduced expressions of germ-cell related markers during PGCLC differentiation. On the other hand, the expressions of DNA methylation-related genes were unaffected upon YAP1 knockout. Our findings demonstrated the importance of YAP1 during early PGC development in human.

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Keywords: Hippo pathway, Primordial germ cell, Expanded potential stem cell

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INVESTIGATING THE ROLE OF NANOG IN HUMAN PRE-IMPLANTATION DEVELOPMENT

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While molecular mechanisms that regulate epiblast and embryonic stem cells (ESC) pluripotency in the mouse have been characterised, this feature is poorly understood in the human. A role for the transcription factor NANOG has been demonstrated in the mouse, showing NANOG is necessary to specify the pluripotent epiblast that allows subsequent mESC generation. We have recently developed methods to understand gene function in human embryos using CRISPR/Cas9 genome editing. This project aims to characterise the NANOG- null mutant phenotype in human embryos using CRISPR/Cas9 genome editing. We hypothesize that NANOG functions to regulate the human pluripotent epiblast based on detailed protein expression characterisation indicating that this protein distinguishes the nascent human epiblast. Using MiSeq next generation sequencing, we have developed a screening pipeline to assay sgRNA efficiency in a quantitative manner in hESCs and identified several highly efficacious sgRNAs leading to NANOG null mutations. Using a novel electroporation pipeline, we targeted the NANOG locus and demonstrated that NANOG-null mutant mouse embryos recapitulate the previously published NANOG-null phenotype. Compared to zygote microinjection, we provide quantitative evidence that electroporation is a viable and efficacious method of delivering CRISPR-Cas9 to mouse embryos. We are now investigating the functional role of NANOG directly in human embryos and in human pluripotent stem cells. We will use marker expression, stem cell derivation and multi-omics to characterise the effect of NANOG loss on the three populations of the pre-implantation human blastocyst: the trophectoderm, epiblast and primitive endoderm. Understanding the role of NANOG in human embryos will provide crucial insights into human epiblast specification, the pluripotency gene regulatory network and the consequences of human zygote genome editing.

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Keywords: Embryology, Genome editing, Developmental genetics

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THE ROLE OF LET-7 IN HUMAN TROPHOBLAST DIFFERENTIATION

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First lineage segregation involves the formation of inner cell mass and trophectoderm. This process is crucial for proper embryo development and subsequent implantation. Because of the scarcity of human embryos for research, our current knowledge on the molecular events in lineage segregation mainly relies on mouse models. Our previous studies demonstrated the importance of a family of microRNA lethal-7 (let-7) in early mouse embryo development. We found over-expression of let-7 during cleavage stage led to lower blastocyst formation rate and embryo dormancy. More importantly, let-7 targeted and downregulated Tead4, an important trophectoderm marker. We hypothesize in this study that let-7 has a conserved role for modulating trophectoderm development in humans and mice. Here we utilized a novel human expanded potential stem cell derived from human embryos (hEPSC-em) as an in-vitro model for study. hEPSC-em was differentiated efficiently into early trophoblast lineage by administration of BMP4, A83-01 and PD173074 in the form of embryoid bodies (BAP-EB). Similar to the primed hESC, BAP-EB from hEPSC-em showed cystic structure at 48H post-differentiation with size and morphology reminiscent of human blastocysts. Moreover, single-cell RNA sequencing data revealed that the BAP-EB formed from hEPSC-em had high expressions of early trophoblast markers (CDX2, GATA2 and GATA3). We detected that the expression of let-7 was significantly downregulated during BAP-EB formation. More importantly, forced expression of let-7 mimic significantly hindered the cystic structure formation of BAP-EB at 48H post-differentiation. We sought to identify the downstream target of let-7 during human early trophoblast development. In-silico analysis predicted YAP1, an important human trophectoderm effector, was downstream target of let-7. We further confirmed the downregulation of YAP1 protein upon let-7 overexpression in BAP-EB. Our results suggested that let-7 has a conserved role in regulating human and mouse trophectoderm and early trophoblast differentiation.

Funding Source: National Key Research and Development Program of China (2022YFC2702503), Shenzhen Science and Technology Program (KQTD20190929172749226), Health@InnoHK from Innovation and Technology Commission.

Keywords: let-7, Trophectoderm, Human expanded potential stem cells

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REPROGRAMMING TOWARDS MULTIPOTENT, PLURIPOTENT AND EXPANDED POTENTIAL STEM CELLS WITH RE-ENGINEERED SOX17

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Homologues of SOX2 (SoxB group) and SOX17 (SoxF group) are found across multicellular animals. What sets SOX2 and SOX17 apart in mammals, is their ability to partner with OCT4 or other POU family factors on distinctive composite DNA elements. The selective dimerization on DNA leads to maintenance of stemness (SOX2) or to specify the germline (SOX17). The partnership with OCT4 is directed by a single amino acid which is conserved in all animals as a lysine in SoxB and a glutamate in SoxF. Mutating this amino acid turns SOX17 into an enhanced 'super SOX2' termed eSOX17 that accelerates pluripotency induction in mouse and human. eSOX17 permits the direct conversion of somatic cells into naïve and expanded potential stem cells. As part of two factor cocktails, eSOX17 directly transdifferentiates somatic cells from mouse or human into induced neural stem cells without detour to pluripotency and without the complete epigenetic reset associated with pluripotency induction. We will discuss how the cooperativity with POU factors, protein interaction networks, binding to nucleosome core particles, regulation of chromatin accessibility, formation of molecular condensates and chromatin scanning determines the outperformance of eSOX17 over SOX2 in stem cell generation and maintenance. eSOX17 could permit reprogramming of challenging tissues and species, advance reprogramming towards stem cells with features of totipotency and enable the decoupling of reprogramming from rejuvenation for studies on age-associated diseases and age reversal.

Keywords: Direct reprogramming, Naive pluripotency, Neural stem cells

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UNDERSTANDING THE ROLE OF TP53 IN THE MALIGNANT BEHAVIOR OF HUMAN PLURIPOTENT STEM CELLS

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The malignant potential of human pluripotent stem cells (PSC) is not fully understood and its evaluation currently relies solely on the assessment of the cells' behavior in vivo upon their engraftment into mouse models (teratoma assay). In vitro however, long-term culture of PSCs can lead to (epi)genetic drift, potentially activating processes that resemble malignant transformation as an adaptive mechanism to the culture conditions. Here we investigated the relevance of TP53, a key gene regulating cell cycle control and (epi)genetic stability and found mutated in various PSC lines. With this goal, TP53 knockout cell lines were generated through CRISPR-Cas9 technology of representatives of an embryonic (H9) and an induced (Lu07) PSC line. We demonstrate that despite the loss of TP53 expression and related depletion of P21 expression (mRNA & protein), the knockout lines did not show an increased expression of the pluripotency and malignancy-associated miRNA-371 and miRNA-373. Similarly, we did not observe changes in the expression levels of pluripotency markers both through qPCR (OCT4, SOX2) and flow cytometric analysis (OCT4, SSEA3, TRA-1-60, NANOG). Additionally, TP53 knockout cell lines displayed proliferation rates similar to those of their isogenic wild-type counterparts, despite showing a greater colony formation capacity. We also explored the effect of the depletion of TP53 to cisplatin exposure, a DNA-damaging agent commonly and successfully used in clinics for the treatment of malignant germ cell tumors, showing partial similarities to PSC-derived (in vivo) tumors. We observed that removal of TP53 led to increase of cisplatin resistance of PSC lines, similar to clinical observations in malignant germ cell tumors. Overall, this study shows that TP53 mutations in PSCs in vitro can lead to a greater resilience of the cells, while not necessarily triggering their oncogenic transformation, supporting the potential value of studying PSCs in the context of pathogenesis and clinical handling of malignant germ cell tumors and vice versa.

Keywords: Human Pluripotent Stem Cell, Malignancy, TP53

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3D ECM-RICH ENVIRONMENT SUSTAINS SINGLE HUMAN NAÏVE IPSCS TOWARD THE GENERATION OF REGION-SPECIFIC NEURAL ORGANOIDS

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The establishment of in vitro naïve human pluripotent stem cell cultures opened new perspectives for the study of early events in human development. The role of several transcription factors and signalling pathways have been characterized during maintenance of human naïve pluripotency. However, little is known about the role exerted by the extracellular matrix (ECM) and its three-dimensional (3D) organization. Here, using an unbiased and integrated approach combining microfluidic cultures with transcriptional, proteomic and secretome analyses, we found that naïve hiPSC colonies are characterized by a self-organized ECM-rich microenvironment. Based on this, we developed a 3D culture system that supports robust long-term feeder-free self-renewal of naïve hiPSCs and allows direct and timely developmental morphogenesis simply by modulating the signalling environment. Naïve hPSCs have the potential to differentiate into all embryonic and extra-embryonic lineages and are defined as an epigenetic "tabula rasa", since their DNA is broadly demethylated. Primed hiPSCs, instead, have been demonstrated to be hypermethylated and to possess heterogeneity in differentiation potential. Thus, we speculated we could recapitulate human early neurulation starting from the pre-implantation stage of pluripotency. Here, we adapted differentiation protocols to our 3D culture system to obtain neural organoids of different anteroposterior identities from naïve hiPSCs. We obtained 3D neuroepithelial cysts that we further differentiated into brain cortical organoids. By adding WNT activation we also differentiated naïve hiPSCs into neuromesodermal progenitors, from which cells of the developing spinal cord can be derived. Our study shows that naïve hiPSCs allow to study critical stages of human early development in 3D starting from a single cell. The clonal nature of our system may permit the study of tissue patterning unconfounded by pre-existing heterogeneity. Starting from the naïve state we may also access earlier state transitions and molecular processes relevant for healthy human embryogenesis or for the pathophysiology of epigenetic-related diseases, including Fragile X syndrome, that can't be studied in primed-derived models.

Keywords: Naïve pluripotency, Neural organoids, 3D morphogenesis

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THE TRANSCRIPTION FACTOR DUXBL IS REQUIRED FOR SUPPRESSION OF ZGA AND TOTIPOTENCY EXIT

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In mammalian embryos, DUX transcription factors drive a cleavage stage-specific transcriptional burst associated with zygotic genome activation (ZGA) during which hundreds of genes and endogenous retroviral elements are transiently expressed. In mice, ZGA sparks in late 1-cell embryos and its shutdown begins immediately after the mid 2-cell (2C) stage. While this transition is accompanied by loss of totipotency, it remains elusive how the toti- to pluripotent transcriptional switch is regulated. Here, we reveal an essential negative feedback loop by which the DUX family member Duxbl suppresses ZGA from the mid 2C stage onward. Consequently, genetic depletion of Duxbl results in sustained expression of ZGA genes, leading to a 4-cell stage arrest. Mechanistically, Duxbl binds Dux-occupied regulatory sites, and thereby counteracts Dux-mediated transcription in an embryonic stem cell based model. Our study reveals that Duxbl orchestrates totipotency exit, enabling the first divergence of cell fates.

Keywords: Zygotic genome activation, Totipotency, DUX factors

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HETEROGENEITY IN THE TIMING OF EMBRYO POLARIZATION BIASES LINEAGE SPECIFICATION IN THE MOUSE

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The first allocation of lineages in the mouse embryo separates an embryonic inner cell mass lineage from an extra-embryonic trophectoderm lineage. Segregation of these lineages is classically attributed to polarization of all blastomeres at the 8-cell stage - the process by which each cell gains an apical domain - as well as the subsequent cell divisions, in which the apical domain is differentially inherited. After division, those cells which remain polarized are specified as trophectoderm, whilst apolar cells are specified as inner cell mass. Recent evidence has shown that heterogeneities between blastomeres at the 4-cell stage also bias first lineage allocation, but the molecular and cellular mechanisms linking polarization and early heterogeneity have remained unclear. Here, we show that heterogeneity in the timing of polarization exists at the 8-cell stage, with a proportion of cells polarizing early, before compaction of the embryo, which is when cell-cell contacts increase and blastomere membranes flatten against one another. These 'early polarizing' cells have been shown to follow the canonical polarization pathway and display known markers of polarization, such as an apical accumulation of Par6, Ezrin and F-Actin, although they have distinct cellular properties such as a shorter apical-nucleus distance. Importantly, lineage tracing has shown that early polarizing cells are biased towards trophectoderm fate. This is consistent with the fact that early polarizing cells have a wider geometry and higher expression of the trophectoderm fate specifier Cdx2 at the end of the 8-cell stage. Moreover, inhibition of the arginine methyltransferase Carm1, whose heterogeneous activity at the 4-cell stage influences cell fate, increases the frequency of early polarization, as does overexpression of its downstream substrate BAF155. Overall, our study provides the first characterization of heterogeneity in timing of blastomere polarization in the mouse embryo. We demonstrate how polarization timing can be altered by early molecular heterogeneities to

influence cell fate, unifying previously separate models of the first lineage allocation.

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Keywords: apical-basal polarity, lineage specification, mouse embryo development

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DISSECTING THE ROLES OF TFS IN EARLY DEVELOPMENT USING CRISPR ACTIVATION AND MULTI-OMIC READOUT

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Early organogenesis involves a rapid diversification of cell transcriptional programs mediated by time- and space-specific activation of transcription factors (TFs) in the embryo. A widely held model in the field of transcriptional regulation argues that co-activation of specific combinations of TFs can interconvert cell types. In pluripotent cells, activation of individual "master regulator" TFs such as FOXA1 frequently leads to robust differentiation along the lineage in which the TF is specifically expressed in vivo. However, there are many lineages without defined master regulators. Our group recently generated a paired scRNA-seq and scATAC-seq (10x Multiome) atlas of mouse embryos from late gastrulation and early organogenesis between Embryonic day (E)7.5 and 8.75. A computational approach called in silico ChIP-seq was developed to assign putative cell-specific regulatory roles to TFs. Here, this method was applied to determine a list of candidate master regulator TFs during mouse early organogenesis. CRISPR single guide RNAs (sgRNAs) targeting five putative master regulators were pooled in a CRISPR-activation screen in pluripotent serum/LIF mouse embryonic stem cells with a 10x Multiome readout. Development of a novel bioinformatic analysis for guide amplicon sequencing libraries allowed sgRNA assignment to individual nuclei. Nuclei were then grouped by sgRNA before further analysis. Most strikingly, differential gene expression analysis demonstrates activation of lineage-specific transcriptional sub-programs together with lineage specific chromatin changes by corresponding master regulators. These experimental and analysis approaches demonstrate feasibility of sgRNA assignment from nuclear guide amplicon libraries, identification of transcriptome- and chromatin-based gene regulatory networks of master regulators, and are scalable to studying hundreds of TFs at once.

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Keywords: CRISPR activation, transcription factor, organogenesis

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UNCOVERING THE PRIMORDIAL GERM CELL TRANSCRIPTIONAL PROGRAM

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Germ cells contain the extraordinary potential to generate every cell in the body. This totipotent potential is established in the germline at the earliest stages of development and must be maintained for the duration of an individual's reproductive lifespan, a task that can last many decades in humans. To overcome this challenge, germline regulators activate the germline transcriptional program while simultaneously protecting germ cells from reprogramming to a somatic cell fate. In the dynamic embryo, a specific program for germ cell transcriptional activation has yet to be described, largely because a 'master-regulator transcription factor' for germ cell fate has not been identified. Here we discover the dynamics of germline gene activation during embryogenesis by identifying six specific, temporally regulated waves of transcriptional activation that define the PGC transcriptional program using single-cell RNA sequencing. Interestingly, two of these PGC transcriptional profiles include transient, zygotically transcribed RNAs that are known to be expressed early in the embryonic soma. We have identified the pioneer factor Zelda as a potential activator of this transcription and are currently probing how loss of Zelda in PGCs impacts germ cell development. Using tissue-specific interference, we are distinguishing between cell-intrinsic and non-autonomous signaling mechanisms regulating the PGC transcriptional program. To identify transcription factors that activate each of these transcriptional profiles, we performed ATAC-seq with footprinting analysis on isolated PGCs. In addition to known transcription factors, we have identified novel cis-acting sequences that are enriched in the open chromatin of PGCs that may regulate germ cell transcription. We are currently working to identify trans-acting factors that interact with these novel sequences to uncover if their activity is sufficient to activate germline transcription. Together, our analyses have generated a quantitative view of the transcriptional transitions that occur during PGC development. Now, with a better understanding of the transcriptional landscape of PGCs, we are beginning to uncover the specific transcription factors that activate the germline genetic program.

Keywords: Germline, Primordial germ cell identity, Transcriptional regulation

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HUMAN AND MOUSE EMBRYONIC GENOME ACTIVATION INITIATES AT THE ONE-CELL STAGE

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In fertilization, the gametes, sperm and oocyte, combine to produce a totipotent one-cell embryo. At this point, their genomes are transcriptionally silent, so they must be activated in the new embryo, a process termed embryonic genome activation (EGA). EGA is a critical developmental event, yet its timing and profile remain elusive in both human and mouse. The prevailing view is that EGA occurs by the eight-cell stage in human embryos, and the two-cell stage in mouse embryos. However, this reflects technical constraints, including transcript signal smoothing and low signal-to-noise ratios, as well as lack of synchronous embryos or, in human, restrictions on sample availability. These proposed timings leave multiple issues unresolved. We here address these limitations via high resolution single-cell RNA sequencing (scRNA-seq) following polyadenylation-independent library preparation. In the case of mouse, we constructed libraries of precisely-synchronous one-cell embryos produced by precisely coordinated sperm injection. Human healthy bipronuclear (2PN) one-cell embryos were subjected to analogous scRNA-seq. This strategy overcame resolution limitations and unveiled previously inaccessible gene expression changes in one-cell embryos. Results showed that human gene expression initiates at the one-cell stage and is disrupted in morphologically abnormal (1PN and 3PN) embryos. The mouse transcriptome profile revealed analogously low-amplitude gene expression that initiated as a program within four hours of sperm-egg union. We refer to the first 12 hours of gene expression as immediate EGA, iEGA. In both human and mouse, transcripts were canonically spliced and most were down regulated apparently coincident with a higher-amplitude wave of expression, termed 'major' EGA. Bioinformatic analyses of both human and mouse datasets identified upstream regulators, whose protein presence in early embryos was confirmed in the mouse. Many of the putative regulators are oncogenes, including MYC. Blocking the mouse ortholog of MYC, c-Myc, precipitated acute developmental arrest and abrogated iEGA. These findings illuminate intracellular mechanisms that regulate onset of mammalian development, totipotency establishment and suggest mechanistic parallels to the onset of cancer.

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Keywords: embryonic genome activation: EGA, human and mouse one-cell embryo, totipotency

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SYSTEMATIC MAPPING OF 3D ENHANCER INTERACTIONS REVEALS STEM CELL LINEAGE SPECIFIC REGULATORY NETWORKS

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Mammalian embryogenesis commences with two pivotal and binary cell fate decisions that give rise to three essential lineages, the trophectoderm (TE), the epiblast (EPI) and the primitive endoderm (PrE). Although key signaling pathways and transcription factors that control these early embryonic decisions have been identified, the non-coding regulatory elements via which transcriptional regulators enact these fates remain understudied. We have characterized, at genome-wide scale, enhancer activity and connectivity in embryo-derived stem cell lines that represent each of the early developmental fates, yielding high-resolution 2D and 3D regulatory maps of the first cell fate decisions. We observed extensive enhancer remodeling and fine-scale 3D chromatin rewiring among the three lineages, which strongly associate with transcriptional changes. In each lineage, high degree of connectivity or "hubness" positively correlates with levels and cell-type specificity of gene expression and enriches for essential genes. Genes within 3D hubs also show a significantly stronger probability of coregulation during cell fate transitions, compared to genes in linear proximity or within the same contact domains. By building and testing various computational models of transcriptional regulation; we found that incorporating specific 3D chromatin features outperforms models using only 2D promoter or proximal variables in predicting cell-type specific gene expression. Importantly, genome-wide in silico perturbations allowed us to nominate candidate functional enhancers in each cell lineage for validation at several relevant genomic loci. Our study comprehensively identifies 3D regulatory hubs associated with the earliest mammalian lineages and describes their relationship to gene expression and cell identity, providing a framework to understand lineage-specific transcriptional behaviors.

Keywords: 3D Chromatin Organization, Cell Fate Decisions, enhancer-promoter interactions

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COMBINATORIAL SIGNAL INTEGRATION AND TRAJECTORY DYNAMICS DURING HUMAN MESODERM AND ENDODERM SPECIFICATION

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Lineage specification is dictated by the complex interplay between combinatorial signals which guide cells along specific trajectories in gene-expression space. How combinatorial signalling cues drive cell-state transitions and when along trajectories cells commit to specific lineage identities remains a mystery. To explore these questions, we used human embryonic stem cell (hESC) differentiation to mesoderm and endoderm, two lineages driven by opposing growth factors BMP4 and Activin. Using

high-throughput quantitative imaging of hESCs challenged with combinatorial inputs of BMP4 and Activin we defined the concentration and duration thresholds required for commitment to mesoderm and endoderm. We found that cells challenged with endoderm-inducing cues exhibit a temporal window in which they can be diverted to mesoderm before reaching a defined endoderm commitment point. By combining live-cell imaging of endogenous cell fate reporters and single-cell RNA-seq we mapped the trajectories of differentiation from pluripotency to mesoderm and endoderm and found that changes in lineage competency corresponded to specific state transitions. Furthermore, we identified that endoderm-fated cells undergo a novel trajectory passing through a mesoderm-like progenitor state before irreversibly diverting to become endoderm. Finally, we identified sets of early driver-genes associated with entry/exit to this progenitor state which when genetically perturbed can extend/delay the window of commitment and rewire the relationships between signalling cues and cell fate. This work adds to our understanding of the interplay between signalling and cell-state transitions and more globally highlights how decision-making events can shape the topology of developmental trajectories.

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Keywords: Human gastrulation, Combinatorial signal integration, Single-cell trajectory dynamics

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IDENTIFICATION OF GENOMIC AND TRANSCRIPTOMIC CHANGES THAT OCCUR DURING IPSC REPROGRAMMING AND GENE EDITING

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iPSCs are a useful tool for modeling human development and disease. The development of gene editing techniques to create isogenic iPSC control lines have further increased the power of these cell lines. However, the potential for genomic and transcriptomic changes that could occur during reprogramming and gene editing have been the subject of much scrutiny. Current best practices suggest that multiple iPSC colonies and multiple cell lines be used in experiments to ensure the results are biologically relevant and not due to genomic or transcriptomic changes that occur upon reprogramming or gene editing. An individual iPSC line may harbor a wide range of genomic variations including aneuploidies, CNV, and SNPs that could affect the results of the functional assays that the cells are used in. These genomic variations may occur due to heterogeneity of the source cells, the reprogramming process, and/or extended culture of iPSCs. Genomic or transcriptome changes could also occur during gene editing of iPSCs as a result off target mutations. Previous studies have focused on comparison of iPSC lines to the source fibroblast when looking at genomic changes. Here, we used whole genome sequencing (WGS) to compare the genomes of multiple iPSC clones generated from erythroblasts isolated from whole blood (WB) to the WB DNA of the subject as well as between the iPSC clones and their isogenic counterparts. Since genomic variations often induce aberrations at transcriptome levels, we also evaluated how genomic events identified in the iPSC and subject samples will produce changes in transcriptomic profiles by performing RNA sequencing (RNA-seq) comparing the transcriptome differences between multiple iPSC clones from the same subject. Additionally, using RNA-seq we will compare the transcriptome of the iPSC clones with their edited counterpart. Comparing the data generated from WGS and RNA-seq of source cell, multiple iPSC clones from the same subject, and edited iPSCs will tell us if the reprogramming or editing process results in significant genomic or transcriptome changes that could affect downstream studies. This data can be used to determine if specific groups of genes or pathways may be perturbed when comparing different clones of the same iPSCs, between wild-type and mutant iPSCs, understanding their importance.

Funding Source: NINDS Human Cell and Data Repository 1U24NS095914

Keywords: induced pluripotent stem cells (iPSCs), whole genome sequencing (WGS), RNA sequencing (RNA-seq)

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DERIVATION OF MURINE TROPHOBLAST STEM CELLS AND GENERATION OF BLASTOIDS FROM EMBRYONIC STEM CELLS FOLLOW COMPETING MOLECULAR TRAJECTORIES

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Mouse embryonic stem cells (ESCs) are pluripotent cells, considered to lack the potential to contribute to trophoectoderm. Here, we report the derivation of trophoblast stem cells (ESTS) under defined conditions from ESCs. The ESTSs show transcriptome similarity to embryo-derived trophoblast stem cells and efficiently contribute to the placenta during development. Further, we generated blastoids (E-blastoid) consisting of all three lineages of blastocyst exclusively from ESCs at very high efficiency. The E-blastoids implant and induce decidualisation in utero. Their transcriptome resembles preimplantation blastocyst. A stage-specific transient activation of 2C-genes in ESCs is essential for the high efficiency of E-blastoid generation. Mechanistically, GSk3b-signalling inhibits trophoectoderm fate but promotes efficient blastoid generation by activating of 2C-gene network in ESCs. In contrast to the paradigm of the restricted potential of pluripotent ESCs, the murine ESCs have the unrestrianed potential for trophoectoderm

lineage and generation of blastoids composed of all lineages of the blastocyst.

Funding Source: Department of Biotechnology, India. CSIR-Centre for Cellular and Molecular Biology

Keywords: Trophoblast stem cells, Blastoid, Mouse embryonic stem cells

TOPIC: HEMATOPOIETIC, IMMUNE, AND ENDOTHELIAL

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INTEGRATIVE TRANSCRIPTOMIC AND EPIGENOMIC ANALYSIS IDENTIFIES BCL6B AS A NOVEL REGULATOR OF HUMAN PLURIPOTENT STEM CELL ENDOTHELIAL DIFFERENTIATION

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Due to the inaccessibility of the early human embryo, little is known about the chromatin status during human endothelial development. In this study, we used the human pluripotent stem cell (hPSC) differentiation system to sketch the epigenomic roadmap of endothelial cell (EC) development. By profiling the open chromatin of stage-specific progenitor cells, we showed that the binding sites of developmentally important transcription factors open up in a highly coordinated manner along the endothelial differentiation continuum. Through integrative analysis of the genome-wide association of key histone marks and accessible chromatin, we characterized broad H3K4me3 domains and three types of cis-regulatory elements (CREs) that correlated with the EC fate specification and differentiation stage. Finally, we identified BCL6B as a novel regulator of EC differentiation and subtype bifurcation. Our findings provide a valuable resource to study the epigenetic regulation of EC development and dysfunction.

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Keywords: human pluripotent stem cells, endothelial differentiation, epigenomic landscape

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ENGINEERING THE MULTICELLULAR NICHE FOR T CELL DIFFERENTIATION

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Induced pluripotent stem cell (iPSC) derived T cells show great potential for off-the-shelf immunotherapy. Methods for differentiating human T cells from iPSCs rely primarily on mouse cocultures, where Notch ligand delta-like ligand 4 (DLL4) are presented on mouse stromal cells (e.g., OP9) that arrest cells mostly at an immature stage. Recent advancements in feeder-free T cell differentiation have generated clinically relevant iPSC-T cells, yet focus on engineering synthetic thymic niches without understanding co-culture systems. Understanding cell-cell communication in the OP9-DLL4 platform can aid in engineering a T cell differentiation niche. To determine how surrounding cells drive T cell development, we modeled cell-cell communication in the human thymus using NicheNet. Ligands that impact the differentially expressed genes between in vitro derived and naïve CD8 T cells were predicted and their regulatory potential was plotted over pseudotime to achieve a temporal prediction of cell-cell signaling. We hypothesized that knocking out or activating Il15, Tnf, Cd80, Cxcl12, Il6, Crlf2, Tslp, Btla, Cd40, Cd40lg, Adam17, H2-d1 Or Il7 in the OP9-DLL4s may improve iPSC-T cell maturation to CD8. To validate our predictions in vitro, each ligand was knocked out in the OP9-DLL4 cells using CRISPR/Cas9. Day 21 T cell progenitors were seeded onto gene edited OP9-DLL4-Cas9-mCherry cells and assayed for CD4 and CD8 one week later. As expected, knocking out H2-d1 decreased the percentage of CD8 over CD4 T cells. Knocking out known targets Il15, Tnf and Cxcl12, and unexpected targets (Inhbb and Ocln) increased the percentage of CD8 over CD4 T cells. These data could uncover previously unknown roles for ligands in T cell differentiation and identify targets for manipulation of the differentiation niche. We present a systems-biology approach to understanding the OP9-DLL4 co-culture through modeling cell-cell communication and performing a CRISPR screen of computational hits. Future work includes further validation of knock outs in vitro and combinatorial addition of targets into a feeder-free T cell differentiation. By learning from current T cell differentiation methods, we can engineer a more biomimetic synthetic thymic niche for manufacturing of more potent iPSC-T cells.

Keywords: pluripotent stem cells, systems biology, T cells

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RUNX1 MODULATOR RO5-3335 INDUCES HEMATOPOIETIC STEM CELL DIVISION

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Hematopoietic stem cell (HSC) expansion and self-renewal are important for stem cell therapy. Expanding HSC ex vivo remains challenging. To identify novel compounds that increase HSCs, we screened 3840 small molecules using a zebrafish blastomere culture screening system with a HSC specific Runx1+23:GFP reporter and identified a Runx1 modulator Ro5-3335 that increases

Runx1+ cells. Using spinning disk confocal time-lapse imaging, we found that Ro5-3335 treatment increases HSPC number as much as 1.79 fold ($p< 0.0001$) and significantly increases HSPC divisions (5 vs 3.5 divisions, $p=0.009$) in the fetal/larval stem cell niche. To determine whether the increased HSPC production during development increases HSC clones in adulthood, we used a brainbow color barcoding system to lineage trace each HSC and its blood progenies. Treatment of Ro5-3335 during definitive hematopoiesis significantly increases HSC clones in adulthood (23 vs 18.6 clones, $p< 0.0001$). To determine whether the effect of Ro5-3335 in HSC expansion is conserved in human, we cultured human CD34+ HSPCs and found that Ro5-3335 promotes CD34+CD38-CD45RA-CD90+ HSPC expansion in 6-day ex vivo culture (20.3 vs 15.8 fold ($p=0.0157$)). RUNX1 is an important transcription factor for HSC proliferation and differentiation. Chromatin immunoprecipitation sequencing (ChIP-seq) of RUNX1 showed that Ro5-335 treatment increased RUNX1 binding to target genes in human CD34+ HSPCs. Motif analysis of the genes with increased RUNX1 binding and increased transcription suggested binding of Elf family transcription factors to RUNX1 target genes upon Ro5-3335 treatment. Elf1 ChIP-seq confirmed that Elf1 has increased binding to the Runx1 target including cell cycle genes CDC45, CDC37L, CCND2, and CCND3 upon Ro5-3335 treatment. Knocking down Elf2b abolished the effect of Ro5-3335 on HSPC expansion in zebrafish ($p=0.0003$). Together, our studies reveal that Runx1 modulator Ro5-3335 alters Runx1-Elf1 complex binding, leading to increased HSPC production and clonality.

Keywords: Hematopoietic Stem Cell Expansion, Stem Cell Clonality, Runx1 modulator

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DEVELOPMENT OF INDUCED PLURIPOTENT STEM CELL-DERIVED IMMUNOSUPPRESSIVE T CELLS AS A NOVEL THERAPEUTIC PLATFORM FOR INFLAMMATORY DISEASES

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Autoimmune- and transplantation-related inflammations occurs both acute and chronic, and lead to long-term disability and life threading disorders. Patients are usually treated anti-inflammatory drugs, biologic therapies, and immunosuppressive drugs but systemic treatment of these medications have side-effects. Although emerging efforts for engineering regulatory T cell (Treg) cell therapy such as chimeric antigen receptor (CAR)-Treg has been reported as new modality, there is still being researched and is not yet widely available as therapy. One of the challenges is autologous Tregs from patients may lose their number and immunosuppressive activity. Another important aspect is cost-effectiveness that must be addressed before cell therapy can be widely used in the clinic. In this study, we tried to develop immunosuppressive T cells from induced pluripotent stem cell (iPSC) to overcome these issues. We successfully established immunosuppressive T cells from iPSC-derived CD4+ T cells and these cells showed in vitro suppressive activity and identified optimized maturation and expansion conditions which are named AMRT. AMRT-conditioned CD4+ T cells expressed FOXP3, a master transcription factor of Tregs, and showed comparable profiles with primary Tregs such as high TSDR demethylation, low IFN-gamma secretion, and suppressive function in vitro. We then prepared human leukocyte antigen (HLA)-A2 CAR-transduced iPSC-derived immunosuppressive T cells an evaluated the ability to suppress xeno graft-versus-host disease (GvHD) in NSG mice treated with HLA-A2+ human peripheral blood mononuclear cells. These cells also suppressed the GvHD progression as much as primary Tregs. We further optimized differentiation process through generating FOXP3 gene-transduced iPSC to gain yield of immunosuppressive T cells. Collectively, iPSC-derived immunosuppressive T cell platform may offer a novel modality for treating wide variety of inflammatory diseases in the future.

Keywords: iPSC, Treg, GvHD

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DEVELOPMENT OF CHEMICALLY DEFINED, ANIMAL-ORIGIN FREE (CD-AOF) SUPPLEMENT FOR HPSC DERIVED αβT CELL DIFFERENTIATION

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Differentiation media for hPSC often contain animal-derived components such as serum or extracted materials from animal tissues. However, differentiation with animal-derived components have some obstacles to develop cell culture process for clinical application because of their lot-to-lot variation of components or risks of viral contamination. Therefore, it is desirable to use chemically defined and animal origin free (CD-AOF) media for developing safe and stable cell differentiation process. In recent years, as immune cell therapy has been approved, a lot of immune cell therapy products are being developed using blood cells such as CAR-T cells. In this study, we developed novel CD-AOF supplement to make αβ T cells from hiPSC. For developing αβ T cells, firstly we differentiated hiPSC to hematopoietic progenitor cells (HPC) with clinically applicable CD-AOF differentiation supplement, StemFit For Differentiation. We sorted CD34+ HPC and cultured for 4 weeks in T cell differentiation medium which composed of basal medium, novel CD-AOF T cell differentiation supplement, and growth factors to successfully induce CD4+CD8+

αβ T cells. We expect our novel CD-AOF T cell differentiation supplement will support development of safe and stable cell culture process for T cell therapy by the use of CD-AOF media for iPSC expansion and T cell differentiation.

Keywords: T cell differentiation, chemically defined and animal origin free, hematopoietic progenitor cells

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HISTONE REMODELLING DURING HUMAN HEMATOPOIETIC STEM CELL DIFFERENTIATION DEPENDS ON MICROTUBULE-DIRECTED NUCLEAR DEFORMATIONS

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The cell nucleus, which is considered the most rigid organelle within a cell, undergoes significant deformation during various biological processes, including cellular differentiation. Nuclear deformations arise from cytoskeletal forces being transmitted from the cytoplasm to the nucleus, inducing a process called nuclear mechanotransduction. Comprehension of this process, particularly in non-adherent cells is limited, despite its significance in development. In this study, we uncovered a novel nuclear mechanotransduction event during myelopoiesis, where hematopoietic stem cells (HSCs) differentiate into myeloid progenitors. In the course of myelopoiesis, the microtubule network forms bundles along the nuclear envelope through dynein tethering and actively deforms the ovoid HSC nucleus. We discovered that these nuclear deformations are microtubule-dependent and irreversible. Furthermore, our immunostaining analysis revealed nuclear envelope and chromatin remodelling at the deformed regions with loss of Lamin B and of both facultative and constitutive heterochromatin marks (H3K27me3 and H3K9me3, respectively). Our CUT&RUN-based epigenome analysis uncovered that microtubule-dependent nuclear deformation specifically triggers the de-methylation of histones associated with key myeloid genes, which is followed by transcriptional activation. Moreover, pharmacological disruption of microtubule forces in HSCs led to retention of repressive histone markers on key myeloid genes, resulting in hindered myeloid identity acquisition during ex vivo expansion, despite myelopoiesis-enhancing culture media. Our results reveal a novel regulatory role of microtubule dynamics in hematopoiesis. HSCs are responsible for maintaining lifetime blood homeostasis through balanced self-renewal and differentiation; a failure in differentiation can result in severe consequences, including leukemia and fatality. Understanding the functional significance of microtubule-dependent nuclear deformations in myelopoiesis is therefore of utmost importance. Our findings advance the comprehension of nuclear mechanotransduction in HSC differentiation and blood maintenance.

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Keywords: Nuclear Mechanotransduction, Hematopoietic Stem Cells, Nuclear Envelope

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ULTRASTRUCTURE AND TOPOLOGICAL PATHS OF NEUTROPHILIC NUCLEAR MORPHOGENESIS

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Differentiation of stem and progenitor cells into specialized mature cell lineages often involves morphological changes in subcellular structures. How the shapes of organelles are modulated during differentiation is often not well understood. We focus on neutrophils, which are derived from hematopoietic progenitor cells and play critical functions in inflammation and cancer. Whereas most cells in the body contain spherical nuclei, mature mammalian neutrophils uniquely have non-spherical, often multi-lobulated or toroid-shaped nuclei. The non-spherical nuclear morphology is evolutionarily acquired in mammalian neutrophils, strongly suggesting that this unusual shape closely relates to the immune functions of these cells. However, the ultrastructural features and topological path by which the spherical nuclei of progenitor cells morph into non-spherical shapes during neutrophil differentiation are poorly known. In this study, we implement focused ion beam scanning electron microscopy (FIB-SEM) and fluorescence expansion microscopy to image the nuclei of primary mouse hematopoietic progenitors and neutrophils at various stages throughout differentiation, yielding three-dimensional reconstructions of nuclear morphology at high resolutions. We observe complex nuclear contours and nuclear membrane expansions that have not been well appreciated. We further report, for the first time, the time-lapse visualization of nuclear morphological changes of in vitro differentiating neutrophils derived from Hoxb8-immortalized murine hematopoietic progenitors. We observe that neutrophils form typical toroid-shaped nuclei via nuclear elongation and circularization. In addition, we observe dynamic switches between toroid-shaped and open-band nuclear morphology for the same cells. Surprisingly, immature neutrophils with toroid-shaped nuclei can further divide into two daughter cells both inheriting the non-spherical nuclear shape enabled by active remodeling of the nucleus after cytokinesis, a finding which suggests an epigene-

tic memory governing nuclear shape. Our work reveals levels of complexity that challenge the pre-existing models of neutrophil nuclear maturation and showcases the dynamic morphological changes during lineage differentiation from stem and progenitor cells.

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Keywords: Neutrophil, Nucleus, FIB-SEM

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DISRUPTED LIPID HOMEOSTASIS AND LOSS OF IRF4 IMPAIRS LYMPHOID PROGENITOR MAINTENANCE IN A MURINE MODEL OF ACCELERATED AGING

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The aging immune system is characterized by increased bone marrow (BM) adiposity, clonal hematopoiesis, myeloid skewing, impaired lymphocyte function and response to infection, and loss of hematopoietic stem and progenitor cell regenerative capacity. Human aging studies have found alterations in lipid metabolism, such as increased saturated fatty acid accumulation and decays in n-3 polyunsaturated fatty acid (PUFA) species leading to cellular membrane rigidity. However, the extent to which impaired fatty acid metabolism contributes to immune system aging and altered hematopoeisis is largely unexplored. Therefore, we characterized BM aging phenotypes in a unique aging model that expresses a mutant inactive form of Elongation of very long chain fatty acids protein 2 (ELOVL2), which is responsible for extending 22-carbon PUFAs to 24-carbon PUFAs. We performed lipidomics analyses, RNA-sequencing, and immunophenotype profiling in BM samples from young (3 months old) and aged (18 months old) wild-type mice, compared with age-matched Elovl2 mutant mice. Lipidomic profiling showed an almost complete depletion of products of ELOVL2 activity in mutant mouse BM, suggesting significant changes in the biophysical properties of cellular membranes. Gene set enrichment analyses (Reactome) revealed that pathways involving B cell receptor (BCR) signaling and lymphopoiesis were among the most disrupted in Elovl2 mutant mouse BM compared to age-matched controls. Key lymphoid lineage maturation markers (Cd19, Cd22, and Slamf7, among others) were downregulated in the BM of aged versus young wild-type mice, and to a greater extent in aged Elovl2 mutant animals. This was accompanied by a significant downregulation of the lymphoid and plasma cell transcription factor, interferon-regulatory factor-4 (Irf4). Immunophenotyping profiles and IRF4 protein expression were also evaluated by flow cytometry. These results provide insights into a link between disrupted lipid homeostasis and aging-related defects in hematopoietic stem cell development, leading to impaired maturation of B cell and plasma cell populations. Thus, lipid catabolism pathways may provide a novel regenerative medicine target for age-related disease modeling and reversing a variety of aging-associated immune deficits.

Funding Source: LAC is a Scholar of the Leukemia & Lymphoma Society.

Keywords: aging, hematopoiesis, lipidomics

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SINGLE-CELL TRANSCRIPTOMICS OF GENETICALLY-MATCHED IN VIVO AND IN VITRO-DERIVED HUMAN HEMATOPOIETIC STEM/PROGENITOR CELLS REVEALS NOVEL ROLE OF MICRORNA-MODULATED REGULATORY PATHWAY GENES

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Researchers have been working for over two decades on ways to develop hematopoietic stem cells (HSCs) in vitro from human pluripotent stem cells (PSCs), given the significant impact such cells could have to both basic research and clinical medicine. Although there have been advances towards this goal, the problem remains that in vitro PSC-derived human hematopoietic stem/progenitor cells (HSPCs), unlike their in vivo HSC counterparts, are unable to fully reconstitute the hematopoietic system. While comparative transcriptomic studies of in vivo HSCs and in vitro PSC-derived populations have led to significant insights, they have also been hindered by a number of potentially confounding effects. The inclusion of non-functional cells in the in vivo HSC population due to the previous lack of a well-defined cell surface marker profile, the effect of individual genetic background variation on differential gene expression in genetically-diverse samples, and the use of different tissue sources and developmental stages might all lead to the detection of differentially-expressed genes that may not be affecting cell function, or the masking of more subtle changes that are critical for cell function. We hypothesized that a more focused view of molecular variation underlying functional differences could be achieved by profiling a pure population of in vivo HSCs and performing comparative analysis on genetically matched in vitro cells. To accomplish this goal, we performed the first single-cell transcriptomic analysis of genetically matched in vivo HSCs and in vitro iPSC-derived HSPCs, and demonstrate the unique advantages provided by removing the influence of genetic background as a source of variability between populations. Comparison of genetically matched samples revealed novel regulatory pathways modulated by miRNAs, many with no currently known strong associations with hematopoietic development. miRNA targeted genes display significant enrich-

ment for hallmark pathways related to cellular function and include highly-expressed genes in both in vivo and in vitro cells, as well as factors previously identified as important for in vitro HSC derivation. We propose that modulation of these identified key miRNAs during in vitro differentiation provides a novel approach to obtaining functional HSCs from human PSCs.

Keywords: hematopoietic stem cell, pluripotent stem cell differentiation, reprogramming

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MECHANISMS OF FLOW-DRIVEN TRANSCRIPTIONAL CONTROL OF HEMATOPOIETIC STEM AND PROGENITOR CELL DEVELOPMENT BY YAP AND TAZ

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Hematopoietic stem and progenitor cells (HSPCs) emerge from hemogenic endothelium (HE) in select arterial niches of the vertebrate embryo that adopt hematopoietic fate during development, driven by the Runx1 transcription factor (TF). A major goal of cellular therapeutics is to derive patient-specific HSPCs from iPSCs for clinical use, yet current differentiation protocols largely fail to produce or expand long-lived multi-potent HSPCs in vitro, suggesting a lack of in vivo regulatory cues. Physical forces of wall shear stress (WSS) and cyclic stretch (CS) produced by hemodynamic blood flow are required to generate HSPCs from HE, but the mechanisms by which these forces are sensed and converted into a "stemness" regulatory module remain unknown. Here, using transcriptomic and genetic approaches in the zebrafish model, we show that the YAP TF is responsible for maintenance, not initiation, of the hematopoietic program in newly specified HE. Using a heat shock-inducible dominant negative YAP zebrafish line, we reveal a role for the YAP paralogue TAZ in hematopoiesis, which can promote CD41+ and Flk+/Myb+ HSPC production upon reduced YAP function. YAP and TAZ, members of the Hippo signaling pathway, initiate transcriptional responses downstream of mechanical stimuli and require DNA binding cofactors to direct activation or repression of target genes. Surprisingly, using luciferase-based YAP- and RUNX-responsive reporter assays in HEK293 cells, we saw a potent synergistic effect of TAZ/RUNX1, but not YAP/RUNX1, in transcriptional regulation at RUNX binding elements. Finally, by pharmacologic and genetic manipulation, we identify the stretch-gated membrane ion channel Piezo1 as a regulator of CS-induced YAP/TAZ mechanotransduction in HE. Stimulation of zebrafish embryos with the Piezo1 small molecule agonist Yoda1 increases HSPC number and YAP target gene expression in a YAP-dependent fashion. A similar modulation of blood and YAP target genes in human iPSC-derived CD34+ HE cells is seen with Yoda1, suggesting that this stretch-Piezo1-YAP axis can be tuned in vitro by pharmacologic agents to enhance HSPC differentiation. These results have broader implications for alternate regulatory effects of mechanically-stimulated Hippo TFs depending on the transcriptional milieu in cell-type specific contexts.

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Keywords: Hemogenic endothelium, YAP/TAZ, hematopoiesis

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DECEPHERING THE ROLE OF EOMES IN EXTRA-EMBRYONIC HAEMATOPOIESIS USING SINGLE-CELL MULTI-OMICS

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Eomesodermin (Eomes) is a T-box family transcription factor (TFs) that is expressed at early stages of mouse gastrulation (E6.5 – E7.5). Eomes is required for the generation of definitive endoderm and a subset of mesodermal derivatives (cardiac and hematopoietic). During mouse development, blood progenitors arise in the yolk-sac in two temporally restricted waves. The first wave (E7.5) gives rise to primitive erythrocytes, while the second wave (E8.5), forms multipotent erythro-myeloid progenitors via an

endothelial intermediate (i.e. hemogenic endothelium). Our previous work suggested that Eomes regulates yolk-sac blood via epigenetic mechanisms as phenotypic defects occur days after transient Eomes expression. Understanding the role and hierarchy of TF regulators during gastrulation helps unveil the complex regulatory networks that guide cell fate decisions in early development. Here, we aim to further understand the specific roles of Eomes during the development of different embryonic lineages using in vitro and in vivo systems in combination with transcriptomic and epigenetic single-cell sequencing. Using scRNA-seq of E8.5 Eomes KO chimeras, we recapitulate known disruptions in the endoderm and mesoderm and additionally observe differential abundances of a subset of extra-embryonic cell types. Interestingly, we observe changes in abundance to a subset of ectodermal lineages in Eomes knockout chimeras, suggesting that Eomes plays a broader an previously reported . To further study the mesodermal role of Eomes, we temporally modulated its protein expression using an ES differentiation model of yolk-sac haematopoiesis and performed single-cell multiomic (RNA+ATAC) profiling. Upon Eomes KD, cells are pushed down alternative, non-haematopoietic, differentiation trajectories. Differential expression analysis reveals which genes are implicated in these dramatic disruptions and suggest that Eomes may play a critical repressive function at the outset of gastrulation. Together, these results further elucidate possible downstream mechanisms by which Eomes plays a crucial role in gastrulation.

Funding Source: Wellcome Trust

Keywords: Embryonic haematopoiesis, Transcription factor regulation, Cell fate decisions

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REJUVENATION: IMPROVING T-CELL ANTITUMOR PROPERTIES THROUGH PARTIAL REPROGRAMMING

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Cellular identity and age determine T-cell function and fitness over an organism's lifespan. Increased T-cell age and differentiation are associated with reduced efficacy of solid tumor cell therapy (Kishton et al., 2022). As such, methods to de-differentiate T cells into induced pluripotent stem cells (iPSCs) that return to embryonic immaturity, but lose their functional identity, have been explored. Early work revealed challenges to re-differentiate iPSCs into T cells with the desired functional phenotype under a complex and time-consuming process. Our novel strategy to counter the impact of aging on T-cell function through cellular rejuvenation is achieved via partial reprogramming of aged T cells by transient expression of transcription factors associated with iPSC reprogramming. Our partial reprogramming methodology reduces epigenetic age and rejuvenates T cells while reacquiring the phenotype and function of conventional T cells. We were the first to illustrate the ability to reduce the epigenetic age of T cells without fully transitioning to pluripotency. In initial studies with PBMC (n=3), we observed a 27.4+/-9.3% years' reduction in age. RNAseq analyses show that rejuvenated and conventional T cells have equivalent transcriptomes, suggesting maintenance of identity. Functionally, the rejuvenated T cells are characterized by improved cell-expansion capacity and increased expression of biomarkers associated with T-cell stemness, including CCR7 and CD62L. In vitro studies reveal that rejuvenated NY-ESO-1-targeted T-cell receptor (TCR) and CD19-targeted chimeric antigen receptor (CAR) T cells exhibit improved antitumor properties compared with non-rejuvenated T-cell controls in sequential cell-killing assays, extending functional tumor-cell killing by 2-3 rounds. We also confirmed enhanced in vivo antitumor efficiency of rejuvenated T cells using NY-ESO-1 TCR in a murine xenograft tumor model. Similar improvements in T-cell stemness phenotype, and cell-expansion capacity were shown when we applied rejuvenation to tumor-infiltrating lymphocytes (TIL). These results highlight the potential application of rejuvenation across all major T-cell therapeutic modalities, which may in turn improve outcomes for patients with solid tumors.

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Keywords: Rejuvenation, T cell, Antitumor

TOPIC: KIDNEY

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FGF9 TREATMENT REMOVES OFF-TARGET CHONDROCYTES FROM IPSC-DERIVED KIDNEY ORGANOIDS

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Chronic kidney disease affects 11–13% of the global population. Worldwide, 9.7 million people need kidney replacement therapy but only 2.6 million are able to receive it, increasing the pressure to find alternative solutions. One regenerative medicine alternative comprises the use of induced pluripotent stem cell (iPSC)–derived kidney organoids as a therapeutic engraftment to the dysfunctional kidney. However, several drawbacks need to be overcome before clinical translation, among which is the presence of non-renal populations such as cartilage. The aim of our study was to prevent the appearance of cartilage using phar-

macological and biomolecular approaches. We modified the culture protocol and maintained kidney organoids in medium containing fibroblast growth factor 9 (FGF9) for one additional week compared to the protocol first described by Takasato et al.. We harvested organoids at several time points to assess the appearance of cartilage and the expression of key renal markers. Whole organoid staining and cryosections showed cartilage forms between day 18 and 25 after aggregation (day 7+18 and 7+25), with a concomitant increased expression of markers such as ACAN, COL2A1, COL1A1 and COL10. Conversely, the FGF9-treated kidney organoids had no cartilage at day 7+25 and had diminished chondrocyte marker expression. Interestingly, epithelial–mesenchymal transition markers such as vimentin and alpha-smooth muscle actin were also reduced in FGF9-treated organoids. Importantly, the renal structures assessed by immunofluorescence were unaffected by the FGF9 treatment. To conclude, we improved the quality of kidney organoids cultured at the air–liquid interface by removing the off-target cartilage. This produces a higher quality kidney organoid that can be maintained longer in culture to improve their maturation for further in vivo work.

Funding Source: This research was funded by RegMed XB Kidney Moonshot consortium

Keywords: Kidney organoids, Cartilage, small molecules strategy

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EXPLORING THE BIOLOGICAL AGING DYNAMICS IN RENAL DEVELOPMENT ORGANOID MODEL

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With the development of omics-based aging biomarkers named aging clocks, one can precisely estimate the biological age of an organism from its tissues. The clocks emerge as promising molecular estimators of biological age based on methylation or transcriptomic profiles, shown to precisely quantify the aging process of humans and multiple other species. Notably, recent studies have tracked the biological aging dynamics during early embryonic development, pointing out a point of the lowest biological age "ground zero". To study the biological age dynamics during human embryogenesis, we established a differentiation culture system that mimics embryonic kidney development from human iPSCs. We applied the aging clocks to this system, and analysed samples at each developmental stage, from hiPSCs to kidney organoids. We performed DNA methylation and RNA-seq analyses and applied various aging clocks. We observed a biological age at its lowest point around the intermediate mesoderm stage in early development, indicating a potential rejuvenation event in renal development. This might be "ground zero" in the phase of embryogenesis. Principal component analysis suggested that the developmental process may comprise two processes with distinct implications for aging. We found that PC1 increased monotonically with development, while PC2 increased in the phase of the primitive streak and decreased after the mesoderm phase. We further compared them with our previously-established transcriptomic signatures of aging and reprogramming. Interestingly, we discovered that PC1 correlated positively and PC2 correlated negatively with signatures of reprogramming. This indicated that PC1 may represent mostly age-related genes and PC2 may represent reprogramming-related genes. PC1 and PC2 were compared with functional pathways involved in aging or reprogramming, to elucidate the relationship in detail between them. Some aging-related pathways are activated in the phase of the primitive streak and then suppressed. Therefore, it can be hypothesized that those pathways promote rejuvenation after aging. Taken together, we identified the "ground zero" in a model of early embryonic development and analyzed the development process in the context of functional signaling pathways and transcriptomic signatures.

Funding Source: This work was supported by JSPS KAKENHI Grant Number JP22K15354, 2020 iPS Academia Japan Grant, The Uehara Memorial Foundation and Takeda Science Foundation.

Keywords: kidney, aging clock, omics

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COMPARATIVE SINGLE-CELL ANALYSES IDENTIFY SHARED AND DIVERGENT FEATURES OF HUMAN AND MOUSE KIDNEY DEVELOPMENT

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Mammalian kidneys maintain homeostasis of tissue fluids and other critical aspects of systemic function. A vascular-derived renal filtrate is processed by the conjoined epithelial networks of the nephron and collecting system. Each epithelium originates from a distinct progenitor cell type, nephron and ureteric progenitors, respectively, through reciprocal cell interactions, over a lengthy period of development. To extend a comparative understanding of these developmental events between human and mouse kidneys, we profiled chromatin organization (ATAC-seq) and gene expression (RNA-seq) in developing human and mouse kidneys. Data were analyzed at a species level then integrated into a common, cross-species multimodal data set. Comparative analysis of cell types and developmental trajectories identified conserved and divergent features of chromatin organization and linked gene activity, revealing species- and cell-type specific regulatory programs. Identification of human-specific enhancer regions linked

through GWAS studies to kidney disease highlights the potential of developmental modeling to provide clinical insight.

Funding Source: National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), (Re)Building a Kidney (RBK), Chan Zuckerberg Initiative (CZI)

Keywords: human and mouse kidney development, multiomic analysis, species-specific

TOPIC: LIVER

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BIOENGINEERED LIVER FOR ACUTE LIVER DISEASES

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Liver transplantation is the only available treatment for patients with chronic and acute liver diseases. Unfortunately, ~20% of these patients die on the waiting list to receive a donated organ. Furthermore, the number of patients who could benefit from liver transplantation is increasing every year. To address this major unmet clinical need, we aimed to develop a cell-based bioengineered liver that can serve as a 'bridge therapy' for acute liver disease until a suitable donor is found or until endogenous regeneration can take place. To achieve this goal, we developed a co-culture system to combine human induced pluripotent stem cells (hiPSCs) derived forward programmed hepatocytes (FoP-Heps) and human umbilical vein endothelial cells (HUVECs). We co-cultured FoP-Heps and HUVECs together in suspension in low attachment U-bottomed 96-well plates. Cells grown in these conditions formed aggregates within 48 hours of seeding. FoP-Heps continued to express albumin (ALB) and HUVEC cells continued to express PECAM1/CD31 until day (D)15 of suspension culture. We then focused on D5 and D10 aggregates for further analyses. Compared to the 2D FoP-Heps, 3D aggregates showed upregulation of hepatic markers-ALB, HNF4a, A1AT; liver zonation markers-GLUL, AXIN2, ASS1, PCK1; cytochrome450 gene-CYP3A4, nuclear receptor genes-PXR, CAR; blood clotting factors-FVII, FIX and liver maturation marker-G6PC, metabolizing enzymes-UTG2A1, UTG1A6. The expression of these markers was comparable in FoP-Heps/HUEVCs aggregates compared to FoP-Heps only aggregates. Interestingly, the FoP-Heps/HUEVCs aggregates showed higher CYP3A4 activity compared to FoP-Heps only aggregates. Overall, these results showed that Fop-Heps and HUVECs maintain their identity and are compatible to support each other during co-culture. Moreover, our co-culture approach improves the level of functional maturity in hepatocytes. These results demonstrate the interest of these bioengineered livers for therapeutic applications in the context of liver diseases.

Funding Source: This work is supported by Wellcome Leap Program in Human Organs, Physiology, and Engineering (HOPE).

Keywords: human induced pluripotent stem cells (hiPSCs), Forward programmed hepatocytes (FoP-HePs), bioengineered liver

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N-METHYL-PROPTOPORPHRIN IX MEDIATES HEPATOCYTE REPROGRAMMIING TO PROGENITOR IN LIVER INJURY

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Liver has been renowned for its remarkable regenerative capacity, we tried to address the potential of reprogramming mature hepatocytes to bipotential progenitors for the purpose of repairing liver injury. Initial efforts had demonstrated that periportal hepatocytes could be reprogrammed into Sox9-expressing progenitor cells in mice treated 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC). However, we discovered ABCG2 deficiency affected reprogramming-mediated liver repairing. To reveal insight into the cellular and molecular mechanism of the liver regeneration and to develop strategies to trigger hepatocyte reprogramming toward bipotential progenitor fate via activating the genetic routes that mimicking natural regeneration machineries, The further analysis revealed, in DDC-treated mice, N-methyl-protoporphyrin IX-(N-methyl-PPIX) produced from the breakdown of Cytochrome P450 and transported through ABCG2 could trigger induction of Sox-9 expression in hepatocyte reprogramming. We found depletion ABCG2 possibly restricted N-methyl-PPIX transportation thus inhibiting Sox9 induction and hepatocyte reprogramming. In contrast, ductular reaction and hepatocyte reprogramming toward Sox9-expressing progenitor cells could be restored either by p53 knockout or by rescuing ABCG2 expression via introduction of pCMS-ABCG2-EGFP into the liver of ABCG2-knockout mice by hydrodynamic injection. Immunohistochemical staining further identified induction of SOX9 was found to associated hepatocyte dedifferentiation together with upregulation of Yap1, pERK and NEATc1. Importantly, we found that treatment of human and mouse primary hepatocytes with N-methyl-PPIX was sufficient to trigger Sox9-expression. We also demonstrated N-methyl PPIX together with EGF/bFGF/HGF can efficiently induce human hepatocyte reprogramming toward expandable Sox9-expressing cells in vitro. And transplantation of reprogrammed Sox9-expressing cells into to the mice treated with carbon tetrachloride (CCL4) could replenish damaged hepatocytes. Hopefully, the findings from the work can lead to understanding the insight of liver regeneration and to development of cell therapeutic strategies for patients suffering from liver injury.

Funding Source: National Science and Technology Council Grant 108-2321-B-010-006 & 110-2740-B-001-005

Keywords: Liver repair, hepatocyte reprogramming, ABCG2

TOPIC: MESENCHYMAL STROMAL CELLS, ADIPOSE, AND CONNECTIVE TISSUE

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ODD-SKIPPED RELATED 1 IS REQUIRED FOR EPITHELIAL AND MESENCHYMAL CELL DIFFERENTIATION DURING BLADDER DEVELOPMENT

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Odd-skipped related 1 (Osr1) is zinc finger transcription factor that regulates mesenchymal progenitor cell survival and differentiation in the kidneys, lungs, and limbs during embryonic development. Our lab identified that Osr1 is expressed strongly in the embryonic bladder upon its formation and continues to be expressed in the bladder into adulthood. The bladder is formed from a layer of epithelial cells derived from the hindgut of the embryo and the surrounding tailbud mesenchyme. Crosstalk between the epithelium and mesenchyme patterns the respective layers through molecules like sonic hedgehog, fibroblast growth factors, Wnts, and bone morphogenetic proteins. However, little is known about how epithelial and mesenchymal cells interpret these signals. Here we show that Osr1 is required for the development of both epithelial and mesenchymal progenitor cells during bladder development. Osr1 knockout mouse embryos at embryonic day (E) 15 have decreased smooth muscle cell differentiation in the bladder seen by histology and immunofluorescent staining for alpha-smooth muscle actin. Other features noted in the bladder include a lack of collagen, loss of subepithelial mesenchymal cells, and impaired epithelial cell differentiation. Interestingly, when we remove Osr1 from the epithelial and mesenchymal progenitor cells using lineage-specific cre drivers, loss of Osr1 in the mesenchyme results in an epithelial defect. In contrast, loss of Osr1 in the epithelium results in a marked decrease in mesenchymal cells within the subepithelial layer. This suggests that Osr1 is required for signaling cross-talk that drives differentiation of epithelial and mesenchymal progenitor cells within the embryonic bladder.

Funding Source: Fonds de Recherche du Quebec Sante (FRQS), Pierre-Lavoie Foundation, Research Institute of McGill University Health Center

Keywords: Transcriptional control of differentiation, Epithelial-Mesenchymal cross-talk, Fetal development

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MIR-29A-3P CIRCUITRY PROMOTES CELL MIGRATION IN HUMAN MESENCHYMAL STEM CELLS

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Mesenchymal stem cells (hMSCs) are multipotent stem cells with a great potential for cell therapy. One of the features that make them an attractive candidate for clinical application is their ability to migrate into injured sites. However, the mechanisms by which microRNAs (miRNAs) regulate hMSCS migration are poorly understood. To systematically understand the role of miRNAs during hMSC migration, we globally depleted miRNAs by silencing the DiGeorge syndrome critical region 8 (DGCR8) gene, an indispensable element of miRNA biogenesis. DGCR8 knockdown hMSCs exhibited severe migration defects. We identified that overexpression of miR-29a-3p restored cell migration defects observed in DGCR8 knockdown hMSCs by affecting the multistep migratory process, including cellular polarization, focal adhesion formation and maturation, actomyosin contractility, and cellular traction forces. Using computational and experimental methods, we demonstrated that miR-29a-3p directly regulates the expression of phosphatases involved in cell adhesion and motility, such as protein tyrosine phosphatase receptor type K (PTPRK) and phosphatase and tensin homolog (PTEN). Furthermore, we found that miR-29a-3p promoted hMSC migration in vivo. Our findings provide a comprehensive understanding of how miR-29a-3p controls hMSC migration both in vitro and in vivo.

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Keywords: Cell migration, microRNAs, Human mesenchymal stem cells

TOPIC: MUSCULOSKELETAL

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REGULATION OF STEMNESS OF CULTURED MEAT USING AUTOPHAGY REGULATION

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Cultured meat is artificial meat grown in a laboratory and provides a promise for future-oriented resources in terms of ethical and environmental concerns raised in the existing livestock industry. Muscle satellite/stem cells, a basic raw material for cultured meat production, are generally procured from the muscle tissue

of slaughtered adult cattle or of the fetus discarded during the slaughter process. Optimized muscle stem cells are required to produce a large amount of qualified cultured meat, but the proliferation and differentiation capacity of stem cells obtained at different ages has not been clearly provided up-to-date. Rather, it has been reported that muscle cells derived from fetal tissue have higher growth capacity than adult. Therefore, in this study, the expression of myogenic marker in fetal muscle cells (FM) and adult muscle cells (AM) was compared with the ability to maintain undifferentiation of muscle satellite cells through autophagy induction to establish appropriate bovine muscle-derived satellite/stem cells for cultured meat. Compared to FM, AM impaired to generate long plurinucleate and contractile structures upon differentiation conditions. Furthermore, transcriptome analysis demonstrated that AM shared gene profiling more close to differentiated status. Here, we found individuals of the appropriate age with optimal conditions to differentiate more muscle cells, and were able to isolate muscle stem cells from the cells. In addition, there was a difference in the level of autophagy according to the age of the individual. Therefore, it was possible to suppress muscle stem cells that spontaneously differentiate into muscle cells by using chemicals that regulate autophagy. Our research provides optimized protocols for the maintenance of muscle stem cells, which are the most basic and important in producing highly qualified cultured meat.

Funding Source: This work was supported by Korea IPET through High Value-added Food Technology Development Program, funded by MAFRA (321025-05) and by the KRIBB Research Initiative Program Grants (KGM4562323)

Keywords: cultured meat, muscle stem cell, autophagy

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CRITICAL ATTRIBUTES INDICATING TRANSLATIONAL QUALITY OF HPSC-DERIVED ARTICULAR CARTILAGE FOR REPAIR

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The ideal cell source for cartilage repair remains elusive, in part due to an incomplete understanding of articular chondrocyte specification during human joint development. We have pioneered directed differentiation protocols to generate articular and growth plate cartilage tissues from human pluripotent stem cells (hPSCs), facilitating the first in depth investigation into human cartilage development. We compared transcriptomic profiles of hPSC-derived chondrocytes isolated from TGFB3-treated articular cartilage or BMP4-treated growth plate tissues to those that are developing in the epiphysis and growth plate of the human femur. Like the fetal epiphyseal cartilage, and despite the lack of mechanical loading, in vitro articular cartilage was zonally organized with both superficial zone chondrocytes that express PRG4, encoding lubricin, and intermediate zone-like chondrocytes that express COL2A1 and CNMD. In contrast, chondrocytes from the BMP4-treated tissues were highly proliferative and in the process of undergoing hypertrophy, like those in the fetal growth plate. With the knowledge that hPSC-derived cartilage tissues behaved similarly to those developing in vivo, we aimed to define the characteristics that enabled the articular cartilage tissues to function as permanent cartilage, an important characteristic for clinical translation. To rigorously test the stability of TGFB3-treated articular chondrocytes to resist hypertrophy, such as that observed in osteoarthritis and in the growth plate, we challenged the tissues with BMP4 and found that they gained resistance to hypertrophy after 8-10 weeks. Importantly, however, they retained their ability to respond to TGFb3, indicating a commitment to the articular chondrocyte lineage, and suggesting that the cells will respond favorably to dynamic shear and compressive loads experienced in weight-bearing joints. Thus, differentially expressed genes we identified between articular and growth plate chondrocytes at this stage likely contain important players in cell fate decisions and lineage commitment, which may be included as critical quality attributes for clinical translation. These results highlight the importance of hPSCs as a model for human cartilage development and the immense translational potential for cartilage repair.

Funding Source: National Institute of Health

Keywords: Human pluripotent stem cells, Articular cartilage, Stability

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CHARACTERIZATION OF SKELETAL MUSCLE PHENOTYPES FROM CYSTINOSIS PATIENTS

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Nephropathic cystinosis is a rare autosomal recessive lysosomal storage disorder, due to mutations in the cystinosin gene (CTNS) located on chromosome 17p13. Patients often manifest with Fanconi's syndrome and failure to thrive in early childhood. Patient survival years following renal transplantation has revealed systemic complications including distal myopathy and dysphagia. Predominantly distal myopathy, including the development of dysphagia when swallowing muscles are weakened, is common in patients with nephropathic cystinosis, even in the absence of clinically overt muscle weakness. Currently, several obstacles exist in the path to implementing clinical trials for the treatment of distal myopathy in cystinosis patients. These include 1) a better understanding of the evolution of distal myopathy symptoms, 2) robust outcome measures, and 3) biomarkers that are sensitive and "responsive" enough to detect meaningful changes in disease status. To better understand the distal myopathy that occurs in Cystinosis patients, we derived primary myoblast lines from patients using a needle punch biopsy from vastus lateralis muscle. We subsequently compared their proliferation capacity to unaffected controls using in vitro live imaging assays in combination

with high throughput immunofluorescent characterization at the single cell level. Overall, we have observed comparable rates of proliferation and total cell numbers generated from patient lines relative to controls. Our future studies include the characterization of key myogenic genes at the protein level and subsequent ability to differentiate into multinucleated myotubes. Our early studies suggest that Cystinosis patients, with active distal muscle weakness, maintain the ability for myogenic progenitors to expand in vitro. A finding that supports the possibility of developing either cell-based therapies or therapies that affect myogenic progenitor function in vivo.

Funding Source: Cystinosis Research Foundation, A generous gift to the Harvard Stem Cell Institute from Dr. Priscilla Winn Barlow, The Research Scholar Initiative at Harvard University, Circle Therapeutics Inc.

Keywords: Myopathy, Characterization, Cystinosis

TOPIC: NEURAL

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SYSTEMS BIOLOGY-GUIDED DESIGN AND MOLECULAR OPTIMIZATION OF TRANSCRIPTION FACTOR CASSETTES FOR ROBUST, HIGHLY EFFICIENT REPROGRAMMING

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Despite substantial advances in reprogramming methods, the rate of reprogramming remains challenging to predict and control. Variability across batches of cells, diverse cocktails of transcription factors, and the variable delivery and expression of those factors contribute to a lack of robustness. This variability across reprogramming replicates limits precise molecular interrogation of the reprogramming process. To improve the controllability, we aimed to reduce the number of viruses required for co-delivery while maintaining reprogramming efficiency. To address this challenge, we capitalized on our recently developed chemical-genetic cocktail that induces a 100-fold increase in direct conversion to motor neurons. Using this cocktail, we identified the requisite transcription factors for generating motor neurons at high efficiency. Surprisingly, we found that reducing the number of transcription factors from the original six to just three (Ngn2, Isl1, Lhx3) increased reprogramming efficiency of mouse embryonic fibroblasts to induced motor neurons (MEF-to-iMN). Minimizing the cocktail allowed us to finely examine the role of each of the three transcription factors. Through systematic titration of the three transcription factors, we demonstrate that MEF-to-iMN direct conversion efficiency correlates with levels of the pioneer transcription factor Ngn2 early in the reprogramming process. We then built an array of multicistronic cassettes based on the minimal set of transcription factors. Through a combination of molecular designs, we optimized the transcription factor cassettes, resulting in a massive 8,000-fold increase in reprogramming over the original six transcription factor cocktail using only two viruses. By optimizing the reprogramming cassettes, we could extend the window of time over which cells reprogram, increasing the yield of motor neurons. Finally, we show that eliminating neurotrophic growth factors does not impair our iMN reprogramming selectivity, reducing cost and eliminating a potential source of variability. Neurotrophic-free reprogramming paves the way for reprogramming in environments lacking robust neurotrophic support, including in vivo.

Funding Source: NBW and BAL are supported by the National Science Foundation Graduate Research Fellowship Program under Grant No. 2141064.

Keywords: motor neuron reprogramming, cell fate engineering, systems biology

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CHAPERONE-MEDIATED AUTOPHAGY CONTROLS NEURAL STEM CELL ACTIVITY DURING PHYSIOLOGY AND PATHOLOGY

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Aging-related diseases are a major cause of mortality and morbidity. These include neurodegenerative and cancer. The determinants that influence aging and the onset of diseases associated with it are multifactorial and recent evidence demonstrated that aging and cancer share common hallmarks but acting differently in a context dependent manner. One of the hallmarks is the activity of adult stem cells. Thus, there is a decline in the number and/or activity of stem cells with aging promoting tissue dysfunction, while encompasses the accumulation of mutations that cause the appearance of tumors. Furthermore, it has been identified a population of cancer stem cells responsible for maintenance, metastasis and recurrence in multiple types of cancer cells. These features are due to their unique characteristics of quiescence and self-renewal. Chaperone-mediated autophagy (CMA) is a homeostatic process essential for the lysosomal degradation of a selected subset of the proteome. CMA activity directly depends on the levels of LAMP2A, critical receptor for CMA substrate proteins at the lysosomal membrane. the implication of LAMP2A, and hence CMA, in neural stem cells (NSCs) and in glioblastoma stem cells (GSCs) remains unknown. In this talk, we will present results revealing that LAMP2A expression is enriched in patient-derived GSC population whereas it is decreased in NSCs aging. Additionally, its knock-down diminishes GSCs tumorigenic activities, whereas its overexpression maintains NSC activity. Furthermore, we identified novel molecular pathways critical for CMA activity. In conclusion, we identified a novel role of CMA directly regulating NSCs and GSCs activity via multiple pathways at proteome and transcriptome level.

Keywords: neural stem cell, chaperone mediated autophagy, cancer and aging

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USING HUMAN PLURIPOTENT STEM CELL MODELS TO ELUCIDATE THE CIS-REGULATORY LOGIC AND TRANSCRIPTIONAL REGULATION OF HUMAN CORTICAL INTERNEURON DEVELOPMENT

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Cortical GABAergic interneurons are known to be a neuronal population particularly sensitive and important to neurodevelopmental disorders including autism spectrum disorder. Despite a heavy focus on this cell type there remains relatively little known about the cis regulatory grammar underlying the specification and development of these cells. A greater understanding of this landscape is critical for understanding the roles single nucleotide polymorphisms or other mutations in intergenic space may have on the development of these cells. We have used our established developmental patterning approach to generate medial ganglionic eminence (MGE) like progenitors, immature and matured GABAergic neurons and have used cut and tag to examine the chromatin landscape changes accompanying these transitions. Examining marks of active chromatin (H3K27ac and H3K4me3) we have identified temporally specific histone mark changes which underlie the specification of MGE-like progenitors and their differentiation to GABAergic neurons. Further comparisons with active chromatin marks in dorsally patterned neuronal progenitors have identified lineage specific cis regulator elements involved in MGE specification which are enriched for mainly bHLH, HMG and Homeobox transcription factor binding sites. Using this novel data set we have selected transcription factors which play either lineage restricting (DLX2) or more general roles in the development of MGE-like progenitors (RFX3/4). Many of which are known risk genes for neurodevelopmental disorders. Examining the effect upon loss in expression of these genes using both constitutive and inducible CRISPR inhibition techniques we have observed specific changes in markers of MGE specification and general changes in markers of proliferation. Overall, this work presents a novel data set which identifies temporally significant cis-regulatory elements involved in the specification of MGE-like progenitors and their subsequent differentiation into GABAergic neurons. This presents an important resource to identify mutations which are likely to effect MGE development and has allowed us to identify and investigate transcription factors playing specific roles in this process.

Keywords: Epigenetics, Interneurons, Neurodevelopment

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UNRAVELING BRAIN AGING ONE CELL TYPE AT A TIME: SINGLE CELL SEQUENCING REVEALS DIVERSE CELL STATES AND LINEAGES IN THE SHORT-LIVED TURQUOISE KILLIFISH

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The African turquoise killifish is an emergent model for aging and neurodegeneration as it has a short lifespan and exhibits mammalian-like aging hallmarks. The killifish telencephalon, subdivided into pallium and sub-pallium, are considered homologous to the primary mammalian neurogenic niches. To investigate the heterogeneity of these neuro-regenerative niches and study the impact of aging, we performed single cell sequencing of young adult and aged telencephali. To improve the sequenced read mapping, we additionally performed Long read sequencing to characterize the full-length transcriptome. Combined analyses identified 22 cell types including neuronal cells (NC), and progenitor cells (PC) of glial and non-glial nature in the adult killifish telencephalon. PC sub-clustering unveiled four radial glia (RG) types, two atypical non-glial progenitor cell types (NGP) and other transition states. Lineage inference analysis of PCs suggests neuroepithelial-like radial glia (NE-RG3) and non-glial progenitor (NGP) to be the start point and intercessor of both neuro- and gliogenesis. We identified one NGP type as a hyper-proliferative mediator cell cluster, connecting different astroglial subtypes and the other more committed towards neurogenesis. NC sub-clustering delineated the neuron types into distinct classes and separated the lineages of excitatory and inhibitory neurons. Validation of our data in situ reveals a distinct spatial setting for the different RG and NC subtypes, reflecting the distribution of morphologically and physiologically distinct cell populations. With regard to the impact of aging on the progenitor heterogeneity, we found important pathways shaping neurogenesis - such as WNT, ribosomal and mTOR - to be altered during aging in the source cell types, NE-RG3 and NGP. The complete catalogue of killifish telencephalon cell types is accessible via an online tool, providing a unique resource to understand adult neurogenesis in healthy brains and upon aging or disease.

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Keywords: Glia and non-glial progenitors, African turquoise killifish, Aging and neuro-regeneration

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SPATIOTEMPORAL DYNAMICS OF HYPOTHALAMIC DEVELOPMENT

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The hypothalamus comprises various nuclei and neuronal subpopulations that control fundamental homeostasis and behaviors. However, the cellular and molecular mechanisms of hypothalamic development remain mostly unclear. We revealed spatiotemporal transcriptome profiles and cell-type characteristics of hypothalamus development and illustrated molecular diversity of neural progenitors and the cell fate decision, which is programmed by different regulator genes (regulons). Overall, this study provides a comprehensive molecular landscape of hypothalamus development at early- and mid- embryonic stages and a foundation for understanding its spatial and functional complexity.

Keywords: neural progenitors, cell fate determination, hypothalamus development

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A SOX10-MORANGE REPORTER IPSC LINE TO STUDY AND ENHANCE IN VITRO DIFFERENTIATION OF OLIGODENDROCYTES AND THEIR PROGENITOR CELLS

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Oligodendrocytes (OLs) are the cells responsible for myelination in the central nervous system. Their main function is to insulate axons through the production of the myelin sheath. OLs and their progenitors, OL progenitor cells (OPCs), are commonly implicated in neurological diseases, suggesting these cells are susceptible to disruptions in homeostasis. While OLs are well studied in murine models, these findings don't always translate to human models. The use of human-derived induced pluripotent stem cells (iPSCs) permits the study of human OLs to model disease and study their function, bridging the gap in knowledge from murine to human. Current growth factor-based iPSC-OL differentiation protocols are characterized by high variability, contaminating astrocytes, and low yield of terminally mature OLs (MBP+), rendering the use of these cells as an in vitro model difficult. To overcome these issues, we developed a gene-edited iPSC reporter line (SOX10mO), in which endogenous mOrange fluorescence is detectable upon translation of the OL-specific transcription factor SOX10. Exploiting this feature, we can employ live cell sorting to purify and categorize our culture according to lineage maturity level. Testing the differentiation efficiency of SOX10mO cells revealed the presence of high number of glial progenitor cells (GPCs), bipotent precursors of astrocytes and OPCs, co-expressing NPC, glial and early OPC markers. Differentiation of GPCs in maturation media identified a subpopulation of late O4+ OPCs, corresponding to an increase in SOX10 expression. The majority of cells failed to reach terminal maturity, suggesting this protocol can produce reliable OPCs but not MBP+ OLs. We next used the SOX10mO system to trial modifications in the differentiation protocol to improve efficiency and reproducibility. We tested growth factor combinations to allow better control of the balance between proliferating progenitors and mature cells, including the subtraction of thyroid factor T3 in the progenitor medium, and the use of a novel maturation medium. We present a robust protocol capable of producing terminally differentiated OLs that lays the foundation for future disease modeling of these cell types.

Keywords: Oligodendrocyte, Protocol, Lineage Development

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MULTIOMIC COMPARISON OF HPSC-DERIVED NEURONS TO HUMAN FETAL NEURONS

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We must compare stem cell derivatives to the relevant primary human tissues to properly identify and understand cellular identity and maturity level. This is particularly true for cells of the human brain, which contains dozens of different cell types with multiple maturation stages. In addition, brain cell maturity levels or neuronal sub-types may be further distinguished by the cell's response to neuronal activity. Neuronal activity from spontaneous firing and sensory input is essential for the proper development of the brain. However, single-cell transcriptional data sets of primary human brain are processed from tissues without intentional stimulation. In this study, we aim to systematically compare primary human fetal cultures of the neocortex to human pluripotent stem cell-derived neocortical neurons derived by a dual-SMAD inhibition approach, before and after neuronal depolarization. We will use single-cell multiomic profiling and Cut&Tag of cell-type marker transcription factor proteins for this comparative analysis of the cell type-specific and activity-dependent transcriptional profiles. This will provide a deeper understanding of how the neuronal cell types made from widely-used neuronal differentiation protocols compare to the correlate cells that developed in vivo. We hope to identify both the strengths and weaknesses of these in vitro model systems, and to reveal the molecular pathways that are appropriate to model with these stem cell derived neurons.

Keywords: neuronal development, gene expression, single-cell profiling

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IDENTIFYING THE MOLECULAR LANDSCAPE OF HUMAN SENSORY INTERNEURON DEVELOPMENT USING EMBRYONIC STEM CELLS

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Cellular replacement therapies use human embryonic stem cells (hESC) or induced pluripotent stem cell (hiPSC)-derived neurons to replace damaged or diseased populations of neurons in patients with neurological conditions. We are developing multiple stem cell protocols that generate functional, bonafide spinal sensory interneurons (INs) to either replace spinal cord tissue after injury (SCI) or use in drug testing platforms. Towards this goal, we use two developmentally relevant factors, retinoic acid in combination with bone morphogenetic protein 4, to generate the entire complement of sensory INs (proprioceptive dI1s, dI2s, mechanosensory dI3s, dI6, and nociceptive dI4s and dI5s) from mouse ESCs. To optimize the differentiation conditions for hESCs and hiPSCs in a 3-dimensional embryoid body/spheroid culture system we have assessed the impact of (1) confluency, (2) the presence of ROCK inhibitor, which prevents cell death, and (3) the day at which spheroids are generated, on the initial expansion of hESC/hiPSC cultures for sensory IN differentiation. With these standardized conditions, we are assessing (1) how the IN-differentiation program differs between protocols that induce INs through different developmental states, i.e neuroectodermal versus neuromesodermal intermediates, and (2) how these alternative routes of differentiation affect IN fate, diversity, and maturation. These studies will both expand our understanding of the pathways involved in human sensory IN differentiation and identify a necessary cellular therapeutic to restore somatosensation in SCI patients.

Keywords: Human Embryonic Stem Cells (hESCs), Human Sensory Interneurons, Differentiation

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PERICYTE REPROGRAMMING FOLLOWING ISCHEMIC STROKE

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Pericytes are cells that wrap around capillary microvessels and are a key component of the blood-brain barrier. An increasing number of studies have demonstrated that brain pericytes can be reprogrammed into various neural and non-neural cells at the lesion site following ischemic stroke. However, it remains unknown what determines pericyte reprogramming potential. Here, we first identified two distinct subtypes of pericytes in the stroke lesion site, marked by two pericyte markers: NG2 and Tbx18. Following this, we performed single-cell RNA sequencing (scRNA-seq) using stroke-injured cerebral cortical tissue isolated from two pericyte lineage tracing mice (NG2Cre-ERT2/Ai14 and Tbx18Cre-ERT2/Ai14) that received intracerebral injections of vasoconstrictors 3 days before scRNA-seq. Both transgenic mice were also treated with tamoxifen 7 days before stroke to enable lineage tracing of Tbx18-Ai14+ and NG2-Ai14+ pericytes. Three groups of Tbx18-Ai14+ and NG2-Ai14+ cells from 1) no injury (naïve), 2) physical injury (saline), and 3) ischemic injury (stroke), were FAC sorted for scRNA-seq. Downstream analysis revealed that Tbx18-Ai14+ cells from naïve brains mostly consisted of pericytes, smooth muscle cells, and endothelial cells, while Tbx18-Ai14+ cells from both physical injury and ischemic injury showed new cell phenotypes which included large quantities of fibroblasts, microglia and to a lesser degree glial precursors and T-cells. Naïve NG2-Ai14+ cells primarily consisted of pericytes, smooth muscle cells, endothelial cells, glial precursors, and mature oligodendrocytes. NG2-Ai14+ cells following physical injury and ischemic injury experienced an expansion of the glial precursor population. Trajectory inference analysis revealed that this glial precursor expansion was generated from pericytes. Immunohistochemical analysis has confirmed that 38% of NG2-Ai14+ cells became Sox2+ induced neural stem cells (iNSCs), 17% of them were DCX+ neuroblasts, while only 5% of Tbx18-Ai14+ cells became Sox2+ iNSCs at the lesion site. Elucidation of underlying cellular and molecular mechanisms that contribute to pericyte reprogramming for neural regeneration after ischemic stroke will pave the road to develop targeted therapeutic strategies to enhance local neural regeneration at the stroke site.

Keywords: Ischemic stroke, Pericyte, Cellular reprogramming

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BDNF-TRKB SIGNALING INDUCES THE GLUTAMATERGIC DIFFERENTIATION FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Glutamatergic neuron, one of the most common excitatory neurons in CNS, resides in various parts of brain, including hippocampus, lymbic system, and neocortex. It produces glutamate as a neurotransmitter, and plays a critical role in cognition, learning, memory, and emotional control. Productions of the healthy glutamatergic precursor cells are essential for the cell replacement therapy and in vitro modeling of various neurological disorders including Alzheimer's disease, mild cognitive impairment, and attention deficit hyperactivity disorder. Brain-derived neurotrophic factor (BDNF) / tropomyosin-related kinase receptor type B (TrkB) signaling is significant in neuroprotection, maturation, and differentiation for the specific neuronal population. Extrinsic morphogens such as Wnt, Notch, Shh, and BMP are also essential for the differentiation, proliferation, and neurogenesis of neural stem cells. In this study, we discovered that the BDNF/TrkB signaling

in addition to the main morphogen stimulation can successfully induce the differentiation of glutamatergic precursor cells from hiPSCs. We characterized the glutamatergic precursor cells and checked the cell viability after the stimulation of BMP and BDNF/TrkB signaling. We found the increased level of cell survivals of the glutamatergic precursors after the stimulation. We also found the increased expression level of synaptic marker proteins after the BDNF/TrkB stimulation. These data suggest that this approach can lead to the efficient protocol to produce the functional precursor cells for the potential cell replacement therapy.

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Keywords: human induced pluripotent stem cells, glutamatergic differentiation, BDNF/TrkB signaling

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EMERGING ROLE OF BAG AS CANCER CELL PLASTICITY REGULATOR IN MEDULLOBLASTOMA

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Abnormal expression of BCL2-associated athanogene (BAG) proteins has been observed in different malignancies. BAG proteins are mainly reported as Hsp70 co-chaperones, as well as regulators of apoptosis and autophagy, while their role in cancer cell differentiation has never been investigated. Medulloblastoma (MB), the most common malignant brain tumor of childhood with unmet therapeutic needs, is characterized by aberrations in diverse developmental pathways (Shh, Wnt, and Myc) but the molecular triggers are still largely unknown.Here we demonstrate that, although MB is characterized by elevated BAG protein levels, its deregulation supports MB progression, chemotherapy resistance and metastasis. Indeed, transcriptomic analysis of BAG-depleted MB cells reveals the downregulation of neuron differentiation program in favour of the upregulation of muscle cell development and differentiation gene ontology terms, especially Mef2 and MyoD1 muscle differentiation transcription factors which have already been described as highly expressed in MB cells, as well as in proliferating granule neuron progenitors (GNP). In particular, BAG depletion modulates the expression levels of both stemness (Sox2, Nanog, Oct4) and neuronal markers (bIII-tubulin, NeuroD1, Nestin) hence possibly blocking MB cells into a transit-amplifying progenitor status. Accordingly, BAG-depleted cells display a proliferative advantage in vivo with increased in c-Myc level, a major driver oncogene in medulloblastoma. In addition, a negative enrichment in genes involved in cell-cell and cell-substrate adhesion underlies the acquisition of epithelial-to-mesenchymal transition (EMT) features, corroborated by the peculiar spindle-shaped morphology observed in BAG-depleted MB cells. Moreover, BAG may be a crucial factor determining sensitivity and resistance in cancer cell since its downregulation induces a drug tolerant phenotype to chemotherapeutic agents used for MB treatment. Collectively, these results demonstrate that BAG is able to control MB cancer cell plasticity acting as bona fide tumour suppressor gene. Future investigation of BAG gene regulation during brain development will prove an emerging role of BAG protein as master regulator of neural cell fate and its possible involvement in medulloblastoma origin.

Keywords: Medulloblastoma, Cancer cell plasticity, Neural cell fate

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HUMAN GLIAL PROGENITOR CELLS UNDERGO EPIGENETIC DYSREGULATION WITH AGE

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White matter loss is a common feature of dementia and healthy aging. Human glial progenitor cells (hGPCs) produce myelinating oligodendrocytes throughout life, but why hGPCs fail to appropriately remyelinate the aged brain remains unknown. We previously noted that unlike fetal hGPCs, adult hGPCs precociously differentiate when engrafted into the dysmyelinated shiverer mouse brain, and fail to expand and colonize the recipient brain. Because oligodendrocyte differentiation is driven by chromatin closure, we sought to identify how the chromatin state of human GPCs changes with age, and how this might lead to a loss of self-renewal and consequent white matter attrition. We characterized single-cell chromatin accessibility and transcriptomes of young and aged human embryonic stem cell (hESC)- and induced pluripotent stem cell (iPSC)-derived human GPCs in vitro at the single-cell level, and found a reduction in cycling cells, lower expression of genes associated with migration and self-renewal, an increase in immune pathway gene expression, and a de-repression of mature neuronal and glial programs. To understand the regulatory mechanisms underlying these events, we used CUT&Tag to profile chromatin marks H3K4me3, H3K27ac, H3K27me3, H3K9me3, and H3K9ac every 30 days throughout the differentiation and subsequent aging of hESC- and hiPSC-hGPCs. We found a 57% decrease in accessible chromatin in aged hGPCs genome-wide, corresponding to an increase in repressed chromatin. We used these data to identify sites of chromatin dysregulation in aged hGPCs, and by that means identified potential targets for epigen-

etic manipulation. These findings advance our understanding of age-related white matter loss and may thereby offer us an operational basis by which to restore GPCs to an expansion-competent and myelinogenic state in the aged human brain.

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Keywords: glia, aging, epigenetics

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CHARACTERIZING THE RELATIONSHIP OF LGL1 AND AKT IN NEURAL STEM CELLS

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Asymmetric cell division and migration are critical for neural stem cell differentiation and brain development. When these processes are dysregulated in neural progenitor cells (NPCs), developmental defects and diseases like glioblastoma multiforme (GBM) can result. Lgl1 is a tumor suppressor gene first characterized in Drosophila neuroblasts that is best known for its regulation of apical-basal cellular polarity through its association with the Par complex. The PI3K/AKT signaling cascade is also regulated by Par signaling and involved in cellular migration. To investigate the role of LGL1 on the regulation of NPC migration, NPCs were cultured from genetically matched Lgl1-/- and Lgl1+/+ primary cell lines isolated from the subventricular zone (SVZ) and corpus collosum (CC) of P30 mice. Spheroid and scratch test migration assays revealed that loss of Lgl1 increased migration rates by up to two-fold, demonstrating that LGL1 regulates NPC migration. To see if loss of Lgl1 could be influencing migration through PI3K/AKT signaling, we used targeted drugs to inhibit mTOR and PI3K in the same cellular migration assays and evaluated the phosphorylation states of AKT in the same genetically matched cell lines and in the subventricular zone of GFAP CRE Lgl1 knock out vs. wild type mice. Our findings suggest that changes in Akt phosphorylation a a key link between LGL1 and the migratory defects we observed. Our results are also consistent with findings that loss of Lgl1 increases rates of cellular migration. Our results contribute to the understanding how loss of cell polarity affects neural cell differentiation, cancer cell properties, and GBM progression.

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Keywords: AKT, LGL1, Neural Progenitor Cells

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INDUCING MAPK SIGNALING PROMOTES DIRECT CONVERSION TO NEURONS BY INCREASING PROLIFERATION

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The MAPK pathway transmits extracellular cues into changes in gene expression to regulate diverse cellular responses including proliferation and differentiation. We recently developed a chemo-genetic cocktail that drives overexpression of a mutant of the MAPK pathway, HRASG12V. This cocktail increases the population of cells that reprogram at near-deterministic rates, which in turn increases the conversion of mouse embryonic fibroblast to motor-neuron reprogramming by 100-fold. While mutant RAS broadly promotes transformation by inducing signaling through the MAPK pathway, the mechanistic contribution to reprogramming remains undefined. Moreover, cell-fate transitions toward highly proliferative cell types, such as iPSCs, may obscure the potential transient role of MAPK signaling and proliferation to promote these transitions. To address the question of how transient MAPK signaling and proliferation influence conversion, we introduced a live-cell reporter of MAPK-activity. We observe that our high efficiency cocktail induces elevated MAPK signaling early in reprogramming. Inhibition of the MAPK pathway decreased proliferation and motor-neuron yield, indicating that signaling supports proliferation and conversion. Different isoforms of RAS induce different transformation rates that vary by cell type. To examine isoform-specific differences in reprogramming, we explored a panel of RAS isoform mutants, which generated reprogramming yields ranging from 25% to 125%. These reprogramming yields correlate with the ability of each RAS mutant to induce proliferation in the reprogramming cells. Overall, we find that MAPK signaling promotes direct conversion to neurons by increasing proliferation. With these insights and tools, we can dissect the molecular paths that cells traverse during reprogramming. Importantly, we aim to develop well-controlled systems for reprogramming cellular identity to generate specific neurons.

Keywords: Reprogramming, MAPK/ERK signaling, RAS mutants

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GENOME-WIDE SCREENS REVEAL ESSENTIAL ROLES FOR HOX GENES AND IMPRINTED GENES DURING NEURONAL DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

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Mapping the essential pathways for neuronal differentiation can assist the development of cellular models for neurodevelopmental disorders and suggest new therapeutic targets. To this aim, we utilized a genome-wide loss-of-function mutant library in haploid human embryonic stem cells, which we differentiated into neurons. This CRISPR/Cas9-based mutant library, which was generated by targeting 18,000 coding genes with over 180,000 sgRNAs, recently allowed us to identify the essential gene networks for the maintenance of pluripotency and early germ layer differentiation in human. Here, we identify the essential genes and pathways for neurogenesis, showing that they are enriched for secreted and membrane proteins, and that a large group of neurological conditions, including neurodegenerative disorders, manifest early neuronal differentiation phenotypes. Moreover, the essential transcription factors that we identified include several HOX genes demonstrating synergistic roles and surprising non-redundant functions for HOXA6 and HOXB6 paralogs during neuronal differentiation. Furthermore, we establish the essentialome of imprinted genes during neurogenesis, showing that the maternally expressed genes are non-essential in pluripotent cells and their differentiated germ layers, yet several of these imprinted genes are essential for neuronal development. These include Beckwith-Wiedemann syndrome- and Angelman syndrome-related genes, for which we suggest a novel regulatory pathway. Overall, our work identifies essential pathways for neuronal differentiation and stage-specific phenotypes of neurological disorders.

Keywords: Neuronal differentiation, Genome-wide CRISPR screening, Disease modeling

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MOLECULAR PROGRAMS OF AREA SPECIFICATION AND NEURAL STEM CELL FATE PROGRESSION IN DEVELOPING MACAQUE CORTEX

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Mammalian corticogenesis involves patterning of the distinct cortical domains along with fate specification of the neural stem cells (NSCs). Here we generated single-cell transcriptome data of 787,418 cells from multiple areas of the prenatal rhesus macaque telencephalon, from a phase before neurogenesis till mid-gliogenesis. We defined the molecular programs of the brain organizers and their cross-talk with cortical NSCs. We found primate-specific signaling active in the antero-ventral domain of the telencephalon implicating GALP and Galanin and further evaluated the function in brain organoids. Regional transcriptomic divergences in the neocortex were evident at early states of the NSC progression and in terminally differentiated neurons and astrocytes more than in intermediate transitions. Finally, we show that neuropsychiatric disease- and brain cancer-risk genes might have early roles in brain organizers' activity and across cortical NSC progression.

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Keywords: cortical neural stem cells cortical organoids, brain organizers primate brain development, neurodevelopmental diseases

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DIRECT NEURONAL REPROGRAMMING FROM THE SKIN CAN BE EXPLAINED BY DIFFERENTIATION OF NEURAL CREST STEM AND PROGENITOR CELLS

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Direct cellular reprogramming involves conversion of one fully differentiated and mature cell type into another through manipulation of gene expression and without returning to an early developmental state. One aspect of this process that is often unexplored is the potential heterogeneity in the starting cell population prior to reprogramming. In this work we examine two key components of potential heterogeneity: the developmental lineage of starting cells, and to what extent there is a mixture of stem and progenitor cells in culture. Our model for this is directly reprogramming cells from the murine skin into neurons, as skin is a common substrate for many reprogramming experiments. The skin contains cells at different levels of maturity (stem cells vs. post-mitotic differentiated cells) and from multiple developmental origins, including the neural crest, surface ectoderm, and mesoderm. We hypothesize that a single developmental lineage in the skin contributes to reprogrammed neurons and that within this lineage there are a smaller subset of elite stem or progenitor cells with intrinsic bias to turn into neurons. This hypothesis would also explain the common feature of reprogramming being an inefficient process. In support of these predictions, we find that nearly all reprogrammed neurons are derived from neural crest cells in the skin. This seems to be a cell autonomous bias in the neural crest compartment, as FACS purified non-neural crest cells show a similar inability to reprogram as in mixed cultures. Neural crest stem and progenitors comprise a small subset of the skin culture, however, when these cells are ablated there is a drastic reduction in the efficiency of direct neuronal reprogramming. These experiments support an elite model of direct reprogramming over a stochastic process where all cells have equal potential to reprogram. Furthermore, these data call into question whether this is truly a direct reprogramming phenomenon as traditionally defined. It was originally thought that skin to neuron reprogramming represents a conversion across germ layers from mesoderm to neuroectoderm. Our alternative interpretation is that this is really directed differentiation of a neural stem cell in the skin that has intrinsic potential to produce neural progeny.

Keywords: Direct Neuronal Reprogramming, Neural Crest Stem Cells, Elite vs. Stochastic Models of Reprogramming

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LIF SIGNALING REGULATES OUTER RADIAL GLIA TO INTERNEURON FATE DETERMINATION DURING HUMAN CORTICAL DEVELOPMENT

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The appropriate development of the nervous system is essential for long-term organization and function where dysregulation of molecular programs during embryogenesis can have devastating neurological consequences. However, the local cues in the cortical niche that pattern discrete cell types have not been explored in depth. Outer radial glial (oRG) cells are specialized neural stem cells that differentiate into diverse populations of neurons and glia. To understand the lineage contribution of oRG we explored the role of the Leukemia inhibitory factor (LIF) signaling. The LIF receptor (LIFR) is uniquely expressed in oRG cells during peak neurogenesis. Utilizing cortical organoids, we identified that LIF activation promotes oRG proliferation with a concomitant decrease in excitatory neurogenesis. However, after LIF activation we observed an unexpected increase in the number of inhibitory interneurons (IN). While IN are predominantly born in the ventral forebrain and migrate dorsally into the cortex, recent studies suggest a subset of IN may be dorsal forebrain-derived. We reanalyzed publicly available cortical organoid datasets compared to primary ventral forebrain reference datasets and identified that after six months in culture a population of IN, that transcriptionally resemble dorsally-derived IN, are observed in organoids. We then evaluated our organoid dataset after LIF activation and identified earlier development of and increased proportions of this IN population. To identify the lineage source of described IN, we isolated oRG using FACS, cultured in the presence of LIF for several weeks and subsequently observed an increase in IN abundance. The studies suggest that LIF signaling is sufficient to promote oRG development into an underexplored population of dorsal forebrain-derived IN.

Keywords: Pluripotent stem cell models, Neurodevelopment, Neural organoids

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INVESTIGATING THE IMPORTANCE OF HUMAN- AND BRAIN-SPECIFIC LONG NONCODING RNAS

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Long noncoding RNAs (lncRNAs) are important cellular regulators as they are involved in chromatin remodeling, transcription modulation, and post-transcriptional regulation through a variety of chromatin-based mechanisms and interplay with other RNA species. Studies have reported the functional implications of lncRNAs in the developing and mature brain, and lncRNA deregulations have been associated with many neurodegenerative diseases. Some lncRNAs are evolutionary conserved, suggesting a critical role across diverse species. The goal was 1/ to identify lncRNAs that are human- and brain-specific, 2/ to investigate whether the expression of these lncRNAs was critical the differentiation of iPSCs into neurons, and 3/to determine whether deregulations of their expression could be associated with neurodegenerative diseases. The functional characterization of these lncRNAs was performed using human induced pluripotent stem cells derived into neurons and brain tissue of patients with neurodegenerative diseases. We performed a bioinformatic analysis on lncRNA expression in different tissues and organisms and identified 8 lncRNAs that are specifically expressed in humans and in a high abundance in brain tissue. Subsequent quantitative PCR analysis validated the expression of these lncRNAs in mature neurons and brain tissues. We focused this study on our lncRNA #1 candidate and demonstrated that its expression is dynamically regulated during neuronal differentiation. The knock-down of this lncRNA impaired the differentiation and maturation of neurons, and transcriptomics analysis confirmed that the absence of lncRNA #1 led to the dysregulation of key signature genes involved in neuronal differentiation. Finally, we showed that the expression of this lncRNA is deregulated in the brain tissue of patients with Alzheimer's and Huntington's disease. We identified human- and brain-specific lncRNAs whose expression is critical for neuronal maturation and deregulations associated with neurodegenerative diseases. Our next step is to identify the molecular mechanisms by which they are important contributors to brain pathophysiology.

Keywords: long noncoding RNAs, neuronal differentiation, brain

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OPTIMISED AND SCALABLE REPROGRAMMING OF HUMAN IPSCS TO GENERATE NOCICEPTOR SENSORY NEURONS FOR THE STUDY OF PAIN MECHANISMS AND NEUROPATHIES

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Nociceptive sensory neurons, a subtype of somatosensory cells of the dorsal root ganglia, respond to diverse noxious and pruritic stimuli, and are critical for the study of pain mechanisms and neuropathies. Many promising drugs with efficacy in animal models of pain have failed in the clinic due to interspecies differences in nociception. Conventional differentiation methods to generate nociceptors from human induced pluripotent cells (hiPSC) offer a more physiologically relevant alternative to animal models, but are limited by complex, inconsistent, and long protocols. Thus, there is a need for a consistent, scalable human in vitro model to study the pathophysiology of nociceptive sensory neurons and develop new, efficacious, and safe pain therapeutics. We used transcription factor mediated precision cellular reprogramming technology, opti-ox™, to rapidly and consistently generate mature, functional and physiologically relevant sensory neurons that display critical features of nociceptors, named ioSensory Neurons, at scale from hiPSCs. Transcriptomic and phenotypic characterisation demonstrated that reprogrammed hiPSCs acquired a sensory nociceptor identity. Within 14 days post-revival, the cells expressed the pan-sensory neuron markers ISL1, POU4F1 and PRPH, and key nociceptor markers NTRK1, TRPV1, TRPM8, and SCN9A. Neurotrophic factors influence subtype specification of sensory neurons, and thus by optimising culture conditions we enriched for cells expressing key sensory genes, including peptidergic nociceptor markers TAC1 and ADCYAP1. Multi-Electrode Array and calcium assays demonstrated asynchronous spontaneous activity and responsiveness to diverse noxious stimuli, revealing the sensory neurons' functionality. In conclusion, with opti-ox precision reprogramming, iPSCs are rapidly converted into functional sensory neurons offering a robust and scalable source of human nociceptors which can be used as a relevant in vitro model to study the biology of pain and to develop novel therapies for neuropathies.

Keywords: nociceptor, pain, reprogramming

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PROMOTING THE DIFFERENTIATION OF NEURAL PROGENITOR CELLS INTO OLIGODENDROCYTES THROUGH THE INDUCTION OF OLIG2 EXPRESSION: A TRANSCRIPTOMIC STUDY USING RNA SEQUENCING AND GENE ONTOLOGY ANALYSIS

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Oligodendrocytes play key roles in myelination and in mediating interactions between glia and neurons within the central nervous system. As such, the loss of these cells is associated with disrupted signal conduction and severe functional deficits. Neural progenitor cells (NPCs) are a promising source of cells for the treatment of oligodendrocyte-related neurological injuries and diseases due to their ability to differentiate into oligodendrocytes, in addition to neurons and astrocytes. However, the differentiation of NPCs into oligodendrocytes is often inefficient, whereby the majority of the cells tend to differentiate into astrocytes within the injury microenvironment. In order to enhance oligodendrocyte differentiation, we aimed to generate inducible oligodendrogenic NPCs (ioNPCs) and to characterize the cells in vitro. Human ioNPCs were prepared by engineering iPSC-NPCs to express Olig2 under the control of the conditional doxycycline-inducible Tet-ON promoter. The cells were then treated with doxycycline for 3, 7 or 10 days in order to identify the optimal doxycycline treatment timeline. qRT-PCR analysis revealed that the expression of several genes involved in oligodendroglial lineage determination, including OLIG1, OLIG2, PDGFRA and NKX6.1, progressively increased with longer doxycycline treatment timelines. Immunostaining showed that following differentiation, the ratio of O1+ oligodendrocytes was significantly higher in the ioNPCs (39.44 ± 16.5%) compared to NPCs (24.73 ± 6.5%). Additionally, bulk RNA sequencing was performed in order to assess the transcriptomic hallmarks of the ioNPCs in comparison to NPCs. A total of 521 genes were differentially expressed between the two groups. These included oligodendroglial genes such as OLIG1, PDGFA, and MYRF which were upregulated in the ioNPCs, as well as neuronal and astrocyte genes such as TUBB3, MAP2 and S100b, which were downregulated in this group. Furthermore, gene ontology analysis identified pathways such as oligodendrocyte cell fate commitment, oligodendrocyte cell fate specification and spinal cord oligodendrocyte fate specification amongst the differentially expressed genes. In conclusion, our study suggests that ioNPCs are a promising source of cells for the replacement of oligodendroglial lineage cells.

Funding Source: Canadian Institutes for Health Research

Keywords: neural progenitor cell, oligodendrocyte, spinal cord injury

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REQUIREMENT OF SULF1 IN HUMAN MIDBRAIN DOPAMINERGIC NEURAL PROGENITOR CELL DEVELOPMENT

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Midbrain dopaminergic (mDA) cells form a uniquely defined cell type that governs motor function and behaviour. The mDA cell fate is specified by the combination of sonic hedgehog (SHH), WNT and other factors, but precisely how concentration gradients of SHH and WNT, in particular, induce the mDA identity is not well understood. Here, we used in vitro human induced pluripotent stem cell (iPSC) induction techniques to precisely control morphogen dose and timing. We conducted an RNA sequencing (RNA-seq) experiment to identify genes that are highly upregulated when iPSCs are induced into mDA progenitors. Our data led to the identification of SULF1 as a remarkably accurate marker of mDA progenitor cells, which we confirmed by single-cell RNA-seq analyses from mouse and human data. Further, we show that SULF1 is highly expressed in mDA progenitors but not in forebrain progenitors or iPSCs, and that engineered heterozygous loss of SULF1 has a dose-dependent effect on levels of the known midbrain marker FOXA2. These results suggest that SULF1 may be essential for the mDA identity. We then demonstrate that SULF1 expression is strongest under an SHH and WNT combinatorial exposure compared to SHH or WNT individual exposures, which, along with our other observations, indicates that SULF1 directly responds to the combination of midbrain inducers SHH and WNT. Currently, SULF1's role in human mDA development is still unknown. Our system would thus allow for the discovery of SULF1's role in human mDA cell fate specification at the mechanistic level, with the hypothesis that SHH- and WNT-driven expression of SULF1 may function in a positive feedback loop to increase SHH and WNT activity in human mDA progenitor cells.

Keywords: midbrain dopaminergic cell fate, SULF1, morphogen patterning

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NAÏVE STATE STEM CELLS PLUS TIME-SENSITIVE ADDITION OF NEUROTROPIC FACTORS PRODUCE SUPERIOR MIDBRAIN DOPAMINERGIC NEURONS

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Pluripotent stem cell derived midbrain dopaminergic (mDA) neurons hold great promise for the treatment of Parkinson's disease, but are still held back by technical challenges. mDA neurons derived from donor embryonic stem cells (ESCs) pose risk of rejection and often require immuno-suppressive treatment. Historically, iPSCs, which may be patient-derived, have not differentiated into mDA neurons as well as ESCs. Both approaches suffer from poor in vivo engraftment rates of only about 10%. Early implantation of stem cell derived mDA neurons improves engraftment, but necessitates implanting the cells before the cellular product can be fully characterized and is typically before the cells secrete dopamine. A newer protocol showed that activating WNT/β-catenin pathway at specific timepoints improved yield and differentiation to mDA neurons. Our studies of human naïve state stem cells versus the primed state showed that naïve stem cells express the highest levels of active β-catenin. To this end we used a published protocol to compare the differentiation of naïve state iPSCs versus primed state iPSCs and ESCs into mDA neurons, with or without addition of agents that activate WNT/β-catenin. We also modified the protocol by introducing into the differentiation media critical factors that may be present in the human brain at the time of neural development. Multiple experiments showed that naïve state iPSCs differentiated into mDA neurons better than primed state iPSCs. mDA neurons differentiated from naïve iPSCs produced up to 10-fold more dopamine than those differentiated from primed state iPSCs and produced it at much earlier timepoints. These results show that dopaminergic neurons derived from naïve state iPSCs could be fully characterized with dopamine production verified before implantation, which would allow for full quantification and characterization of the cellular product to satisfy regulatory agencies. The addition of specific neurotropic factors, at specific timepoints, resulted in improved mDA neuron-like morphology with appropriate expression of molecular markers and a dramatic increase in the inter-connectivity of the neurons, which could be a predictor of improved engraftment.

Keywords: Parkinson's Disease, Dopaminergic Neurons, iPSCs

TOPIC: PANCREAS

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UNRAVELLING HUMAN ENDOCRINE PROGENITOR CELLS

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Cadaveric islet transplants are the best option for the treatment of autoimmune Type 1 diabetes. This treatment is however severely restricted by the supply of donor islets and the immune response. To date, many groups globally have pursued the derivation of insulin-secreting beta cells in vitro from the directed differentiation of human pluripotent stem cells (hPSC) with extensive and costly protocols. An alternative is to expand islet-progenitor cells to generate islet-like structures in vitro. It is therefore imperative to have a deeper understanding of human pancreas development and the signalling pathways that govern the emergence of the different endocrine subtypes – alpha, beta, delta, epsilon and PP cells that are all derived from a common, ancestral progenitor, transiently expressing the transcription factor NGN3. Using a human hPSC NGN3.T2A.dTOMATO knock-in reporter cell line, we monitored in vitro the development and differentiation from endocrine progenitor to hormone-positive cell types. With the reporter cell line referenced against anti-NGN3 antibody staining, we determined the kinetics of NGN3 expression in vitro with the goal of identifying time points representative of NGN3 and post-NGN3, day 16 and 22 of culture respectively. We found that NGN3 and post-NGN3 positive cells expressed ki67 suggesting a potential to proliferate. We next investigated whether these divisions are symmetric or asymmetric. Preliminary data of differentiated adjacent daughter cells showed either the same or different hormone expression. To further explore this observation, we sorted NGN3+ cells at these two stages and differentiated them into 3D islet-like cells with no apparent difference in proportion of INSULIN+ and GLUCAGON+ cells. These data strongly suggest that endocrine commitment is likely either predetermined before/during NGN3 expression. Further research aimed at determining whether cell-cell interactions also play a role in the endocrine cell

fate is ongoing, as 2D culture of post-NGN3 sorted cells were viable and of those that differentiated chiefly expressed INSULIN.

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Keywords: Human Pluripotent Stem Cells, Endocrine Progenitor, NGN3, Pancreas Development

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OVEREXPRESSION OF CTCF PROMOTES OFF-TARGET CELL FATE IN STEM CELL-DERIVED ISLETS

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Insulin-dependent diabetes mellitus is an incurable disease in which the body loses the ability to control its blood glucose levels due to the death or dysfunction of insulin-producing beta cells. Currently, transplantation of stem cell-derived islets (SC-islets) is being explored as a potential treatment and cure. However, current protocols to generate SC-islets produce both on-target stem cell-derived beta (SC-beta) cells and off-target stem cell-derived enterochromaffin (SC-EC) cells, which negatively impact SC-islet function. Single-nuclei multiomic sequencing was performed to concomitantly characterize gene expression and chromatin accessibility in SC-islets. Comparative analysis of gene expression and chromatin accessibility in SC-beta and SC-EC cells resulted in a list of potential transcription factors that regulate cell fate specification between SC-beta and SC-EC cells. From this list, the highly conserved CCCTC-binding factor (CTCF) had the largest enrichment of DNA-binding motifs in SC-EC cells. On this basis, we sought to explore the effects of CTCF expression on cell fate in SC-islets cells using a doxycycline-inducible dCas9-VPR stem cell line. To achieve this, we first performed a plasmid assembly procedure of the CTCF guide RNA (gRNA) by utilizing a Type IIS restriction endonuclease enzyme and T7 DNA ligase enzyme to efficiently ligate our target CTCF fragments in a defined order into a Lenti sgRNA (MS2) Puro vector backbone of plasmid DNA. We then generated CTCF-lentivirus, infected the dCas9-VPR stem cell line and differentiated this line using our established SC-islet differentiation protocol. We found that CTCF, a well-known chromatin-architectural protein, when overexpressed in SC-islets, decreases the expression of beta-cell identity genes while also increasing the expression of EC-cell associated genes. These data have demonstrated the utility of virus-mediated gene transfer methodology in studying the effects of chromatin remodeling regulators during SC-islet differentiation.

Keywords: SC-islets, Virus-mediated gene transfer, Lentivirus

TOPIC: NO TISSUE SPECIFICITY

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NOVEL ROLE OF B-CATENIN IN DECISION TO SELF-REPLICATE OR DIFFERENTIATE

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The literature is replete with conflicting reports as to whether the Wnt/β-catenin pathway induces human stem cell differentiation or pluripotency. Recently scientists showed that human stem cells expressing low levels of active β-catenin preferentially differentiate down neuroectoderm lineage, whereas cells expressing high levels favor differentiation to mesendoderm. However, these results appear to contradict two other studies, where researchers improved differentiation to both neuroectoderm and mesoderm by increasing levels of active β-catenin at the start of differentiation. Here, we show that stem cells cultured in medium containing naïve growth factor, NME7AB, express the highest levels of active β-catenin, yet readily differentiate into neuro-ectoderm cells and mes-endoderm, without lineage preference. This raised the interesting question of whether activation of the Wnt/β-catenin pathway could itself play a role in maintaining or inducing a naïve-like state. To answer that question, the β-catenin agonist WNT3A was added to stem cells, in the absence of any other growth factors, including those present in adhesion layers. Surprisingly, WNT3A induced the concurrent emergence of two segregated populations: an OCT4+, XaXa naïve-like population and an OCT4- population. This finding could explain the apparently conflicting reports in the literature as to whether β-catenin induces pluripotency or differentiation, while raising a new set of intriguing questions. Notably, does the naïve-like sub-population, devoid of cell fate decisions, contribute to an increased differentiation potential of the overall population? Conversely, could the OCT4- cells differentiate better because they are poised to differentiate? To address these questions, we compared the differentiation of primed state stem cells, with or without pre-treatment with WNT3A, to that of naïve state stem cells. Pre-treatment of primed state stem cells with WNT3A improved their differentiation potential, whereas the addition of WNT3A to naïve stem cells had no effect. In each case, the differentiation of the naïve cells was superior to the primed state cells, even after pre-treatment with WNT3A, which is consistent with the idea that the improved differentiation is due to the sub-population of the WNT3A induced naïve-like cells.

Keywords: β-catenin, naïve stem cells, differentiation

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ELUCIDATION OF THE ROLE OF P-CADHERIN AND A C/EBP BETA ISOFORM LAP AS MEDIATORS OF THE EFFECTS OF EXTRACELLULAR SYNTAXIN4 IN ES CELLS

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Pluripotent stem cells possess differentiation potential into all three germ layers and hold great promise for regenerative medicine. In the presence of GSK3β inhibitor and MEK1/2 inhibitor (2i), together with a leukemia inhibitory factor (LIF), these cells maintain undifferentiated stats, exhibit cuboidal morphology and grow as tightly-packed rounded colonies. Upon removal of 2i, however, a cell population spontaneously appears, that displays flattened morphology, moves apart from the colonies, and loses stemness signatures. We previously showed that the removal of 2i led to the membrane translocation of syntaxin4, a t-SNARE protein, which subsequently induced these changes in ES cells even in the presence of LIF. Recently, these effects appeared to be primarily mediated by up-regulation of P-cadherin: extracellular syntaxin4 induces P-cadherin expression and an artificial overexpression of P-cadherin induced the aggressive phenotype resembling those in extracellular syntaxin4-expressing cells. Herein, we identified liver-enriched activator protein LAP, an isoform of CCAAT enhancer binding protein β (C/EBP-β), as an alternative downstream effector of extracellular syntaxn4. We found that expression of extracellular syntaxin4 up-regulated not only P-cadherin but also LAP protein, which led us to pursue the possible involvement of LAP protein in syntaxin4's effects. C/EBP-β gene is known to be translated into several isoforms through in-frame translation initiation, and long isoforms LAPs exert transcriptional activity whereas a short isoform LIP that lacks the N-terminal transactivating domain behaves as an antagonist of LAPs. We prepared the doxycycline/tetracycline-inducible expression construct for LAP, in which an ATG codon for the translation initiation of LIP was replaced to CTG. The ES cells stably introduced with this construct expressed LAP protein and induced morphological changes in ES cells as observed in P-cadherin-expressing cells. While P-cadherin is known as a transcriptional target of C/EBP-β, overexpression of LAP did not affect the amount of P-cadherin, suggesting that extracellular syntaxin4 independently up-regulates P-cadherin and LAP but signals propagated by these elements might converge into common regulators for changes in ES cell behaviors.

Keywords: Differentiation, Syntaxin4, P-cadherin, Liver-enriched activator protein LAP, an isoform of CCAAT enhancer binding protein β (C/EBP-β), as an alternative downstream effector of extracellular syntaxin4., Relationship between P-cadherin and C/EBP-β

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COMPREHENSIVE ANALYSIS OF TRANSCRIPTIONAL VARIABILITY OF INDUCED PLURIPOTENT STEM CELLS DURING CULTURE

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Human induced pluripotent stem cells (hiPSCs) are supposed to be a source of regenerative medicine for a wide range of diseases. The cells used for clinical applications are required to be manufactured under strict quality control for the warranty of safety and efficacy. Although transcriptome analysis is a useful tool to understand the characters of the cells, it is more difficult to determine the critical quality attribute of hiPSCs from those data than to do that for compound drugs due to their dynamic alteration and heterogeneity. We investigated the temporal dynamics of gene expression of hiPSCs during colony development and found that transcriptional variations attributed to the duration after cell seeding were the largest among other factors such as the difference of lines (donors of somatic origin) and the number of passages in the repeated same culture condition. Therefore, it might be better to consider the difference in the phase of culture duration for the evaluations of cell characters rather than to determine by the expression of several marker genes. In addition, since the temporal regulations of gene expressions during colony development were tend to along the axis from endoderm to ectoderm, the timing to induce differentiation into any lineage may be critical for the efficient differentiation protocol.

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Keywords: human induced pluripotent stem cells, Quality control, Transcriptome analysis

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DIFFERENTIAL SHIFTS IN GLUTAMINE NITROGEN USAGE CONTRIBUTES TO EARLY CELL FATE TRANSITIONS

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Shifts in metabolite levels and fluxes help determine whether human pluripotent stem cells (hPSCs) self-renew or undergo differentiation. Upon exit from pluripotency, glutamine (Gln) requirements distinguish ectoderm, mesoderm and endoderm germ lineages. Gln has pleiotropic roles in cell function and provides five carbon and two nitrogen atoms, as amide and amine groups, for biosynthetic reactions and energy generation. In mesoderm and endoderm, Gln carbons may form an alpha-ketoglutarate precursor to fuel TCA cycling and catalyze histone modifications for fate specification. However, ectoderm preferentially shunts carbon atoms towards lipid biosynthesis and gluconeogenesis to support

proliferation. How the three germ lineages differentially use Gln carbons for cell fate specificity was recently described, although how the germ lineages apportion Gln nitrogen groups during hPSC differentiation remains unknown. To examine the fate of Gln nitrogens during hPSC differentiation, we profiled metabolite changes using mass spectrometry and quantified shifts during tri-lineage fate transitions with relative abundance measurements and stable-isotope tracing. We report that, similar to Gln carbons, ectoderm uses Gln nitrogen groups to support proliferation by building cell biomass. Metabolite changes during hPSC differentiation to ectoderm revealed that by the end of differentiation the abundance of 15 amino acids significantly increased compared to the start of differentiation. In addition, Gln-nitrogen amide and amine fluxes were increased for amino acids and metabolites of the hexosamine and nucleotide biosynthesis pathways at the start of ectoderm differentiation but decreased by the end of differentiation. The fact that these biosynthetic pathways were sustained by Gln nitrogens throughout the period of ectoderm differentiation specifically suggests that such biomass accumulation supports ectoderm fate commitment. These preliminary data provide insight into how differential Gln nitrogen disposition and utilization during hPSC differentiation may contribute to different hPSC fate transitions.

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Keywords: cell fate, pluripotent stem cell, metabolism

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LSD1-MEDIATED FOXA2/AP-1 TRANSCRIPTION PROGRAM DRIVES LINEAGE PLASTICITY IN PROSTATE CANCER

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Prostate cancer (PCa) initially responds to the standard androgen deprivation therapies (ADTs) that block androgen receptor (AR) activity, but eventually relapses to a more aggressive stage, called castration-resistant PCa (CRPC). One critical ADTs resistance mechanism is that tumor cells can progress to AR-indifferent stem cell-like (CRPC-SCL), neuroendocrine-like (CRPC-NE), or Wnt signaling pathway-dependent CRPC (CRPC-WNT) through lineage plasticity. However, the underlying molecular basis driving this progression remains to be determined, and clinical treatment options for these aggressive CRPC subtypes are currently limited. FOXA1 and FOXA2, which are members of the FOXA (Forkhead Box A) protein family, are pioneer transcription factors that function to maintain accessible nucleosomes at tissue-specific enhancers. While FOXA1 is well known for its function as a critical pioneer factor of AR and pivotal for maintaining CRPC-AR lineage, the molecular function of FOXA2 in PCa cells is poorly understood. We found that FOXA2 silencing decreased the growth and metastasis of CRPC-SCL/CRPC-NE cells. Importantly, we discovered that FOXA2 chromatin binding is tightly associated with binding of JUN family proteins (primarily c-Jun), and FOXA2 silencing dramatically interrupts the global chromatin binding of c-Jun and FOSL1. The transcription targets of FOXA2/AP-1 are highly enriched for plasticity associated genes and associated with poor clinical outcomes. Furthermore, we also found that FOXA2 chromatin binding is globally enhanced by an epigenetic factor Lysine-specific demethylase 1 (LSD1), possibly through demethylating FOXA2 protein, and LSD1 inhibition can repress FOXA2/AP-1 activity in multiple CRPC models. Overall, our data indicate that FOXA2 functions to maintain tumor growth and metastasis in CRPC-SCL/CRPC-NE. Mechanistically, FOXA2 can function as a pioneer factor of AP-1 (c-Jun/FOSL1) and reprogram AP-1 transcription activity to maintain the tumor lineage. This FOXA2/AP-1 axis is regulated by LSD1 via demethylating FOXA2 protein and LSD1 inhibition represses FOXA2-dependent CRPC tumor progression. Our study provides novel mechanistic insights into the molecular mechanisms for PCa lineage plasticity and treatment resistance.

Funding Source: NIH (R01 CA211350 to C.C.) and DoD (W81XWH-19-1-0361 and W81XWH-21-1-0267 to C.C.). Z.W. is supported by CSM graduate student fellowship from University of Massachusetts Boston.

Keywords: LSD1, FOXA2/AP1, Lineage plasticity

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NEW REGULATORY MECHANISM FOR THE BALANCE BETWEEN LINEAGE SPECIFICATION AND STEMNESS

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It is important to detoxify cytotoxic byproducts formed during carbohydrate, protein and fatty acid metabolism, which lead to nucleotide and protein modification. Excessive byproducts cause increased reactive oxygen species, caspase-dependent cell death and damage to many tissues or organs. To eliminate toxic byproducts, various detoxification systems works together, however, their roles in human pluripotent stem cells have not been clearly defined yet. Here, we reveal new regulatory role that one of metabolism modifiers has ability to control equilibrium between self-renewal versus differentiation potential of hPSCs. An abolished expression of modifier using gene editing limits lineage specification in three-germ layer in vitro while highly maintaining pluripotent transcription factors, Oct4 and Nanog. We uncover the identification of Clathrin Heavy Chain (CLTC), which regulates the early steps of autophagosome formation, as a direct interaction partner for the modifier. It also results in stabilizing the self-renewal capacity of hPSCs at the expense of differentia-

tion potential. In conclusion, we address a novel functional role of modifier independent of metabolite detoxification in maintaining CLTC-mediated pluripotency.

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Keywords: human Pluripotent Stem Cells;hPSCs, Lineage specification and stemness, Clathrin Heavy Chain;CLTC

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SINGLE-CELL MAPPING THE HUMAN AMNIOTIC FLUID CONTENT ACROSS GESTATION AT GENE AND PROTEIN EXPRESSION LEVEL

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The amniotic fluid (AF) surrounds and protects the human fetus during gestation. The AF contains multiple cell populations, shed from multiple fetal tissues. Consequently, its cellular composition is highly heterogenous, with relevant changes occurring across pregnancy. The generation and dynamics of AF are complex, and its cellular content follows the developmental progression of a multiplicity of fetal organs, such as gut, kidney, lungs, skin and placenta. AF cells have been mostly ascribed to the epithelial, mesenchymal, and haematopoietic linages. However, a comprehensive, dynamic and detailed map of the human AF cells has yet to be compiled. The AF is rich in dead cells, debris and urea crystals, with viable cells forming just ~1% of the total events present. Hence, we developed a method for isolating live cells from the AF. By combining centrifugation, filtration, and live/dead cell sorting, we consistently obtained representative AF cells with 95% viability. This made it suitable for scRNAseq and CITEseq analyses. Single cell transcriptomic data was generated for 23 AFs (10-37 gestational age weeks), with proteomic data determined for a selection of 6 fluids (16-24 GA). Overall, we analysed over ninety thousand human single cells, annotating each with its identity, as well as tissue of origin wherever possible. This has allowed us to track the development of the fluid over gestation and to identify cells of clinical interest. A range of immune cell types were identified (B-cells, T-cells, NK-cells, Dendritic Cells, Monocytes, Myelocytes, Macrophages, Neutrophils), as well as hematopoietic progenitors. In addition to these, a large epithelial population (~60% of cells present) was shown to contain cells originating from the skin, kidney, lung, small intestine and placenta. Within this, we identified previously unreported tissue-specific epithelial progenitors originating from the developing lung, kidney and intestine. These cells have been prospectively isolated from the fluid, and are currently being used to develop organoid-based developmental and disease models of these tissues.

Funding Source: Marie Skłodowska-Curie Postdoctoral Fellowship Great Ormond Street Hospital (GOSH) Children's Charity National Institute for Health and Care Research (NIHR) Biomedical Research Centre (BRC)

Keywords: Amniotic Fluid Cell Atlas, single-cell RNA CITEseq sequencing, Developmental mapping of the amniotic fluid

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INVESTIGATING THE DYNAMICS OF CELL STATES USING FLUORESCENTLY TAGGED HUMAN INDUCED PLURIPOTENT STEM CELLS (HIPSCS) GENERATED USING NOVEL METHODS

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The Allen Institute for Cell Science aims to understand the principles by which human induced pluripotent stem cells (hiPSCs) establish and maintain robust dynamic localization of cellular structures, and how they transition between states during differentiation and disease. Cellular differentiation is a dynamic state change that can be characterized by a switch in cellular organization and behavior, making these processes attractive for investigation. Our approach utilizes CRISPR/Cas9 to fluorescently tag proteins that localize to cellular organelles and 3D live imaging and visualization tools to interrogate cell behavior. Here, we discuss the novel methods we have been employing to improve the efficiency of gene editing in studying differentiation-specific changes in cellular organization. For example, we have tagged several genes specific to hiPSC differentiating into cardiomyocytes (TTN, MYL7, MYL2, TNNI1, and ACTN2), using a multistep tagging method where a GFP fusion tag and a constitutively expressed mCherry fluorescence selection cassette were delivered simultaneously to a gene of interest. Following selection, the mCherry cassette was excised via microhomology-mediated end-joining and non-homologous end-joining. We have also used an updated, selection-free AAV-mediated CRISPR/Cas9 method

to fluorescently tag genes associated with EMT regulation (TBXT, TBR2) and endothelial cell fate (CDH5). The CDH5-mEGFP hiPSC line maintained the ability to differentiate into endothelial cells, exhibited morphology similar to untagged endothelial cells and will allow us to visualize morphological changes in response to shear stress using live cell microscopy. These hiPSC lines with differentiation-specific markers not only facilitate live imaging to study the organization and principles of cell state transitions but also serve as valuable tools for others in the scientific community. The Allen Cell Collection, the plasmids used to create these lines, and the image analysis tools are openly available (allencell.org).

Keywords: Gene editing tool development, Differentiation, Microscopy

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PERTURBATION OF MECHANICAL CONTRACTILITY ENABLES ROBUST LINEAGE CONVERSION FROM FIBROBLASTS TO NEURONS AND ADIPOCYTES

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Direct cell reprogramming, also called transdifferentiation, is valuable for cell fate studies and regenerative medicine. Current approaches to transdifferentiation are usually achieved by directly targeting the nuclear functions, such as manipulating the lineage-specific transcriptional factors, microRNAs, and epigenetic modifications. Here, a robust method to convert fibroblasts to neurons through targeting the cytoskeleton followed by exposure to lineage-specification surroundings is reported. Treatment of human foreskin fibroblasts with a single molecule inhibitor of the actomyosin contraction, can disrupt the cytoskeleton, promote cell softening and nuclear export of YAP/TAZ, and induce a neuron-like state. These neuron-like cells can be further converted into mature neurons, while single-cell RNA-seq shows the homogeneity of these cells during the induction process. Finally, transcriptomic analysis shows that cytoskeletal disruption collapses the original lineage expression profile and evokes an intermediate state. More recently, fibroblast transdifferentiation into adipocytes has been achieved by disrupting cytoskeletal contraction and binding signals that promote mesoderm development. In combination with the above findings, disruption of cytoskeletal contraction combined with an environment conducive to the specialization of different lineages can achieve the transdifferentiation of fibroblasts to different lineages, which suggested that the cytoskeleton may serve as a new dimension for regulating cell fate in addition to lineage-specific transcription factors and epigenetic modifications.

Keywords: Cytoskeleton, Mechanical Contraction, Lineage Conversion

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INVESTIGATING THE ROLE OF HUMAN UTF1 IN CELL REPROGRAMMING AND MAINTENANCE OF IPSC IDENTITY USING A CRISPR/CAS9 TOOLBOX

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Undifferentiated embryonic stem cell Transcription Factor 1 (UTF1) was first identified in embryonic stem cells (ESCs) as a pluripotent cell-specific transcription factor. Although it was one of the members of the reprogramming cocktail tested for the generation of the first induced pluripotent stem cells (iPSCs), it was not amongst the four transcription factors (Yamanaka factors) identified to derive the iPSCs. However, its importance was soon revealed in a study, which showed that the inclusion of UTF1 in the reprogramming cocktail greatly enhanced the efficiency and quality of human iPSCs generated. Our study aims to elucidate the role of human UTF1 in reprogramming and maintenance of iPSC identity. We first investigated the role of UTF1 in reprogramming human adult dermal fibroblasts by knocking out the UTF1 gene using a CRISPR/Cas9 toolbox. The depletion of UTF1 resulted in a considerable decline in the reprogramming efficiency and the few iPSC-like colonies formed showed spontaneous differentiation eventually. Further, we also deleted the UTF1 gene in an established iPSC line to determine its role in the maintenance of iPSC identity. Interestingly, the results showed drastic cell death upon UTF1 depletion in these cells. Our results established an important role of UTF1 in iPSC generation and in the maintenance of iPSC identity. These promising insights of UTF1 could enrich our present knowledge of the regulatory systems actively involved in establishment and maintenance of pluripotency.

Keywords: Reprogramming, Knockout, Pluripotency

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DNA DAMAGE SIGNALLING ACTIVATION IN PLACENTA-DERIVED MESENCHYMAL STEM CELLS IN RESPONSE TO AGING AND SARS-COV-2

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The human placenta has been considered attractive alternative source for non-invasive isolation of human mesenchymal stem cells (PDMSCs). However, donors age affects PDMSCs regenerative and immunomodulatory potential. Previously, we reported a correlation in proliferative capacity and telomere shortening in PDMSCs of 5 maternal donor age groups. Short telomeres activate the DNA damage signalling. Dysregulation in DNA repair pathways can predispose MSCs to senescence, apoptosis and reduced stemness and self-renewal. In this study, we compared the gene expression of DNA damage response and repair pathways in PDMSCs of 3 maternal donor groups (18-28, 29-35 and 36-42). We isolated PDMSCs from human placental tissues of healthy full-term donors and used RT2 profiler array. Data showed altered expression in 28 genes when compared to controls. Maternal age group of 36-42 presented 24 genes with more that ~2 fold up-regulation involved in ATM/ATR signalling, nucleotide excision repair, base excision repair, mismatch repair, double-strand break repair and other DNA repair genes. Proteins involved in telomere maintenance and rolling circle amplification where up regulated in 36-42 PDMSCs group correlating with our previous reports showing longer telomeres in PDMSCs from 36 and older maternal donor age. Activating DNA damage checkpoint help maintain MSC function including regenerative secretome release to activate tissue repairing mechanisms. Further, we isolated PDMSCs from COVID19 positive donors. We performed in vitro infection of SARS-CoV-2 in PDMSCs and COVID19-PDMSCs to analyse and compare gene expression using RT2 profiler array. Data showed 18 genes altered expressions in infected PDMSCs and infected COVID-19 PDMSCS when compared to un-infected controls. Interestingly, genes involved in ATM/ATR signalling pathway were more than ~2 fold down regulated in infected PDMSCs, but stably expressed infected COVID19-PDMSCs. It is known that viral infection induces DNA damage via different mechanism such as, host cell replication stress, however it has been shown that SARS-CoV-2 is not able to infect MSCs due to the lack of ACE2 receptor. The regenerative capacity and the evasion of SARS-CoV-2 infection of PDMSCs conferred the potential for the treatment of COVID19 and the decrease of de inflammatory cascade

Funding Source: Sistema Nacional de Investigación de la SENACYT.

Keywords: DNA damage response, mesenchymal stem cells, COVID19

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MIR-214 MEDIATED EPIGENETIC REGULATION OF HIPSC GENE EXPRESSION TO ENHANCE COMMITMENT TO MESODERM CELLS THAT GIVE RISE TO ECFC

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Endothelial colony-forming cell (ECFC) enhance vascular repair and improve blood flow. The reparative ability of ECFCs are unique cell properties and provide an innovative reagent for cell therapy. We published a novel protocol for deriving ECFCs from mesoderm derived from human induced pluripotent stem cell (hiPSC). While mesoderm formation from differentiating hiPSC requires global epigenetic changes, the identity and roles of microRNA (miR) in differentiation of hiPSC into SSEA5-KDR+NCAM+APLNR+ mesoderm cells (S-KNA+) are completely unknown. We hypothesized that certain miR play a critical role in regulating mesoderm formation from hiPSC. We performed RNA-seq and concomitant miR analysis on RNA samples isolated from undifferentiated hiPSC and hiPSC-S-KNA+ and mapped the top 12 miR with validated and published downstream target genes, to the differentially expressed genes from the RNAseq analysis. Since our miR-expression analysis identified that miR-214 was highly expressed in S-KNA+, we generated a lentiviral construct to over-express miR-214 in hiPSC undergoing mesoderm differentiation. MiR-214 overexpression significantly enhanced formation of S-KNA+. We next examined the list of miR-214 targets that have been validated and are relevant to the differentially expressed mRNA. We identified claudin 6 (CLDN6) as one of 12 miR-214 targets differentially expressed, identified CLDN6 as a direct target of miR-214, and validated significantly higher levels of CLDN6 expression in miR-214 overexpressing cells. We are currently confirming whether loss of CLDN6 expression is necessary and sufficient for mediating the changes in hiPSC differentiation into S-KNA+ identified when miR-214 is overexpressed. We anticipate the enhancement of S-KNA+ formation by differentiating hiPSC will be blunted by CLDN6 over-expression and that CLDN6 deletion alone will significantly enhance S-KNA+ formation by diminishing commitment of differentiating hiPSC into endoderm.

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Keywords: Mesoderm, CLDN6, miR-214

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QUALITY CONTROL FOR PLURIPOTENT STEM CELLS AND EMBRYOID BODIES BASED ON DNA METHYLATION

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The trilineage differentiation potential toward endoderm, mesoderm, and ectoderm is a hallmark of pluripotent stem cells. Gene expression analysis and immunofluorescence of specific marker proteins are most commonly used for qualitative and quantitative assessment of such differentiation, but reliable quality control of iPSCs remains a challenge. Here, we describe an alternative approach based on a combination of site-specific DNA methylation (DNAm) assays that serve as biomarker for early germ layer specification. Overall, the DNAm changes during differentiation were in line with gene expression data. Notably, directed differentiation towards endoderm and mesoderm resulted in closely related molecular profiles in some datasets. Initially, we identified CG dinucleotides (CpGs) with the new R package CimpleG that reveal characteristic DNAm at pluripotent state. Thereby, we derived a pluripotency score based on three CpGs that tracks reprogramming and may reflect differentiation capacity. In analogy, we established differentiation scores to track endodermal, mesodermal, and ectodermal differentiation in monolayers. The lineage-specific differentiation scores could also reflect the self-organized multilineage differentiation in embryoid bodies (EBs). This is crucial, since directed differentiation and undirected differentiation do not necessarily follow the same epigenetic tracks, and the later may better reflect intrinsic differentiation bias. Germ layer specific epigenetic modifications were in line with flow cytometric measurements, such as up-regulation of PAX6 in undirected ectodermal differentiation in EBs. Furthermore, we established pyrosequencing assays for fast and cost-effective analysis, which are continuously further validated on cell-preparations from different labs. Taken together, GermLayerTracker can provide quality control of pluripotent stem cells and indicate lineage-specific commitment during early differentiation events.

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Keywords: DNA methylation, Embryoid Body, Biomarker

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PARP1 INVOLVES IN ZSCAN4-MEDIATED DNA DOUBLE-STRAND BREAK REDUCTION IN MOUSE EMBRYONIC STEM CELLS

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ZSCAN4 is expressed in mouse 2-cell embryos and is sporadically expressed in 2-5% mouse embryonic stem cells (mESCs). It is known to regulate genome stability by promoting the homologous recombination pathway. PARP1 is involved in DNA double-strand break (DSB) repair and plays a key role in the alternative non-homologous end joining (alt-NHEJ). It is not known whether there is any interaction between ZSCAN4 and PARP1 in the DSB resolution processes. In the present work, we first show that overexpression of ZSCAN4 in mESCs reduced the protein levels of γH2AX, a marker of cellular DSB extent, by both western blot assay immunofluorescence staining. Interestingly, PARP1 inhibitor 3-aminobenzamide (3-AB), abolishes the DSB-reducing benefits by ZSCAN4 overexpression in mESCs. Consistently, overexpression of ZSCAN4 in Parp1 knockout mESCs had no effect on reducing the overall DSB extent. These data suggest that PARP1 interacts with ZSCAN4, and sheds light on the molecular mechanisms by which ZSCAN4 reduces DSB in mESCs.

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Keywords: ZSCAN4, PARP1, DNA double-strand breaks

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EPIGENETIC CONTROL BY IκBα DRIVES PLURIPOTENCY EXIT AND DIFFERENTIATION INDEPENDENTLY OF NF-κB

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Understanding how pluripotency is maintained and acquired is crucial to obtain stem cells with high differentiation performance. Although several pathways have been identified to regulate pluripotency, many players that regulate the transition from naïve to primed pluripotency and control pluripotency exit are mainly unknown. Inflammatory signals such as the NF-kB pathway have paradoxical functions in different types of stem cells. Previous work in our laboratory identified a novel nuclear function for the main NF-kB pathway inhibitor, IκBα, in regulating homeostasis and differentiation potential of many somatic stem cells in vivo and in vitro. We now have investigated whether IκBα has also a stem cell function in pluripotent stem cells. We have observed that IκBα has a nuclear distribution specifically in pluripotent mouse embryonic stem cells (mESCs), and depletion of the protein at that stage causes a blockade of the pluripotency exit upon differentiation in vitro (by embryoid bodies differentiation) and in vivo (teratomas). Further analysis of IκBα-depleted mESCs demonstrated a global loss of DNA methylation at different genomic regions, which is consistently detected in imprinted genes and pluripotency factors upon EB differentiation. This loss of DNA methylation in IκBα-depleted mESCs positively correlates with a reduction in H3K9me3 levels that is more pronounced in imprinted control regions, as it has been previously demonstrated in prolonged 2i/LIF culture of mESCs. Finally, the detection of a physical interaction of IκBα with the main H3K9 methyl transferase SETDB1 further suggests a direct role of IκBα in facilitating H3K9me3 deposition at specific loci in mESCs. To investigate the possible contribution of NF-kB pathway to the altered chromatin function observed in IκBα-depleted cells, we have developed separation-of-function (SOF) mutants of IκBα that specifically lost the NF-kB-inhibition or the chromatin-linked function. We have demonstrated that IκBα SOF NF-kB is sufficient to reestablish the DNA methylation levels in ES cells and to rescue the pluripotency blockage of IκBα-depleted ES cells in a NF-kB-independent manner. Altogether, these results place IκBα as a key player in regulating pluripotency exit by controlling epigenetic mechanisms that involve H3K9me3 and DNA methylation.

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Keywords: Epigenetic regulation of pluripotency, Inflammatory pathways, DNA methylation

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THE CZ CELLXGENE DISCOVER SUITE IS AN ANALYTICAL PLATFORM AND THE LARGEST REPOSITORY OF STANDARDIZED SINGLE-CELL DATA

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CZ CELLxGENE Discover (cellxgene.cziscience.com) is a free-to-use online data portal hosting a growing corpus of more than 700 single-cell datasets comprising over 38 million unique cells from the major human and mouse tissues. As of today the portal hosts single-cell data from modalities that include gene expression, chromatin accessibility, DNA methylation, and spatial transcriptomics. All data have been standardized to include raw counts, gene IDs and symbols, as well as cell metadata, such as cell type, tissue, and donor age and disease, each standardized to a community ontology. All data are easily searchable and can be downloaded in both AnnData and Seurat formats via web or by programmatic API calls. UI-based tools allow for exploration of single datasets without requiring download. New features for the analysis of the entirety of the data are available and under active development. The CELLxGENE explorer displays an interactive 2-dimensional representation of cells in a dataset and allows users to color cells by metadata (e.g. cell type, disease, metadata features etc.) or gene activity. Users can also subset and analyze subgroups of cells, perform differential gene expression and create scatter plots of gene expression. The Gene Expression feature allows querying the expression of any gene across all human and mouse cell types available in the portal, and enables lookup of differentially enriched genes for any cell type. Lastly APIs enable download of individual datasets and cell-based slicing and

download of data subsets. CELLxGENE is continuously improving usability and adding new features tailored to the needs of cell and computational biologists. CELLxGENE is a tool intended for community use and contributions, and aims to maximize rapid reuse of high quality data describing the phenotypes of cells and tissues. To date, CELLxGENE supports data sharing from labs around the world as well as consortia such as the CZ Biohub Tabula projects, LungMap, BICCN, Allen Institute for Brain Science, KPMP, HTAN and the Human Cell Atlas. New collaborations and contributions are welcome. Groups interested in submitting their own data can inquire about the inclusion of your data and the submission process by contacting the CELLxGENE team at cellxgene@chanzuckerberg.com.

Keywords: Single-cell data, Data repository, Data exploration

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ELEVATED ERK ACTIVITY PROVIDES RESISTANCE TO SINGLE-CELL DISSOCIATION-INDUCED CELL DEATH IN DUSP6 KNOCKOUT HUMAN INDUCED PLURIPOTENT STEM CELLS

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Human induced pluripotent stem cells (hiPSCs) are pivotal for regenerative medicine, disease modeling, and drug discovery but are hindered by cell death during single-cell dissociation. Understanding the molecular mechanisms underlying this vulnerability is crucial for improving hiPSC survival. In this study, we investigated the role of dual specificity phosphatase 6 (DUSP6), a negative regulator of extracellular signal-regulated kinase (ERK) activity, in mediating single-cell dissociation-induced cell death in hiPSCs. We generated DUSP6 knockout (KO) hiPSC lines using CRISPR/Cas9 technology and confirmed the successful knockout via genotyping and immunoblotting. DUSP6 KO cells exhibited elevated ERK activity compared to wild-type control cells. We subjected wild-type and DUSP6 KO hiPSCs to single-cell dissociation using EDTA and assessed cell viability and proliferation rates. DUSP6 KO cells showed significantly higher viability, indicating increased resistance to dissociation-induced cell death. DUSP6 KO cells also showed a differentiation propensity toward mesoderm and endoderm comparing to WT cells. Enhanced proliferation in DUSP6 KO cells suggested a potential benefit in large-scale expansion efforts.

Funding Source: 2023-NI-009

Keywords: resistance, dissociation, DUSP6

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GENOME-WIDE QUANTIFICATION OF RNA FLOW ACROSS SUBCELLULAR COMPARTMENTS REVEALS DETERMINANTS OF THE MAMMALIAN TRANSCRIPT LIFE CYCLE

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Dissecting the myriad regulatory mechanisms controlling eukaryotic transcripts from production to degradation requires quantitative measurements of mRNA flow across the cell. We developed subcellular TimeLapse-seq to measure the rates at which RNAs are released from chromatin, exported from the nucleus, loaded onto polysomes, and degraded within the nucleus and cytoplasm. These rates varied substantially, yet transcripts from genes with related functions or targeted by the same transcription factors and RNA binding proteins flowed across subcellular compartments with similar kinetics. Verifying these associations uncovered roles for DDX3X and PABPC4 in nuclear export. For hundreds of genes, most transcripts were degraded within the nucleus, while the remaining molecules were exported and persisted with stable lifespans. Transcripts residing on chromatin for longer had extended poly(A) tails, whereas the reverse was observed for cytoplasmic mRNAs. Finally, a machine learning model identified additional molecular features that underlie the diverse life cycles of mammalian mRNAs.

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Keywords: subcellular RNA flow, RNA dynamics, nuclear RNA degradation

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STOCHASTICITY IN CELL CYCLE TRANSCRIPTIONAL PROGRAMS IS A METRIC OF FATE POTENTIAL

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Since the advent of single cell RNA-sequencing (scRNA-seq), computational methods have been invented that quantify cellular differentiation potential. To the extent that such methods are accurate and broadly applicable, they are highly valuable because they identify rare progenitor populations, they order cells along a pseudo-temporal axis and thus reveal regulatory cascades underpinning development processes, and they measure the impact of genetic or other perturbations on fate potential. However, current

state-of-the-art methods possess notable failure rates and some are too computationally burdensome to be routinely used. Here, we exploited the relationships between cell cycle gene expression, cell cycle length, and transcriptional stochasticity to devise a more accurate, computationally tractable, and robust metric of differentiation potential. Our method, StemFinder, computes differentiation potential based on expression stochasticity of cell cycle genes in single cells, such that undifferentiated cells exhibit highly stochastic cell cycle gene expression. We compared StemFinder to the two current best performing methods based on a gold standard compendium of 24 datasets from 4 species, 9 sequencing platforms, and 11 organ systems in which the ground truth stage of differentiation was known. StemFinder is more accurate and sensitive than its competitors, and its run time is not significantly longer than that of the fastest competitor. We highlight the utility of StemFinder to aid in revealing new biological insights in two case studies: (1) the delineation of progenitor cell subpopulations in the murine dentate gyrus, and (2) the impact of culture conditions on differentiation potential of human pluripotent stem cells. Finally, by application to hematopoietic stem/progenitor cells, we show that this class of computational techniques accurately measures fate potential as defined by the number of distinct lineages reached by the progeny of lineage-traced clones.

Funding Source: This work was supported by the National Institute Of General Medical Sciences of the National Institutes of Health under Award Number R35GM124725

Keywords: Computational biology, Cell fate potency, Cell cycle

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CRITICAL CHARACTERIZATION STEPS OF PLURIPOTENT STEM CELLS FOR DOWNSTREAM APPLICATIONS

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Human induced pluripotent stem cells (hiPSCs) have been established as a powerful tool for modeling human biology both diseased and healthy, developing new cell therapies, and screening potential therapeutic drugs. As the ability of these cells to support such a variety of applications has led to an expanding reliance on hiPSCs, one of the major challenges is ensuring the cell lines used are properly characterized to consistently generate accurate and reproducible results. Furthermore, there has been a recent shift in the hiPSC field from purely research-oriented work towards the downstream process development and manufacturing in a cGMP or clinical setting, and this is leading to a much higher level of scrutiny surrounding the profile of the stem cell cultures that are being used. To address this need, we detail rigorous characterization steps used to comprehensively validate our hiPSC lines. We use the generation of a new iPSC line via the reprogramming of primary fibroblasts to exemplify where characterization is key, in which we qualify the genomic stability, pluripotency, and differentiation potential of the hiPSCs. Specifically, we ensure the cell line contains a genome with a clean karyotype, one free of any aberrations to do with the major cancer related genes, and that compares directly to that of the parental fibroblasts. We demonstrate that this cell line expresses the genes relevant to self-renewal, while down-regulating those related to germ-layer cell types and having a overall gene expression profile equating to a reference set of pluripotent cell lines. As a functional landmark of pluripotency, we further detail the ability of this line to model human systems via embryoid body formation and directed differentiation to multiple target cell types, some of which commonly used in current therapeutic efforts. In summary, we cover a wide variety of hiPSC relevant assays to properly characterize hiPSC banks that in our view are critical when implementing hiPSCs in disease modeling and early development, in screening for potential novel drugs, and as a source for allogeneic cell therapies.

Keywords: Characterization, hiPSCs, Reprogramming

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EXAMINE THE DYNAMICS OF THE PLURIPOTENCY REGULATORY NETWORK BY ENHANCER PERTURBATION AND SINGLE CELL RNA-SEQ

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Enhancers regulate gene expression and have a crucial role in enabling the faithful execution of lineage-specific transcriptional programs during development. Global enhancer activity in human pluripotent stem cells (hPSCs) is dictated by core transcription factors (TFs) OCT4, NANOG, and SOX2. The expression of these TFs is in turn regulated by their own enhancers through the binding of themselves and other such core TFs. However, how the enhancers of these core TFs ensure their proper expression in hPSCs and during differentiation has not been systematically investigated. To address this question, we performed a CRISPR interference (CRISPRi) screen in hPSCs to identify pluripotency enhancers. The hits include a novel NANOG enhancer. HPSC lines heterozygous for this enhancer (no homozygous deletion line was obtained) had reduced NANOG and OCT4 expression. Heterozygous lines also showed accelerated downregulation of pluripotency markers and the upregulation of lineage-specific markers during differentiations. These results implicate that this enhancer safeguards the robustness of pluripotency. To study the impact of core pluripotency enhancers on global gene regulatory network (GRN), we perturbed a selection of enhancers in hPSCs with CRISPRi and profiled their gene expression with single cell RNA-sequencing. The perturbations resulted in a spectrum of transcriptional changes in both the enhancers' target genes and these genes' downstream targets, suggesting the data can be used to quantitatively associate various transcriptomic profiles with different degrees of enhancer perturbations. Furthermore, hPSCs containing gRNAs targeting on NANOG and OCT4 enhancers formed separate clusters, with both overlapping and distinct differentially expressed genes, suggesting the downregulation of different pluripotency factors could lead to different trajectories for exiting pluripotency. Taken together, these experiments will help to decipher enhancer network in development modelled by hPSCs.

Keywords: Enhancers, CRISPR interference, scRNA-seq

TRACK: MODELING DEVELOPMENT AND DISEASE (MDD)

Session 1: Odd

6:00 PM - 6:45 PM

TOPIC: CARDIAC

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MULTIPARAMETRIC IMAGING FOR ENHANCED PREDICTIVE DRUG-INDUCED CARDIOTOXICITY USING HPSC-CARDIOMYOCYTES

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Unexpected cardiotoxicity underlies high levels of late-stage attrition and post-market withdrawals, accounting for up to 45% of the liability. This can, in part, be attributed to the scarcity of physiologically relevant in vitro models capable of predicting cardiovascular safety liabilities early in drug discovery. With the pressing need to integrate human-relevant platforms at an early stage, human pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have become an attractive platform for capturing the effects of chronic modulators or toxicants and could complement existing assays to improve cardiac safety assessments. Several phenotypic methods to measure cardiomyocyte contraction have been developed however, these (i) are not currently scalable and do not provide a single pipeline to deliver actionable data, (ii) require cellular manipulation that can interfere with cell function, and (iii) provide limited mechanistic insights for annotated and unannotated compound classes. We profiled a panel of 90 compounds with known cardiotoxic effects that target different signalling pathways using a novel label-free assay, CardioMotion, to measure alterations in cardiomyocyte contractility; and a morphology-based profiling assay, Cell Painting, to detect differences in the architecture of cellular components and identify bioactivity. We report that the CardioMotion assay is capable of flagging both functional (sensitivity = 70.2%, specificity = 82.1%) and structural cardiac liabilities (sensitivity = 75%), in comparison to the widely used Calcium Flux assay (66.2% sensitivity and 63.9% specificity). Alongside this, Cell Painting was able to effectively cluster small-molecule hits and reveal differences among hit classes in terms of mechanism, capturing distinct information about cellular state. We show the value of integrating functional cardiotoxicity assessments and morphological profiling into small molecule screening cascades to improve the prediction of drug-induced cardiotoxicity.

Funding Source: GlaxoSmithKline Biotechnology and Biological Sciences Research Council (BBSRC)

Keywords: Cardiotoxicity, High-throughput Imaging, Drug discovery

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CARDIAC TISSUE ENGINEERING WITH GENE-EDITED HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES ENABLES DETECTION OF EARLY-STAGE MECHANISMS THAT GOVERN HYPERTROPHIC CARDIOMYOPATHY

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Hypertrophic cardiomyopathy (HCM) is the most common monogenic cardiac disease, affecting up to 0.2% of the global population. As HCM-causing mutations mainly exist in the gene loci encoding sarcomeric proteins, cardiac functions could be directly disturbed by this disease. Further, HCM is associated with sudden cardiac death in young people and competitive athletes. Unfortunately, detailed mechanistic understanding of HCM, especially at the early stages of disease progression, is very limited. To investigate early-stage HCM triggers and potential therapeutic strategies to delay or prevent the HCM onset and progression, we evaluated the impact of myosin heavy chain (MHC) mutations on HCM pathogenicity in 2D culture system as well as an engineered heart tissue (EHT) model by comparing mutant hiPSC-derived cardiomyocytes (hiPSC-CMs) with isogenic control hiPSC-CMs at multiple time points. In 2D, we first verified that gene-edited hiPSC-CMs with the dual MYH7 R723C and MYH6 R725C mutations exhibited phenotypic characteristics consistent with later-stage HCM including hypertrophy, multinucleation, altered calcium handling, and irregular beating. Through bulk RNAseq analysis, FACS, and Western blotting, we identified early extracellular matrix (ECM) changes that precede later-stage physiologic defects associated with this disease. Further in 3D, we generated fibrin-based EHTs with hiPSC-CMs and fibroblasts to study cardiac function changes caused by HCM mutations. Intercellular communication between hiPSC-CMs and fibroblasts was identified as the key to support cardiac function of MHC-mutant EHTs. Contraction force and TGF-beta 1 secretion were significantly increased in the mutant EHTs, and after blocking TGF-beta 1 signaling, the contraction force of mutant EHTs returned to a level comparable to control EHTs. The result of our 2D study is the first to link ECM dynamics with HCM onset and therefore provides a new avenue for HCM therapeutic discovery. The tissue-level 3D model further enabled study of the interplay between hiPSC-CMs and fibroblasts in the context of HCM and suggested hiPSC-CMs impact on the function of fibroblasts very soon after specification. Altogether, these findings lay the foundation for novel strategies to treat HCM at early stages of disease.

Funding Source: This research was supported by American Heart Association (#21Predoc836194 to Jeanne Hsieh).

Keywords: hypertrophic cardiomyopathy, hiPSC-derived cardiomyocyte, engineered heart tissue

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BET INHIBITION BLOCKS INFLAMMATION-INDUCED CARDIAC DYSFUNCTION

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Cardiac injury and dysfunction occur in COVID-19 patients and increase the risk of mortality. Causes are ill defined but could be through direct cardiac infection and/or inflammation-induced dysfunction. To identify mechanisms and cardio-protective drugs, we use a state-of-the-art pipeline combining human cardiac organoids with phosphoproteomics and single nuclei RNA sequencing. We identify an inflammatory 'cytokine-storm', a cocktail of interferon gamma, interleukin 1β and poly(I:C), induced diastolic dysfunction. Bromodomain-containing protein 4 is activated along with a viral response that is consistent in both human cardiac organoids and hearts of SARS-CoV-2 infected K18-hACE2 mice. Bromodomain and extraterminal family inhibitors (BETi) recover dysfunction in hCO and completely prevent cardiac dysfunction and death in a mouse cytokine-storm model. Additionally, we determined that vascular cells within cardiac organoids determine the magnitude of diastolic dysfunction caused by inflammatory factors and identify a paracrine role of endothelin driving dysfunction. Taken together, we demonstrate the importance of multi-cellularity and cellular-crosstalk for disease-modelling and that BETi are promising candidates to prevent COVID-19/inflammation mediated cardiac damage.

Keywords: Heart, Inflammation, Bromodomain and extraterminal family inhibitors

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ISOGENIC ALLELIC SERIES OF LMNA-MUTATED HIPSC LINES GENERATED USING THE NOVEL AND HIGHLY-EFFICIENT TARGETING PLATFORM, STRAIGHT-IN

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Human induced pluripotent stem cells (hiPSCs) have demonstrated their value for in vitro disease modelling. Genetically modified hiPSCs carrying disease-associated variants are particularly useful tools for this purpose but strategies for efficient and rapid integration of large DNA payloads (>10 kb) into specific genomic regions are still limited. To overcome this, the STRAIGHT-IN platform (Serine and Tyrosine Recombinase Assisted Integration of Genes for High-Throughput INvestigation) was developed that combined different classes of site-specific recombinases with CRISPR-Cas9 mediated homologous recombination and allowed stringent site-specific replacement of large genomic fragments in hiPSCs. Here, we use STRAIGHT-IN to simultaneously generate a library of genetically matched hiPSC lines carrying multiple heterozygous mutations in LMNA gene. Mutations in LMNA, encoding Lamin A/C protein, have been associated with 5-10% cases of dilated cardiomyopathy (DCM) with conduction defects. Through detailed online searches of the LMNA database and literature, we identified and selected 11 LMNA mutations to insert into a wild-type hiPSC line based on the following criteria: (i) mutations associated with cardiac abnormalities, in terms of structural (DCM/heart failure) and arrhythmic phenotypes; (ii) mutations with known familiar/pedigree-relationship; (iii) different types of mutations (missense and nonsense). We then applied the workflow of STRAIGHT-IN consisting of: (1) replacing the entire LMNA genomic locus (33 kb) on one allele with a Landing Pad (LP) cassette containing Serine Recombinase (Bxb1) recognition sites; (2) reintroducing LMNA gene into the LP cassette through integration of Bxb1-Donor plasmids carrying the LMNA variants of interest via Bxb1 expression; (3) expressing a tyrosine recombinase (Cre) to excise the majority of the auxiliary exogenous DNA sequences. STRAIGHT-IN allows simultaneous generation of a panel of 11 isogenic hiPSC lines carrying selected LMNA mutations rapidly (5-6 weeks), efficiently and cost-effectively, thus facilitating the production of specific mutated hiPSC lines for disease modelling and personalized medicine applications.

Funding Source: The Netherlands Organisation for Health Research and Development ZonMW (PSIDER project no. 10250022110004); The Novo Nordisk Foundation grants (NNF21CC0073729); European Research Council (ERC-CoG Mini-HEART no. 101001746).

Keywords: site-specific recombination, CRISPR/Cas9, human pluripotent stem cells, disease modelling

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OVEREXPRESSION OF MELUSIN ENHANCES CONTRACTILITY IN STEM CELL-DERIVED ENGINEERED HEART TISSUES

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Melusin is a muscle-specific protein that binds to β1 integrin and is involved in hypertrophic growth via ERK and AKT pathways in response to pressure overload in the heart. Previous work on melusin and its role in cardiac hypertrophy has been with transgenic mice, so its relevance to human pathophysiology is unclear. For this reason, we generated human induced pluripotent stem cell (hiPSC) lines that overexpress melusin to understand its role in human cardiac biology. Specifically, we targeted the insertion of the melusin gene (ITGB1BP2) to the ROSA26 safe harbor locus

in the WTC11 hiPSC line and drove its cardiac-specific expression using a CK8m promoter, which is a small cassette derived from the creatine kinase promoter. We differentiated these cardiac-specific, melusin-overpressing hiPSCs to stem cell-derived cardiomyocytes (hiPSC-CMs) with high purity and cast them as engineered heart tissues (EHTs). EHTs provide a three-dimensional microenvironment that represents spatial aspects of the native myocardium and also allows for the direct measurement of the contractile function of hiPSC-CMs. Compared to EHTs cast with isogenic controls, melusin-overexpressing EHTs generated greater and faster contractile forces at two weeks after casting. By three weeks, isogenic control EHTs matched the contractility of melusin-overexpressing EHTs. Through western blots, we confirmed that there is increased ERK signaling in melusin-overexpressing EHTs as compared to their isogenic controls, which confirms previous in vivo findings in mice. Our results suggest that melusin may have a role in driving the development of contractile function in cardiomyocytes. Thus, melusin may be viable as a molecular target for novel therapeutics for treating heart failure.

Keywords: EHT, melusin, cardiomyocyte

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GENERATION OF HEART AND VASCULAR SYSTEM FROM STEM CELLS BY BLASTOCYST COMPLEMENTATION

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Organ shortage for clinical transplantation is a worldwide issue. In order to address this sanitary burden, the blastocyst complementation approach is a promising method to experiment the generation of humanized organs in farm animals. To generate rejection-free organs, both the parenchyma and the vascular compartment of an organ must be complemented, given their key role in graft rejection. Here, we used the Nkx2.5-Cre; DTA and Tie2-Cre; DTA cell ablation systems to target both the cardiomyocyte and endothelial cell lineages in host mouse embryos, and by intraspecies blastocyst complementation we rescued both heart and vascular development. Complemented chimeras were viable and reached adult stage, showing normal cardiac function and no signs of histopathological defects in the heart. Furthermore, by rat-to-mouse interspecies blastocyst complementation, we rescued heart formation at E10.5, obtaining hearts composed completely by rat cardiomyocytes. These results represent an advance in the experimentation towards the in vivo generation of transplantable organs.

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Keywords: Blastocyst complementation, organogenesis, cardiovascular development

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USING HIPSC-CMS TO INVESTIGATE CARDIOMYOCYTES MATURATION

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The human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) have the potential for vast applications toward a personalized approach in the treatment of cardiovascular diseases as the leading cause of mortality. Unfortunately, hiPSC-CMs being immature cannot represent the mature adult CMs, limiting their use in drug screening and disease modeling. Studies addressing this limitation have only partially been effective emphasizing the need for future investigations. Moreover, despite the lack of a complete picture of signaling pathways involved in CM maturation, mTOR signaling has been proposed to be a key pathway involved in early stages of CM differentiation, maturation, and regeneration making it an interesting candidate for a deeper investigation in CM maturation. The impact of maturation-inducing factors (MIFs) on the regulation of mTOR pathway during CM maturation has not been evaluated to date. In this study, hiPSCs were differentiated to CMs and subjected to maturation treatment after metabolic purification. The Seahorse assay was performed to measure mitochondrial respiration, glycolysis, and mitochondrial staining was conducted in parallel. Mass spectrometry (MS) proteomics was performed to gain a deeper understanding of the mechanisms underlying maturation. To investigate the changes in mTORC1 signaling in CM maturation, hiPSCs were transduced with lentiviruses expressing an mTOR biosensor and its negative control. The biosensor expressing hiPSCs were differentiated to CMs and were treated with MIFs. A Bioluminescence Resonance Energy Transfer (BRET) assay was used to monitor the potential changes in mTORC1 activity. The results of this study show maturation promoting effects of MIFs at the functional level. In addition, MS allowed for a wide quantitative analysis of the proteomic

changes and posttranslational modifications that are both novel and previously known to be involved in the maturation process. In addition, the corresponding changes in mTORC1 signaling were recorded for the first time using live hiPSC-CMs. The results of this study will potentially accelerate the groundwork for the use of hiPSC-CMs as a representative model in cardiovascular regenerative medicine.

Keywords: Cardiomyocyte maturation, mTOR pathway, Cardiovascular regenerative medicine

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COMPLEX GENE-ENVIRONMENT INTERACTIONS IN FAMILIAL DILATED CARDIOMYOPATHY CAUSE VARIABLE PENETRANCE AND EXPRESSIVITY

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Dilated cardiomyopathy (DCM) is a leading cause of death worldwide, and we have identified a family with an history of DCM in which novel Tropomyosin-1 (TEK, TPM1c.97G>A/+) and Vinculin (VFS, VCLc.659dupA/+) heterozygous variants co-segregate. Patient populations containing both variants, i.e. “TV-Dhet” or TEK/VFS double heterozygotes, exhibit both variable penetrance and clinical presentations of DCM. To systematically interrogate the role of environmental stress on disease penetrance and expressivity, we individually introduced the TPM1c.97G>A/+ variant and a VCLc.74del7 frameshift variant, which recapitulates the premature stop codon and haploinsufficiency, into the H9 hESC background. Patient-derived hiPSC-TV-Dhet and isogenic hESC single variant CMs were subjected to DCM-relevant microenvironment stressors. Under fibrotic-like substrate stiffening, TV-Dhet CMs adversely remodeled, showing decreased sarcomere organization, increased eccentric hypertrophy, and increased cell strain energy compared to WT familial hiPSC-CM controls. In single variant hESC-CMs, only the VFS CMs show contractile and sarcomere dysfunction at physiological conditions; these effects are further exacerbated on fibrotic-like conditions. In contrast, the TEK CMs do so only under microenvironment stress. The adverse remodeling of TEK and VFS CMs under stress suggests a sensitivity to environmental insult that can help explain varying expression of DCM in patient carriers. TV-Dhet CMs also have reduced expression of key vinculin-interacting proteins enabling mechanosensitive-protection against microenvironment stress (e.g. FAK, talin 2, α-actinin). Analysis of calcium ion dynamics on stiff microenvironments shows DCM-like dysfunction: prolonged Ca2+ release, slowed Ca2+ reuptake, and lower transient amplitude in TV-Dhet CMs. Voltage imaging also reveals TV-Dhet CMs have reduced full-width, half-maximum and prolonged action potential decay. TV-Dhet CMs have significantly lower expression of key CM ion channel genes SCN5A and KCNQ1. Taken together, these data suggest TPM1 and VCL variants impair structural and electrophysiological CM responses under environmental stress.

Funding Source: American Heart Association National Institutes of Health

Keywords: Gene-Environment Interactions, Mechanosensation, Gene Editing

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IPSC MODEL OF DILATED CARDIOMYOPATHY ASSOCIATED WITH HETEROZYGOUS MUTATION IN ZMPSTE24 GENE

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Cardiomyopathy is a disease of the heart muscle associated with a disorder of its function. This is a heterogeneous group of diseases with various clinical signs that can ultimately lead to heart failure. Subset of cardiomyopathies are genetically conditioned. iPSC derived from patients or engineered with specific genetic defects found in patients serve as a unique model of the disease for pathogenetic studies. We have focused on a group of cardiomyopathies with defect genes associated with lamin A/C processing. One of the proteins needed for functional maturation and localization of the lamin A is metalloprotease ZMPSTE24. Without ZMPSTE24, the final processing step that releases mature lamin A cannot occur, resulting in an accumulation of a farnesylated and methylated prelamin A. The enzymatic defect then leads to various diseases like progeria, muscular dystrophy and heart malfunctions. We have produced iPSC lines from one patient with heterozygous mutation in ZMPSTE24 gene. The iPSC lines were than differentiated to cardiomyocytes and subjected to standard characterization such as differentiation efficiency, response to isoprenylation inhibitors, morphological characterization of nuclei. We have observed decreased differentiation efficiency of patient derived lines. The cardiomyocytes were morphologically different with abnormal nuclei.

Funding Source: The project was supported by research grant from Agency for Medical Research of Czech Ministry of Health, reg. number NV19-08-00122.

Keywords: Cardiomyopathy, ZMPSTE24, iPSC

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CAPTURING THE MECHANISMS OF TRANSCRIPTION FACTOR HAPLOINSUFFICIENCY UNDERLYING CONGENITAL HEART DEFECTS

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Congenital heart defects (CHDs) are the most common birth defect, and haploinsufficient mutations resulting in altered dosage of transcriptional regulators, such as the cardiac transcription factor TBX5, are a pivotal underlying cause. The haploinsufficiency of TBX5 mutations suggests that TBX5 regulates the expression of its target genes in a dose-dependent manner. Using a human iPSC allelic series including TBX5 WT, heterozygous and null lines, and differentiating to cardiomyocytes has allowed us to understand the dose-dependent changes in gene expression that may underlie TBX5-dependent CHDs. However, we still don't understand mechanistically how and why some genes are sensitive to changes in TBX5 dose. This research program investigates the hypothesis that target genes are sensitive to reduced TBX5 levels due to differential binding of TBX5, and the assembly of transcription-regulating chromatin-associated proteins around dose-sensitive genes. To uncover these molecular mechanisms, we have generated a biotin-tagged TBX5 allelic series that we will use for ChIP-exo to determine the binding of TBX5 across the cardiac genome during development and in contexts of reduced TBX5 dosage. To understand the transcriptional machinery that may influence TBX5 dosage-sensitive gene expression, we have optimized a protocol called CAPTURE to isolate locus-associating proteins using biotinylated dCas9 pull-down followed by mass spectrometry. Our initial work targeting the SOX2 promoter in iPSCs has demonstrated that we can isolate transcriptionally-relevant regulatory proteins, including chromatin remodeling complexes and transcription factors. Current work at the TBX5 dosage-sensitive target gene NPPA promoter and enhancer in iPSC-derived cardiomyocytes will allow us to determine the changes in transcriptional machinery that may underlie altered gene dosage in CHDs.

Keywords: cardiovascular development, transcriptional regulation, chromatin

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A TISSUE ENGINEERED MODEL OF HUMAN MYOCARDIAL ISCHEMIA REPERFUSION INJURY RECAPITULATES MYOCARDIAL DYSFUNCTION AND FIBROTIC REMODELING

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Ischemic heart disease is one of the leading causes of death worldwide and few treatment options exist beyond the timely restoration of blood flow following myocardial infarction. Although numerous therapeutic candidates have shown promising results in preclinical animal models, none have successfully translated to improved clinical outcomes. The significant differences between animal and human cardiovascular physiology likely contribute to this discrepancy, and there is an urgent need for better model systems to recapitulate human pathophysiology more accurately. To address these shortcomings, we have developed a new bioengineered model of ischemia reperfusion injury (IRI) and subsequent remodeling that incorporates human iPSC-derived cardiomyocytes and supporting cells and matures these cells via a combination of metabolic and electromechanical conditioning. Following tissue maturation, ischemia is simulated by providing an anoxic culture environment with increased lactate and decreased pH. Electrical stimulation of the tissues applied during maturation is continued during the simulated ischemia to model demand-perfusion mismatch. After 6 hours under ischemic conditions, tissues are returned to standard culture conditions to simulate reperfusion and maintained in culture for up to an additional month to allow observation of post ischemic function and remodeling. Supernatant analysis reveals a significant increase in LDH release following ischemia and then an even larger increase following reperfusion, suggesting that the model recapitulates the cell damage seen in IRI. Additionally, cell viability assays demonstrate approximately a 50% decrease in cellular viability that remains stable during remodeling. Tissues have a marked decrease in function following simulated IRI that is sustained for at least one month of culture. Histological analysis revealed substantial collagen deposition throughout the tissues. Taken together, our data show that the model recapitulates the pathological remodeling and development of tissue fibrosis that occurs following IRI.

Keywords: cardiac tissue engineering, myocardial ischemia reperfusion injury, myocardial fibrosis

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ESTABLISHMENT OF A CARDIOVASCULAR MODEL DERIVED FROM HUMAN PLURIPOTENT STEM CELLS (HIPSC) FOR ANALYSIS OF OXIDATIVE STRESS IN HYPERTENSION

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Hypertension is an important risk factor for various cardiovascular diseases such as stroke, acute myocardial infarction and chronic kidney disease. It is a complex, multifactorial disease whose cellular mechanisms are not well understood. Recently, several studies have focused on the role of oxidative stress in the development of hypertension. Increasing evidence over the last decades indicates an association between reactive oxygen species (ROS) and arterial hypertension. Among the different ROS sources present in the cardiovascular system, NADPH oxidases (NOXs) are particularly important because they are involved in many features of cardiovascular dysfunctions. Particularly in human hypertension, redox signaling regulation and the role of NOXs in cardiovascular pathologies await clarification. In this scenario, human induced pluripotent stem cells (hiPSC) may be a powerful tool for understanding cellular mechanisms of response to oxidative stress in hypertension–induced cardiovascular dysfunction. We used hiPSC-derived cardiomyocytes, endothelial cells and vascular smooth muscle cells from normotensive and hypertensive patients to dissect the redox mechanisms of NOX regulation of cardiovascular function. We generated 9 hiPSC lines from hypertensive (HT) and normotensive (NT) subjects; these cells were differentiated into cardiomyocytes, endothelial and vascular smooth muscle cell, and RT-qPCR analysis of NOXs expression and ROS quantification were performed. We observed different NOX-expression profiles in differentiated cells between groups. HT cells, have increased expression of NOX1 and 2, associated with hypertension, and a decreased expression of NOX4 which has a protective role in redox regulation. In addition, HT cells had decreased expression of antioxidants genes, which means that these cells are in fact producing an oxidative environment. The results suggest that HT cells produce more superoxide, which means that their basal environment is already more stressful than NT cells, indicating that these cells maintain the phenotype related to hypertension in vitro. Our results indicate that this model may bring new perspectives to the understanding of oxidative stress related to hypertension, in addition to opening new possibilities in modelling the disease in vitro.

Funding Source: FAPESP - Fundaçao de Amparo a Pesquisa do Estado de São Paulo; CNPq - Conselho Nacional de desenvolvimento Científico e tecnológico;

Keywords: hiPSC, cardiovascular differentiation, hypertension

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CARDIAC MYOFIBRILLOGENESIS IS SPATIOTEMPORALLY MODULATED BY THE MOLECULAR CHAPERONE UNC45B

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Sarcomeres are the fundamental component responsible for manipulating contraction in both cardiac and skeletal striated muscle. Their impairment can elicit cardiomyopathies, leading causes of death worldwide. However, the molecular mechanism underlying the stepwise process of sarcomere assembly remains obscure. Uncoordinated mutant number 45 (UNC45) is a key molecular chaperone exerting a critical role in myosin assembly during sarcomerogenesis. However, the molecular mechanism by which UNC45B regulates sarcomere assembly spatiotemporally remains unclear. We ablated UNC45B from human embryonic stem cells (hESCs) using a CRISPR/Cas9 technique. Although hESCs with homozygous ablation of UNC45B (UNC45B-/-) still differentiated into CMs, they displayed essentially no contractility. Phenotypic analysis further revealed that: 1) binding of the Z-line anchor protein ACTN2 to protocostameres was perturbed due to impaired protocostamere formation, resulting in ACTN2 accumulation in the premyofibril stage; 2) F-ACTIN polymerization was suppressed; and 3) MYH6 became degraded, so muscle myosin could not replace non-muscle myosin MYH10. Moreover, our mechanistic study demonstrates that UNC45B facilitates expression of the protocostamere marker KINDLIN2 (KIND2), with disruption of UNC45B resulting in impairment of the initiating site of sarcomere assembly. Thus, UNC45B directs human cardiac myofibrillogenesis by spatiotemporally modulating protocostamere formation and muscle myosin folding.

Keywords: Molecular chaperone, Sarcomerogenesis, Human embryonic stem cells

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EXOCYST COMPLEX COMPONENT 2 IS A HOST FACTOR FOR THE SARS-COV-2 INFECTION

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The coronavirus disease 2019 (COVID-19) pandemic has been caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and remains a severe threat to public health with millions of deaths. Despite angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease2 (TMPRSS2) have been studied widely, the analysis of host factors other than receptors and proteases has not been sufficiently performed. Hence, we attempted to identify and characterize novel host factors essential for SARS-CoV-2 infection using induced pluripotent stem cells (iPS) cells and airway organoids. Based on previous CRISPR screening and RNA-seq data, we found that exocyst complex component 2 (EXOC2) is an important host factor for the SARS-CoV-2 infection. The intracellular SARS-CoV-2 nucleocapsid (N) expression level was decreased to 3.7 % and the virus copy number in the cell culture medium was decreased to 1.6 % by EXOC2 knockdown, and SARS-CoV-2 infection was also increased by EXOC2 overexpression. Moreover, EXOC2 knockdown downregulates SARS-CoV-2 infection by regulating interferon omega 1 (IFNW1) expression. In conclusion, controlling the EXOC2 expression level prevents SARS-CoV-2 infection in iPS cells and airway organoids, which may contribute to the development of potent COVID-19 therapeutic agents.

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Keywords: SARS-CoV-2, iPS cells, EXOC2

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EXPRESSION OF ALPHA-1 ANTITRYPSIN AND RESULTING CELLULAR STRESS IN ALPHA-1 ANTITRYPSIN DEFICIENT TYPE 2 ALVEOLAR EPITHELIAL CELLS

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Alpha-1 antitrypsin deficiency (AATD) is a monogenic protein misfolding disease that leads to both emphysema and fibrotic liver disease. While AATD liver disease is the result of misfolded mutant “ZAAT” accumulation and resulting proteotoxicity in hepatocytes, AATD emphysema has classically been attributed to reduced circulating AAT levels and associated protease/antiprotease imbalance in the lung. There is evidence for ZAAT-driven proteotoxicity in extrahepatic cells, such as circulating monocytes, that express SERPINA1, the gene encoding AAT. Although it has recently been shown that type 2 alveolar epithelial cells (AT2s), facultative progenitor cells in the distal lung, express SERPINA1, the consequences of ZAAT protein expression in AATD patient cells has not been examined. We hypothesize that ZAAT accumulates intracellularly in AATD AT2s, inducing gain-of-function toxicity that contributes to emphysema, the most commonly observed disease phenotype in AATD. To confirm AAT protein expression in AT2s, explant lung tissue from patients with AATD, as well as healthy and COPD control tissue, was stained for AT2s and AAT and analyzed via fluorescence microscopy. We identified co-localization of AAT and AT2s in AATD but not in healthy lung tissue, consistent with the possibility that intracellular AAT protein levels might be elevated in AATD AT2s. To determine the impact of ZAAT expression in AT2s, AATD patient-derived induced pluripotent stem cells, along with CRISPR/Cas9-corrected syngeneic controls, were differentiated into AT2-like cells (iAT2s). iAT2s were plated at an air-liquid interface and exposed to cigarette smoke injury before undergoing single cell RNA sequencing. Analysis of DEGs identified enrichment of Hallmark gene sets indicating cellular stress, such as TNFa signaling via NFkB, hypoxia, and inflammatory response in AATD iAT2s compared to their gene-corrected counterparts. Overall, our findings identify heterogeneous AT2 intracellular AAT protein in AATD explant lung tissue, and transcriptional evidence of cellular stress in AATD iAT2s. Future studies will investigate the functional impacts of ZAAT expression on AT2s, and how this expression contributes to emphysema.

Funding Source: Grifols ISR/SRA

Keywords: alpha-1 antitrypsin deficiency, type 2 alveolar epithelial cell, emphysema

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VASCULAR DAMAGE CAUSED BY INFECTION OF SARS-COV-2 TO BRONCHIAL ORGNOIDS IN 3D MPS

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We developed 3D MPS system which evaluate the effect of SARS-CoV-2 infection to co-cultured bronchial organoids and self-organized vasculature. COVID-19 is caused by the infection of SARS-CoV-2 by entering the body through respiratory epithelial cells. Vascular symptoms such as micro thrombosis or endothelialitis are frequently seen in critical cases, making the disease unconventional compared to existing respiratory virus diseases. Although a huge number of studies have been made to elucidate the mechanism of vascular damage caused by SARS-CoV-2, many questions remain, such as virus dynamics among multiple tissues and the direct source of endothelial cell damage. Here we established the co-culturing system of bronchial organoids created from primary bronchial epithelial cells and vasculature formed by primary endothelial cells to evaluate the direct and indirect effects caused by SARS-CoV-2 infection. Experimental results showed that virus mRNA was detected in epithelial cells and not detected in endothelial cells, suggesting that the virus selectively infected bronchial organoids. Despite a negligible amount of virus infection, damage to the vasculature and an increase of virus RNA in the vasculature channel were observed 3 days after infection. These results suggest that the virus infected with Bronchial organoids damaged vasculature via the paracrine effect, along with the upregulation of innate immune response-related genes.

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Keywords: SARS-CoV-2, Vasculature, MPS

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TOWARDS MODELLING BIRT-HOGG-DUBÉ SYNDROME USING HIPSC-DERIVED ALVEOLAR EPITHELIAL CELLS

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Birt-Hogg-Dubé syndrome (BHD) is a rare disease caused by mutations of the FLCN gene which encodes for folliculin. This disease is characterized by formation of fibrofolliculomas, renal cancer, cysts, emphysematous changes and pneumothorax. A robust high expression of FLCN is observed in alveolar type II epithelial cells (AEC2), but it is unknown how this mutation affects alveolar epithelial cell biology. By introducing a mutation in FLCN in human induced pluripotent stem cells (hiPSCs), differentiating them into AEC2 organoids and exposing them to different triggers, we aim to understand the mechanisms governing alveolar epithelial cell dysfunction and the BHD phenotype in the lungs. A CRISPR/Cas9 knock out (KO) of the FLCN gene was generated in hiPSCs. Subsequent differentiation of these cells into iAEC2 organoids was performed. Primary BHD-AEC2 were isolated and cultured as organoids to allow validation of observed results in our hiPSC model. In primary cells, formation of alveolar cysts was modelled by culturing the organoids for 20 days and adding forskolin at 5 μM for the last seven days. In addition, primary BHD-AEC2 were seeded into a Lung-on-Chip system (Emulate Inc.) to mimic breathing-related stresses by stretch application. Genome editing of the hiPSC line generated a heterozygous (HET) and a homozygous (HOM) FLCN KO line, and a HET truncated protein. Further differentiation into alveolar progenitors showed similar percentages in the KO (16%)/truncated (14%) against the control (18%). In parallel, BHD-AEC2 organoids with a HET FLCN KO variant showed an increase in organoid lumen size upon forskolin addition compared to control organoids (1.97 times larger versus 1.1, respectively). Bhd-AEC2 were seeded in the microfluidic system and stretched at 8% for three days. The forskolin and the microfluidic chip experiment are currently being repeated using the iAEC2s. In conclusion we have successfully generated FLCN KO HET and HOM hiPSC lines and derived iAEC2s. Parallelly, primary BHD-AEC2 displayed significant alveolar enlargement upon forskolin addition, when compared to alveolar organoids from a control. Further characterization of its phenotype is currently

ongoing, and includes assessment of tight junctions, forskolin-induced swelling, and responses to stretch.

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Keywords: alveolar epithelial, Birt-Hogg-Dube, stretch lung chip

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COCHLEAR ORGANOIDS FOR THE STUDY OF HAIR CELL DEVELOPMENT AND REGENERATION

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Cochlear hair cells (HC) are susceptible to noise exposure, ototoxic treatments, and age. Currently, no treatment exists to promote HC regeneration in the cochlea and deafness is therefore irreversible once HCs are lost. Many efforts are put on identifying genes, pathways and molecules that would make cochlear HC regeneration possible. However, the low number of cochlear HC as well as the size of the cochlea and its inaccessibility limit in vivo studies. Thus, there is a critical need for a reliable model to examine HC development and to screen for treatments that could stimulate hair cell regeneration. To develop such a model, we generated a mouse strain expressing mCherry under the Atoh1 promoter and combined it with a mouse strain expressing GFP under the Lgr5 promoter. We obtained mice in which a subset of supporting cells and all HCs were labeled in different colors. Application in this new mouse strain of a previously established protocol that allows for clonal expansion of HC progenitors and subsequent HC differentiation, results in labeled cochlear cells in organoids: Lgr5-expressing supporting cells which include hair cell progenitors are labeled in green and HCs in red. Using flow cytometry from dissociated organoids, we can track HC differentiation from Lgr5+ cells to Atoh1+ cells and assess both the number of HC progenitors and the number of HCs at progressive differentiation times. Finally, our model helps to track HC differentiation and to easily assess the number of HC progenitors and HC in the organoids. Additionally, we can sort these populations and assess gene expression (RNA-sequencing), protein expression (immunostaining), and protein-chromatin interactions (CUT&RUN). As the effect of treatments during proliferation or differentiation can be easily determined, the two-color model provides an easy-to-use and reliable tool for assessing the effect of treatments on HC generation, and we are currently using the model to screen small molecules and genes critical for HC development and regeneration.

Keywords: Hair cell, Organoid, Development and Regeneration

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DEFINING NKX2-1-DEPENDENT TRANSCRIPTOMIC SIGNATURES IN LUNG DEVELOPMENT

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NK2 Homeobox 1 (NKX2-1) is a critically important transcription factor in lung development, with all lung epithelia derived from an NKX2-1+ progenitor pool. Nkx2-1-/- mice have severely hypoplastic lungs whereas Nkx2-1+/- mice do not have a lung phenotype. Humans with heterozygous NKX2-1 mutations develop respiratory insufficiency, hypothyroidism, and neurological problems, a disease known broadly as brain-lung-thyroid syndrome. Despite its known importance, the role of NKX2-1 in human development, and how this differs from mouse, is not fully defined. To study the role of NKX2-1, we generated induced pluripotent stem cells (iPSCs) from 3 patients with brain-lung-thyroid syndrome and selected a donor with a frameshift mutation in exon 3 of NKX2-1 for initial study. We corrected this mutation using CRISPR/Cas9 while also inserting a GFP reporter, generating two isogenic lines that differ only at the NKX2-1 locus. scRNA-seq across three timepoints in both airway and alveolar differentiations revealed broad transcriptomic differences between mutant and corrected cells that increasingly diverged over developmental time. We observed more differences between mutant and corrected cells in the alveolar differentiation versus the airway differentiation. Mutant cultures showed strong downregulation of alveolar type II (ATII) differentiation markers in addition to upregulation of non-lung endoderm markers, pointing towards NKX2-1's role in both fate acquisition and maintenance. Additionally, transcription factor activity analysis identified upregulation of SMAD3 target genes in mutant distal lung cells. Inhibition of TGFB signaling in mature mutant alveolospheres display upregulation of AT2 markers, pointing towards a co-regulatory relationship between NKX2-1 and SMAD3. In conclusion, we used an iPSC-based in vitro model

of lung development to identify transcriptomic profiles associated with NKX2-1 mutations, giving insight to the NKX2-1-dependent developmental program. Future directions will use this platform to further identify the core genetic programs by which NKX2-1 works in both normal and aberrant lung development.

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Keywords: Transcription Factors and Development, Lung Development, Developmental Modeling

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A 3D HUMAN IPSC-DERIVED LUNG ALVEOLAR MODEL FOR CRISPR SCREENING AND TARGET VALIDATION

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Lung alveoli are the primary site of gas exchange in the body and are affected by several respiratory tract diseases. To develop efficient therapies, new models mimicking the three-dimensional (3D) organization of the cells in the alveoli are needed. Here, we describe the development of a scalable CRISPR screening platform with human induced pluripotent stem cells (iPSCs)-derived alveolar epithelial type 2 cells (AEC2), grown as 3D alveolar-like organoids (or lung alveolospheres, LAs). We validated our 3D-AEC2 model by confirming the expression of the critical transcription factor NKX2-1 during lung progenitor formation and mature AEC2 markers such as the surfactant protein C (SFTPC) in the later phases of differentiation. In-depth characterization of this complex model by single cell RNA (scRNA) seq profiling provided insight into the different cell subpopulations represented in the organoids and their respective gene regulatory networks. Scalability of the 3D lung model was obtained by leveraging the steps of single cell dissociation and cryopreservation in the AEC2 differentiation protocol. Thawed cells re-assembled into proliferating LAs that maintained expression of maturation markers, making them suitable for alveolar-specific functional read-outs. Next, we aimed to develop a CRISPR screening platform to identify and deconvolute new targets. By using an internally generated doxycycline-inducible Cas9 iPSC line, we were able to induce gene knockouts at high efficiency by electroporating sgRNAs into the single cell-dissociated LAs. The deriving gene edited-LAs can be used for high content phenotypic screens and validation assays, e.g. probing mechanisms that control cell proliferation or apoptosis. In conclusion, we describe here a scalable 3D platform that recapitulates alveolar cell lineages and can be used to interrogate the function multiple genes in alveolar biology and identify/validate new targets for respiratory disease drug discovery.

Keywords: CRISPR, Lung, scRNAseq

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GUIDING SELF-ASSEMBLY OF HAIR-BEARING HUMAN SKIN ORGANOIDS-ON-CHIP

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Our group recently reported a human pluripotent stem cell (iPSC)-derived skin organoid model comprising skin appendages, including neurons, adipocytes, and hair. Our model could self-organize and has in vivo-like morphology tracking normal skin developmental processes; however, it is challenging to track individual cell behavior in floating organoids, which reach sizes of 1-2 mm. Here, we used a microfluidic chip approach to cage organoids, limiting the thickness of the organoid and immobilizing the

orientation, facilitating live tracking of specific cell differentiation from the very early stage to the later time point. Also, the microfluidic chips provide physical confinement that can precisely control the organoid shape, creating an image-based quantifiable format. The chip bottom layer is composed of thin glass, optimal for a higher magnification imaging of fluorescently tagged cells. By locating different appendages of skin organoids in the close focal plane, we could image organoids more clearly throughout skin and hair development in a time-lapse manner. In addition to clear fluorescence images, the chip could create more meaningful data out of brightfield images. Conventional organoids make slightly different brightfield images each time due to the difference in light transmittance depending on the shape of the organoid, the shadow pattern, and the wall of the culture dish, making it difficult to set an automatic imaging analysis workflow. Using the chip, we obtained more reliable image analysis results via standardized image quality at different time points. Our organoid chip could visualize the skin organoids throughout the culture so that we could quantify and improve culture conditions guiding iPSC differentiation with fewer off-target cells. Also, we confirmed the potential of the chip platform on organoid culture by quantifying the effect of different factors that can be used to study human developmental processes.

Keywords: organ-on-a-chip, pluripotent stem cells, hair-bearing skin organoids

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GENERATION OF HUMAN ALVEOLAR EPITHELIAL TYPE I CELLS FROM PLURIPOTENT STEM CELLS

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In the distal lung, alveolar epithelial type I cells (AT1s) are uniquely flattened to allow for the diffusion of oxygen into the capillaries. This structure has made them particularly challenging to study and isolate. As a result, there are no established models for the study of human AT1 biology, and little is known about the mechanisms regulating their differentiation. We sought to engineer a human in vitro model of AT1s through the directed differentiation of induced pluripotent stem cells (iPSC). The Hippo signaling pathway has been implicated in mouse AT1 program maintenance and development, so we tested the effect of the Hippo pathway effector protein YAP in self-renewing mature iPSC-derived alveolar epithelial type II cells (iAT2s). Cells were transduced with a lentivirus encoding constitutively active nuclear YAP (YAP5SA) or a control WT YAP lentivirus. We performed single cell RNA sequencing on the lentiviral-transduced cells and observed that those expressing YAP5SA clustered separately from the control iAT2s by UMAP projection and had downregulated AT2 genes while upregulating AT1 specific genes, including AGER, CAV1, and PDPN. In addition, we developed a reporter iPSC line containing a knock-in AGERtdTomato that is detectable post YAP5SA transduction allowing for the tracking and isolation of these AT1-marker expressing cells. Lastly, using this reporter line, we developed an iAT1 induction medium containing a LATS inhibitor, thus avoiding the need for forced lentiviral-based over-expression to activate YAP signaling. This serum-free defined medium generated AGERtdTomato+ cells from iAT2s with 70% efficiency when grown in 3D cultures and resulted in upregulated expression of AT1 marker transcripts such as AGER, PDPN, CAV1, and CLIC5 by qRT-PCR. Additionally, when plated in 2D air-liquid interface cultures in this medium, iAT2-derived cells formed flat cell monolayers containing AGERtdTomato+ cells. Our results suggest a role for the Hippo effector protein YAP in the differentiation of human AT1s and establishes an iPSC reporter cell line able to serve as a potential human AT1 in vitro model system.

Keywords: Lung, Alveolar epithelial type I cells, Hippo signaling

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IN VITRO CELLULAR DEVELOPMENT AND IN VIVO TISSUE RECONSTITUTION WITH HUMAN PSC DERIVED MELANOCYTE STEM CELLS

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Many human diseases involve internal cells that cannot be directly observed, and induced pluripotent stem cells (iPSCs) offer the possibility of studying them in vitro. The renewal state of human melanocyte stem cells is not fully understood, and their expansion and differentiation potential may be relevant to diseases such as vitiligo, melanoma and juvenile grey hair. However, due to a lack of surface markers, it has not been possible to precisely isolate human melanocyte stem cells, let alone dynamically observe the cellular differentiation process, nor to characterize the changes in melanocyte stem cells in these diseases. MITF is a key transcription factor that determines melanocyte fate and maintains the expansion or differentiation of melanocyte stem cells into melanocytes. Some studies have suggested that MITF is a marker for melanocyte stem cells, but this conclusion is still controversial. Using CRISPR/Cas9 gene editing, we were able to visualize MITF gene expression using EGFP in real time during the differentiation of hiPSC into melanocytes. We also found that MITF-positive cells have a high expansion rate, suggesting partially that MITF is a marker for melanocyte stem cells. We then compared the clonal colony-forming capability based on the single cell assay and cell differentiation of MITF-expressing and non-expressing cells, with MITF-expressing cells showing good expansion and mature properties. We then transplanted MITF-positive and MITF-negative subpopulation cells into the intradermal part of immunodeficient mouse NCGs and compared them with the non-fractionated cells

from hiPSC-initiated melanocytes, indicating a renewable subpopulation that promotes human melanocyte stem cell characteristics. Our study creates a convenient in vitro and in vivo dynamic observation model with great potential to track the migration and differentiation of melanocyte stem cells, breaking down barriers to tracking melanocyte stem cells; our study also suggests that MITF may be a marker of melanocyte stem cells, which may shed light on patients with depigmentation disorders in the near future.

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Keywords: hiPSCs-MITF^GFP, Melanocyte stem cells, cell fate

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TUBULAR ORGANOID-DERIVED GUT-ON-A-CHIP MODEL SUITABLE FOR DRUGDEVELOPMENT

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Adult stem cell derived organoids are the most physiologically relevant cell source used in in vitro research, as they are composed of multiple differentiated cell types and resemble biology of an organ they were established from. In the standard culture protocols, organoids are grown as polarized cystic structures embedded in an ECM with limited apical access. Microfluidic techniques are increasingly recognized as an important toolbox to add physiologically relevant cues to traditional cell culture. There is a need to develop better models that capture the 3D morphology, heterogeneity, and boundary aspects of tissues while having potential to be implemented in high throughput screening pipelines. We established a human gut-on-a-chip model composed of intestinal organoids derived epithelial cells patterned inside of the microfluidic channel of an OrganoPlate®. Such tubular epithelial model of the intestinal tract shows rapid cell polarization, tight junction formation, functional transporters and expression of major intestinal markers. Reproducible barrier formation has been shown with the measurement of transepithelial electrical resistance (TEER) of tubules generated from different organoid lines' donors. Developed barriers proved to be sensitive to the pro-inflammatory triggers. The model is suitable for high-throughput screening of compound effects through real time imaging and rapid barrier integrity assessment. Complexity can be added to the miniaturized gut tubules model by incorporation of the other cell types (i.e. endothelial) into the system to mimic in vivo immunological responses. These next generation gut-on-a-chip models allow mimicking disease phenotypes such as inflammatory bowel disease (IBD) and support screening for potential drug targets. By combining adult human stem cell derived intestinal organoids with the microfluidic technology we provide a powerful platform to study physiology and disease mechanisms in patient specific gut models.

Keywords: Tubular Organoid, organ-on-a-chip, 3D modeling, disease model, drug development

TOPIC: GERMLINE AND EARLY EMBRYO

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PARTHENOGENETIC POTENTIAL OF DISCARDED HUMAN EGGS FOLLOWING AMBIENT TEMPERATURE TRANSPORT

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The clinical practice of cryopreservation of eggs from young women for use in fertility treatment has markedly increased in the last decade. Strict morphological criteria for metaphase II (MII) eggs deemed most likely to support a pregnancy following thawing and fertilization lead to discarding a number of eggs collected each cycle of controlled ovarian hyperstimulation. We studied the developmental potential of such discarded eggs following ambient overnight transport in Hepes-buffered medium containing cyclic AMP and caffeine. A total of 1,557 discarded eggs, 19 to 74 per group, twice a month for 12 months, were studied. Forty nine percent were at metaphase II on arrival. Following overnight culture, an additional 9% progressed to MII. Ninety six percent of the remaining 654 eggs remained viable in culture for 5 days, but did not resume meiosis nor activate. Treatments during the 5 days with estrogen, progesterone, IGF1, serum replacement, cycloheximide, and anti-sense cMos oligonucleotides failed to promote progression to MII. In contrast, 42% of the 903 MII eggs underwent activation as judged by the appearance of pronuclei, or cleavage to 2-cells. Thirty eight percent of the 380 activated eggs continued cleavage to the six- to 8-cell stage during the 5 days of culture. Similar percentages of activation and cleavage were observed following treatments with SrCl2 or A23187 without or with the inclusion of cycloheximide, cytochalasin D, -B and anti-sense cMos oligonucleotides. Co-staining nuclei with Hoechst and actin filaments with phalloidin revealed blastomeres with multiple pronuclei as well as blastomeres with no nuclei or actin filaments, independent of activation conditions. Eggs that failed to activate did not appear to die. Time lapse video recordings of eggs undergoing activation revealed numerous aberrant cleavage events, including re-annealing back to a single cell stage as well as immediate fragmentation. These preliminary studies high-

light the innate viability of human eggs under a variety of culture conditions, presenting the possibility of using this system to further explore the developmental potential of human eggs normally discarded.

Funding Source: Funded by the Bedford Research Foundation

Keywords: Human, Eggs, Parthenogenesis

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IN VITRO MATURATION OF HUMAN PRIMORDIAL GERM CELL-LIKE CELLS (HPGCLCS) IN THE FEMALE HUMAN SOMATIC NICHE

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The female reproductive reserve is finite due to the limited number of oocytes that are generated only during embryonic development. Hence recapitulation of human oogenesis in vitro using induced pluripotent stem cells (hiPSCs) could be a useful model to investigate female fertility. Currently hiPSCs can be differentiated into primordial germ cell-like cells (hPGCLCs), but the maturation of hPGCLCs into oocytes has not been achieved. Different from the mouse, human fetal germ cells develop asynchronously in the gonads, with some of the germ cells entering meiosis I and forming primordial follicles, while some remain at the PGC stage expressing POU5F1 and NANOS3 at any given time during development. Due to limited access to human fetal material and lack of in vitro models, it is not well understood what biochemical cues trigger fetal germ cells to progress further in the development. Here we demonstrate that by reaggregating single cell suspension of human fetal gonads and encapsulating them in 3D hydrogel, we are able to preserve the somatic microenvironment necessary for fetal germ cells to proliferate and mature. In these 3D aggregates, we observed FOXL2+ pre-granulosa cells interacting with DDX4+ germ cells. After 10 days in culture, the number of DDX4+ fetal germ cells increased up to 5-fold and more than 25% of those entered meiosis I, indicated by the presence of SYCP3 and pH2AX(Ser139). Using this platform, we investigated the roles of retinoid acid and ascorbic acid in fetal germ cell maturation. Furthermore, to test whether this platform can be used to mature hPGCLCs derived from hiPSCs, we reaggregated hPGCLCs with human fetal ovarian cells and observed progression of hPGCLCs after 25 days of culture. Our study demonstrated the importance of the somatic niche interaction in the survival and maturation of female germ cells, and the potential of the somatic niche to support in vitro female gametogenesis.

Funding Source: Dutch Research Council (NWO) and Novo Nordisk Foundation Center for Stem Cell Research (reNEW)

Keywords: in vitro Gametogenesis, Fetal Germ Cell Development, Germ Cell Niche

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MODELING SACROCOCCYGEAL TERATOMA FORMATION USING HUMAN PRIMORDIAL GERM CELL-LIKE CELL REPROGRAMMING AND MOUSE IN UTERO INJECTIONS

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Sacrococcygeal teratomas (SCTs) are the most common tumor in newborns, have significant perinatal morbidity and mortality, a 35% recurrence rate, and lack good biomarkers to predict recurrence risk. SCTs are believed to originate from embryonic gamete-precursors known as primordial germ cells (PGCs). In mice, PGCs can revert to pluripotent cells called embryonic germ cells (EGCs) when exposed to a specific cocktail of growth factors, but establishing an in vitro model of human EGCs has been technically and ethically challenging. To understand the process by which PGCs lead to teratoma formation, our approach includes human induced pluripotent stem cell-derived PGC-like cells (PGC-LCs) and EGC-like cells (EGC-LCs), an innovative mouse embryonic injection model, and referencing the first single cell atlas of SCTs. First, using an established PGC-LC reprogramming method to derive EGC-LCs, we created a time course of transcription across the first several days of reprogramming to understand the mechanisms by which human PGC-LCs re-acquire pluripotency. Second, we developed an in utero injection model to introduce EGC-LCs into the developing mouse sacrum environment as a model for SCT development. To date, we have found that cells can survive in the sacrum for at least 24 hours. Lastly, we performed single cell/nuclear RNA sequencing of fresh SCT tissues to understand their heterogenous cell type composition. That sequencing showed the tumor is composed of populations of fibroblasts, macrophages, T-lymphocytes, and endothelial cells. These studies are the first to identify the important developmental trajectories that occur with reprogramming PGCs towards pluripotency, model this in utero, and create the SCT atlas. These results add insight to aid biomarker development for recurrences of PGC-derived tumors and provide potential targetable pathways to treat aggressive/recurrent SCTs.

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Keywords: germ cell tumors, primordial germ cells, teratomas

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DISCOVERY OF CHROMATIN COMPETENT REGIONS IN HESCS USING CRISPRA SCREENS

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The mechanism that enables embryonic stem cells (ESCs) to swiftly establish the chromatin regions that regulate the expression of lineage genes at the earliest differentiation stages remains to be defined. It has been suggested that in ESCs the regions that will become tissue-specific enhancers are pre-marked with permissive chromatin features which support their ability to induce gene expression in response to the binding of lineage-specific transcription factors (TF). However, a functional link between the pre-marking of the chromatin in ESCs and its transcriptional competence upon TF binding has not been established. To address this, we leveraged a CRISPR-activation(a) system to identify the chromatin regions that have the competence to support the transcription of lineage-specific genes in hESCs. Through multiple screens we discovered 42 chromatin competent regions (CCRs) able to induce the transcription of 5 developmental genes upon CRISPRa interrogation. Chromatin conformation experiments revealed that CCRs are exclusively found inside the topologically associated domains of the related genes, although most of them do not have pre-established chromatin contacts with the gene promoters. Characterization of the DNA features and the chromatin-associated factors revealed that CCRs are enriched in POU motifs and have a higher binding of OCT4, NANOG, TET1, QSER1 and HDAC1-family members when compared to non-CCRs. Importantly, none of the hallmarks of active or primed enhancers were found to be enriched at the CCRs. Using hESC-KO lines we discovered that QSER1 and TET1 safeguard CCRs from becoming heavily methylated. In addition, we found that HDAC1-family inhibition boosts the transcriptional competence of the CCRs. The concomitant presence of functional chromatin activators and repressors suggest CCRs have a bivalent structure. In summary, our results indicate that CCRs are a novel type of regulatory regions which are established before enhancer formation in ESCs. We are currently developing prediction models to discover genome-wide CCRs. This study sheds light on the regulatory mechanisms that sustain cellular plasticity in ESCs and improves the understanding of gene expression regulation during development, reprogramming, and pathological conditions such as cancer.

Keywords: Chromatin premarking, CRISPR activation, Developmental competence

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ROLE OF NODAL SIGNALING REGULATORS IN NODAL DYNAMICS AND CELL FATE DECISIONS IN A STEM CELL MODEL OF HUMAN GASTRULATION

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The morphogen Nodal is essential for embryonic development and its loss results in severe gastrulation defects with loss of mesendoderm fates. Studies in model organisms have shown that Nodal signaling is regulated by a co-receptor (Cripto), two co-ligands (GDF1 and 3), and by the extracellular inhibitors Lefty1 and 2. However, consensus is still lacking regarding how these factors shape Nodal signal propagation in space and time in the embryo. Moreover, there are key developmental differences between human and other model organisms that make studies in human systems necessary. We have shown that in human pluripotent cells, Nodal signal only reaches the immediate neighbors of producing cells, and Nodal positive feedback is essential to propagate the signal via a relay mechanism. Therefore, to further investigate the role of Nodal regulation in human gastrulation we used micropatterned 2D-gastruloids, a model for the cell fate decisions that occur at this stage in the human embryo. As in vivo, the development of the mesendoderm in this model requires endogenous Nodal signaling. With genetically modified cell lines, we observed that in the absence of Lefty, maximum Nodal levels are not significantly increased, however, the spatial range of Nodal production as well as of mesoderm differentiation are expanded. This indicates that Lefty is important to restrict Nodal signaling range, and consequently delimit the mesoderm territory. However, loss of Lefty did not change the range of Nodal diffusion demonstrating that Lefty affects Nodal signaling range through the modulation of the relay mechanism, slowing its progression, and restricting it in space. Interestingly, overexpression of Nodal co-factors also locally increased Nodal levels but restricted Nodal signaling range resulting in a thinner mesoderm territory, with a more defined boundary. Surprisingly, even though overexpression of GDF1 and Cripto increased Nodal levels, it decreased expression of endoderm markers FOXA2 and SOX17 indicating that high levels of Nodal are not sufficient for endoderm formation. Taken together, these results reveal important aspects of Nodal regulation and their impact on cell fate decisions in a human patterning system.

Funding Source: NSF MCB-2135296

Keywords: Nodal regulators, Cell fate decision, 2D-gastruloids

TOPIC: HEMATOPOIETIC, IMMUNE, AND ENDOTHELIAL

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MINIATURIZED ISOGENIC IPSC-DERIVED 3D NEUROIMMUNE ASSEMBLOIDS FOR HIGH-THROUGHPUT DRUG SCREENING

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NeuCyte, Inc. (NeuCyte) is a provider of cryopreserved, ready-to-use, induced NGN2 excitatory neurons and ASCL1/DLX2 GABAergic neurons. The SynFireÒ platform has been utilized to demonstrate efficacy in assessing drug seizurogenic risk and to study epilepsy and neurodevelopmental disorders due to inherited or de novo mutations. Recently, our cells were incorporated into a 3D Microphysiological System (MPS) to interrogate neuroinflammation and the blood-brain barrier within the Emulate Organ-on-a-Chip platform. Microphysiological systems (MPS) with defined cellular compositions provide scalable and reproducible brain models that better recapitulate the in vivo environment, in which preclinical drug discovery efforts can translate to a higher success rate for identified targets and compounds. Robust differentiation methods to generate neurons, astrocytes, and microglia from any genetic background enables generation of SynFireÒ isogenic NeuroImmune Assembloids (NIA) in which the 3D microenvironment recapitulates salient ex vivo brain phenotypes enabling improved translatable high-throughput preclinical drug discovery. NIAs use very few cells and yield a defined reproducible ratio of mature cells making them scalable and assay-ready. Here we describe our isogenic platform for high-throughput drug-screening. Because the platform is also modular, the impact of a mutation can be studied in a cell-type specific manner to model non-cell autonomous phenotypes mimicking the cellular complexity of the human brain. Our platform can be adapted for high-throughput drug-screening yielding a cost-effective CNS-relevant drug discovery platform.

Keywords: Assembloid, Organoid, Microfluidic drug-screen

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A HIGH EFFICIENCY, SINGLE CELL DIFFERENTIATION METHOD FOR THE GENERATION OF FIBROBLASTS, ENDOTHELIAL AND MULTIPLE MYELOID CELL TYPES FROM HUMAN INDUCED PLURIPOTENT STEM CELLS FOR DISEASE MODELLING

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Macrophages (MΦ), fibroblasts (FBs), and endothelial cells (ECs) are present in all tissues and play important role in tissue homeostasis, repair, immunity, and angiogenesis. MΦ-FBs-ECs interactions contribute to the development of diseases such as fibrosis, cancer, and chronic inflammation. Recruited MΦ and ECs secrete cytokines and profibrotic mediators which activate FBs into MyoFBs to produce more extracellular matrix (ECM). Robust methods for generating these cell types will be important for developing more complex stem-cell-based disease and developmental models. Currently protocols to produce haematopoietic cells rely on either formation of EBs or differentiation using clumps of stem cells as the starting material. As a result differentiation is often inefficient, has low reproducibility and requires undefined media components that limit clinical application. To address this issue, we have optimised a chemically defined, xeno-free differentiation platform that can generate MΦ, FBs, and ECs from the same starting population of human induced pluripotent stem cells (hiPSCs). Starting with a minimal seeding density we induce mesoderm differentiation over 2 days and then by changing growth factor composition can drive the mesoderm towards fibroblasts, vascular endothelial cells or haemogenic endothelial cells and monocytic cells. At each stage, conversion of cells to the desired cell type is >80% limiting the need for enrichment steps throughout the protocol. We have validated the resulting cell types via mRNA-seq as well as using cell type specific functional assays including polarisation of MΦ (iMΦ) into M1 and M2 cells endocytosis assay. activation of FBs into MyoFBs using TGF-ß stimulation and tube formation assay of hIPSC derived ECs. In summary, we generated isogenic MΦ, FBs and ECs in a chemically defined, xeno free, single cell differentiation protocol that have gene expression and functional profiles similar to their primary equivalents. These cells will are an important step towards development of more complex in-vitro developmental and disease models and could also be applicable to translational clinical applications

Keywords: hIPSCs-derived Myeloid cell types, hIPSCs-derived Fibroblasts, hIPSCs-derived Endothelial cells

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LARGE SCALE DIFFERENTIATION OF HUMAN PSCS INTO ENDOTHELIAL CELLS IN A CLOSED CULTIVATION PLATFORM UNDER XENO-FREE CONDITIONS

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The vascular system, lined by endothelial cells (ECs), provides a barrier to tissue and influences blood homeostasis. Among other processes endothelial cells are involved in neovascularization, which is essential for the growth and metastasis of tumors. In this context ECs transport nutrients and remove metabolic waste from tumor cells. To understand and influence these interactions in more detail as well as to engineer vessels and organ grafts, an unlimited amount of ECs is required. Pluripotent stem cell derived endothelial cells (PSC-ECs) can be produced in unlimited number without ethical concern, under standardized environment and thus provide an optimal source for studying processes mentioned above. Here we present a standardized, scalable and closed system to produce functional PSC-ECs in serum- and xeno-free conditions. Endothelial differentiation of PSCs was performed within seven days using subsequently two different serum- and xeno-free cell culture media. For scalable PSC-EC production we used the GMP compliant CliniMACS Prodigy® Adherent Cell Culture System. Resulting PSC-ECs revealed standard endothelial markers, showed Dil-acetylated LDL uptake and tube formation capacity.

Keywords: iPSC derived Endothelial cells, Closed cell culture system, Serum-free and xeno-free

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ELUCIDATION OF SARS-COV-2 INFECTION-MEDIATED AIRWAY EPITHELIAL-ENDOTHELIAL BARRIER DISRUPTION MECHANISM USING AIRWAY-ON-A-CHIPS

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In the initial process of coronavirus disease 2019 (COVID-19), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infects respiratory epithelial cells and then transfers to other organs via the blood vessels. It is believed that SARS-CoV-2 can pass the vascular wall by altering the endothelial barrier using an unknown mechanism. In this study, we investigated the effect of SARS-CoV-2 on the endothelial barrier using an airway-on-a-chip that mimics respiratory organs and found that SARS-CoV-2 produced from infected epithelial cells disrupts the barrier by decreasing claudin-5 (CLDN5), a tight junction protein, and disrupting vascular endothelial cadherin (VE-cadherin)-mediated adherens junctions. Consistently, the gene and protein expression levels of CLDN5 in a COVID-19 patient's lungs were decreased. CLDN5 overexpression or fluvastatin treatment could rescue the SARS-CoV-2-induced respiratory endothelial barrier disruption. We therefore concluded that the downregulation of CLDN5 expression is a pivotal mechanism for SARS-CoV-2-induced endothelial barrier disruption in respiratory organs and that inducing CLDN5 expression is a novel therapeutic strategy against COVID-19.

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Keywords: SARS-CoV-2, Claudin-5, airway-on-a-chip

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A83-01 IMPROVES MURINE HEMATOPOIETIC STEM AND PROGENITOR CELL EXPANSION FOR PRECLINICAL BIOSAFETY ASSAYS AND GENE THERAPY

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Preclinical biosafety assessment of retroviral vectors is instrumental prior to clinical application. Genotoxicity assays not only demand a constant supply of mouse hematopoietic stem and progenitor cells (HSPCs) but also often an ex vivo cultivation for vector testing. During culture, low proliferation and undesired differentiation can hamper transduction efficiency and assay readouts. We aim to improve HSPC expansion protocols by reducing unwanted differentiation before gene correction. Our standard cytokine cocktail (S3F11) for HSPC culture contains the bone marrow (BM) niche factors SCF, FLT3-L and IL-11. The addition of IL-3 promotes sufficient proliferation for viral transduction but also drives myeloid differentiation specifically to mast cells. By intervening TGFβ signaling with the ALK5/4/7 inhibitor A83-01, mast cell differentiation was attenuated significantly while HSPCs were expanded by 20,000-fold. Murine BM-derived LSK SLAM (lin-Sca1+ckit+CD48-CD150+) cells were expanded in low-cell density for two weeks. S3F11+A83-01-treated cells expressed significantly less of the mast cell surface marker FcεR1α. Transcriptomic analyses confirmed decreased enrichment in mast cell activation, differentiation and proliferation gene sets compared to S3F11-only. Further, we expanded LSK SLAM cells in S3F11+A83-01 and added pomalidomide and UM171 to the medium. Expanded cells were transduced as efficient as freshly isolated lin- cells with lenti- and gammaretroviral vectors to assess the risk of retroviral vector-induced insertional mutagenesis. A mutagenic LTR-driven gammaretroviral vector integrated nearby the typical high-risk locus Mecom and promoted insertional transformation in expanded cells. Immune phenotyping of these transformed samples demonstrated a differentiation block of early progenitors, absent in samples transduced with a safer lentiviral vector. Finally, the surrogate assay for genotoxicity assessment (SAGA), a more sensitive and robust assay, revealed enrichment of oncogene and stemness-associated genes in gammaretroviral-transduced expanded cells. In sum, the S3F11+A83-01 expansion medium ameliorated myeloid differentiation and maintained HSPCs longer in a stem cell-like stage, needed for genotoxicity studies and ultimately, gene therapy.

Keywords: Hematopoietic Stem Cell Expansion, Gene Therapy, Genotoxicity Risk Assessment

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INTERROGATION OF HUMAN IPSC-DERIVED MICROGLIA FOR MODELING MICROGLIA-MEDIATED IMMUNE RESPONSES IN LRRK2 PARKINSONISM

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Recent studies implicate the brain immune system in the onset, development, and progression of neurodegenerative diseases, with microglia gaining more attention as a potential contributor and modulator. The complex interplay of environmental and genetic causes and the synergistic effects of molecular changes in the brain suggest neuroinflammation as a key contributor to neuropathology in Parkinson's Disease (PD). Human induced pluripotent stem cell (iPSC)-derived microglia represent a promising model for studying these mechanisms and the specific reactions of the brain immune system to common stimuli involved in neurodegeneration such as amyloid beta and tau oligomers, while modulation of LRRK2 kinase activity with specific inhibitors can reverse phenotypes and establish causality. Our objective is to measure and find overlapping immune response signals in iPSC-derived microglia with LRRK2 G2019S mutation, a strong genetic risk factor of PD, and compare them to matched controls. We derived microglia from patient and healthy control iPSCs by driving cells towards hematopoiesis and culturing them with cytokines that push cells towards homeostatic microglia. Using flow cytometry, SYBR Green qPCR array, Western blot, and immunocytochemistry, we fully characterized our microglia and confirmed the expression of key markers. An independent preliminary treatment of microglia with LRRK2 inhibitors showed a significant reduction of LRRK2 activity as measured by reduced phosphorylation of its key substrates. Fully differentiated cells were stimulated with ag-

gregated proteins to induce an intracellular signaling response. Conditioned media was analyzed using Luminex Human 80 Plex panel for the release of specific cytokines and chemokines to determine microglial responses. This study results point toward reduced chemokines such as CCL3, CCL22, and CXCL9 and interleukins IL6, IL10, and IL1A in LRRK2 mutant microglia, suggestive of a dysregulation of the JAK-STAT anti-inflammatory pathway. By subjecting microglia to this treatment and measuring their responses, we seek to better understand the role of immune cells in onset and development of LRRK2 Parkinsonism, uncover new signaling patterns and immune markers implicated in the disease, and establish a potential rescue strategy in the PD spectrum.

Keywords: Parkinson's Disease, hiPSC-derived microglia, Neurodegeneration

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GINS4 DEFICIENCY SELECTIVELY IMPEDES PROLIFERATION FOLLOWING COMMITMENT TO THE NATURAL KILLER CELL LINEAGE

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Cell proliferation is a ubiquitous process required for organismal development and homeostasis. However, individuals with partial loss-of-function variants in DNA replicative helicase components, including MCM and GINS proteins, often present primarily with immunodeficiency due to specific loss of natural killer (NK) cell immune subsets in peripheral blood. Such lineage-specific disease phenotypes reveal cell type-specific characteristics of cell cycle and proliferation and raise important questions about the regulation of helicase function and cellular responses to its impairment. We aimed to understand NK cell-specific proliferative dynamics and vulnerability to impaired helicase function using induced pluripotent stem cells (iPSCs) from individuals with NKD due to compound heterozygous GINS4 variants. Characterization of NK cell differentiation in healthy donor lines defined two primary waves of cell proliferation, one in the early hematopoietic precursors and one following commitment to the NK cell lineage. GINS4 protein expression was highest in pluripotent cells and decreased significantly with differentiation in all lines. As predicted by the destabilizing effect of disease-causing variants, GINS4 (NKD) lines had significantly lower GINS4 expression at all time points measured. While cell cycle progression was unaffected in pluripotent cells, CD34+ precursors demonstrated mild elongation of the S phase in NKD lines. More prominently, the second wave of proliferation, occurring in CD45+ lymphocytes, was specifically impaired in the NKD lines. As a result, NKD cell lines showed significantly lower frequency and number of mature NK cells, recapitulating the clinical phenotype of affected individuals. Other lineages, including granulocytes and T cells, were unaffected, underscoring the specific vulnerability of NK cells. Impairments in cell proliferation and generation of NK cells were rescued with correction of the variant alleles. Studies of mature NK cells show that NK, but not T cells, undergo apoptosis when subjected to mild replication stress or decreased helicase protein expression. Our results identify a novel proliferative stage of NK cell differentiation that is particularly sensitive to reduced GINS4 expression.

Funding Source: R01AI137275

Keywords: Natural Killer Cells, Proliferation, Helicase

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TRANSCRIPTION FACTOR MEF2C REGULATES ANGIOGENESIS IN THE ENDOTHELIAL CELLS DERIVED FROM HUMAN IPSCS

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Vascular dysfunction is a major cause of severe illness and death worldwide. Understanding the mechanisms that regulate vasculogenesis and angiogenesis is critical for identification of potential targets to treat vascular malfunction-associated diseases. Myocyte enhancer factor 2 (MEF2) family are known to be key transcription factors for vascular smooth muscle, cardiac muscle, and neural tissue during development. However, the role of MEF2s in human vasculogenesis and angiogenesis is not fully understood. Using human induced pluripotent stem cells (hiPSCs) as a model for understanding vasculogenesis and angiogenesis, we reported that myocyte enhancer factor 2C (MEF2C), but not any other members of the MEF2 family, was robustly upregulated during the differentiation of vascular progenitors and endothelial cells (ECs) from hiPSCs. The upregulation of MEF2C during the commitment of endothelial lineage was induced by vascular endothelial growth factors (VEGF) and dependent on the extracellular signal regulated kinase (ERK). Using shRNA approach to knockdown MEF2C in hiPSCs and their EC derivatives, we found that MEF2C did not affect the differentiation of ECs from hiPSCs, but greatly reduced the migration and tube formation capacity of the hiPSC-derived ECs. Through a chromatin immunoprecipitation-sequencing, genome-wide RNA-sequencing, quantitative

RT-PCR, and immunostaining analyses, we identified TNF-related apoptosis inducing ligand (TRAIL) and transmembrane protein 100 (TMEM100) as novel targets of MEF2C. This study demonstrates an important role for MEF2C in regulating human angiogenesis and highlights MEF2C and its downstream effectors as potential targets to treat vascular malfunction-associated diseases.

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Keywords: induced pluripotent stem cells, endothelial cells, angiogenesis

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ADAPTIVE ROUTES OF HEMATOPOIETIC STEM CELL DIFFERENTIATION TO DISEASE CONDITIONS AND AGE IN GENE THERAPY PATIENTS

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In Lentiviral Vector (LV) -based Hematopoietic Stem Progenitor Cell (HSPC) gene therapy (GT), transplantation of genetically modified HSPCs results in the fully reconstitution of the patients' hematopoietic system and provides therapeutic benefit in a variety of genetic diseases. However, it is still unclear how the underlying genetic disease, as well as other factors, may impact the reconstitution process, lineage specification and patients' safety. Here we analyzed the clonal repertoire and dynamics of hematopoietic reconstitution by studying >4 millions of vector integration sites, a surrogate of clonal identity, from purified myeloid, lymphoid, erythroid cell lineages and HSPCs from 53 patients treated by lentiviral-based HSPC-GT for metachromatic leukodystrophy (MLD) - a neurodegenerative lysosomal storage disorder, Wiskott-Aldrich syndrome (WAS) - an immunodeficiency with thrombocytopenia and β-thalassemia (β-Thal) - a hemoglobinopathy (up to 8 years of follow-up). From this analysis we observed that patients had a polyclonal repertoire without sings of insertional mutagenesis. We then dissect the hematopoietic reconstitution over time in terms of HSPC numbers, output and commitment of HSPCs toward the different linages. We estimated the number of active HSPCs in patients using capture-recapture statistics over time ranged from 770 to 35,000 and remained stable long-term. A large fraction of clones estimated in the early phase were exhausted within 12 months and were replaced by a smaller, yet substantial, number of long-term IS stably sustaining steady-state hematopoiesis. About 50% of hematopoietic clones had multilineage potential, in all disease conditions, while the remaining clones showed a preferential lineage output and long-term linage commitment which was specific to the disease condition: MLD patients showed a long-term commitment towards myeloid lineages, while in patients treated for WAS had a preferential output and commitment towards lymphoid lineages and β-Thal for erythroid lineages, especially in adult patients. Our results showed for the first time that the long-term output as well as the lineage commitment of HSPCs is strongly modulated by the patients' genetic background to better compensate for the demands posed by the specific clinical condition.

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Keywords: Hematopoietic stem cells, Gene therapy, lineage commitment

Clinical Trial ID number: NCT01560182; NCT01515462; NCT02453477.

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MODELING PRIMITIVE AND DEFINITIVE ERYTHROPOIESIS WITH INDUCED PLURIPOTENT STEM CELLS TO STUDY HUMAN DEVELOPMENT

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During development, red blood cells (RBCs) are produced through at least two hematopoietic waves (primitive and definitive), which generate RBCs with different functional characteristics. Induced pluripotent stem cells (iPSCs) can be differentiated into RBCs and allow modeling of pathological and developmental processes that would be virtually impossible to study in humans. While primitive erythropoiesis has been widely modeled using iPSCs, the definitive program has not been fully characterized as protocols that generate pure definitive progenitors were only recently described. To compare in vitro systems with human development, we generated iPSCs from a fetal liver (FL) sample, and produced iPSC-derived primitive and definitive red cells which were then compared to RBC generated directly from the FL. Functional assays confirmed qualitative differences between the programs, with primitive RBCs showing reduced proliferation compared to definitive red cells, larger cell size and a higher expression of embryonic globins. Expression of erythroid antigens was also different; although all RBCs robustly expressed Kell, only FL and definitive RBCs displayed Duffy on their cell surface. Transcriptome profiling by sc-RNAseq showed high similarity between FL and iPSC-derived definitive RBCs and a distinct gene expression pattern for the primitive RBCs. Gene and regulon analysis identified shared erythroid pathways, but also highlighted developmental differences, including fatty acid metabolism, glucocorticoid signaling and cell cycle mediators. To model perturbations of erythropoiesis with iPSCs, we introduced pathological mutations in KLF1, a master regulator of red cell fate and globin switching. While aspects of the disease phenotype were captured both by primitive and definitive RBCs, globin changes and reduced expression of ACKR1 were only recapitulated by definitive KLF1-edited RBCs. Our studies provide new insights into how the primitive and definitive programs differ regarding their transcriptome and highlight the importance of ontology when using iPSCs to model genetic hematologic diseases. Moreover, the generation of fetal liver-like erythroblasts further expand the potential applications of iPSC-derived RBCs as diagnostic reagents and, eventually, transfusion products.

Keywords: Erythropoiesis, Primitive and definitive hematopoiesis, KLF1

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BLOOD VESSEL ORGANOIDS CAN BE USED AS 3D ANGIOGENESIS MODEL

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Coronary artery disease (CAD) is the major cause of deaths worldwide. Until today more than 300 CAD risk genes or loci have been identified to be associated with CAD in genome-wide association studies (GWASs). Mirroring the complex disease, these risk loci are involved in different pathways or mechanisms contributing to onset and progression of the disease including blood pressure, mitosis & proliferation, neovascularization or neoangiogenesis, and vascular remodeling. Neovascularization, or angiogenesis, is a common feature in atherosclerosis and leads to disease progression and plaque instability. Stem cell derived 3D models can be generated, allowing a more physiological situation that can be analyzed in vitro. Blood vessel or vascular organoids have first been described in 2019 by Wimmer et al., derived from iPSCs as well as embryonic stem cells. We use blood vessel organoids as a new stem cell-derived angiogenesis assay in a 3D format. After embedding vascular aggregates into a collagen/matrigel matrix we either stimulate or inhibit the growth of vessel sprouts for approximately 1-2 weeks, using different substances like VEGF-A or CXCL4 respectively. We here demonstrate, that the treatment with stimulators such as VEGF-A enhances vessel sprouting, while inhibition with sunitinib malate or CXCL4 blocks it. Therefore, this model is usable to study the effect of CAD risk genes on angiogenesis in a 3D in vitro system.

Keywords: Organoids, Cardiovascular disease, iPSCs

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PD-L1 BLOCKADE IMMUNOTHERAPY TARGETS THE HSPC COMPARTMENT IN THE BONE MARROW REPROGRAMMING EMERGENCY MYELOPOIESIS DURING CANCER

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Immune checkpoint blockade (ICB) immunotherapy has revolutionized cancer treatment, demonstrating exceptional clinical responses in cancer patients. Despite the success, still a significant proportion of patients fail to respond, highlighting the existence of unappreciated mechanisms of immunotherapy resistance. Therefore, the delineation of such mechanisms is of paramount importance to minimize immunotherapy failures and optimize the clinical benefit. Herein, we reveal that immunotherapy with PD-L1 blockage (aPDL1) crosses the bone marrow (BM) and targets the hematopoietic stem and progenitor cells (HSPCs) in tumor-bearing mice, mediating their exit from the quiescent state and promoting their proliferation. Notably, PD-L1 blockade induces a transcriptomic reprogramming of HSPCs in tumor-bearing animals towards an inflammatory state which functionally alters emergency myelopoiesis. In support, aPDL-treated HSPCs exhibit a reduced potential of myeloid-derived suppressor cell (MDSCs) generation compared to control-treated HSPCs upon in vivo transplantation. Overall, our findings shed light on unrecognized mechanisms of action of ICB immunotherapy in cancer which entails targeting of BM-driven HSPCs and reprogramming of emergency myelopoiesis.

Keywords: Hematopoietic stem and progenitor cells, Immunotherapy, Cancer

TOPIC: KIDNEY

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CREATING A KIDNEY ORGANOID-VASCULATURE INTERACTION MODEL USING A NOVEL ORGAN-ON-CHIP SYSTEM

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Kidney organoids derived from human induced pluripotent stem cells (iPSCs) have proven to be a valuable tool to study kidney development and disease. However, the lack of vascularization of these organoids often leads to insufficient oxygen and nutrient supply, creating a necrotic core. Vascularization of organoids has previously been achieved by implantation into animal models, however, the vasculature arises largely from animal host tissue. Our aim is to transition from an in vivo implantation model towards an in vitro model that fulfils the advantages of vascularization whilst being fully human-cell derived. Our organ-on-chip system supported culturing of kidney organoids, which presented nephron structures (glomeruli, proximal and distal tubuli). We also showed that organoids cultured on chip presented increased maturation of endothelial populations based on a colocalization analysis of endothelial markers CD146 and CD31. To induce vascularization, we coated the microchannels of the chip with umbilical vein endothelial cells (HUVECs), creating synthetic 3D vessels. Upon co-culture, we observed migration and proliferation of these HUVECs inside the organoid tissue, where they interconnected with endogenous endothelial cells and formed structures presenting an open lumen resembling vessels. Our results establish for the first-time vascularization of kidney organoids in HUVEC co-culture conditions using a microfluidic organ-on-chip. Our model therefore provides a useful insight into kidney organoid vascularization in vitro and presents a tool for further studies of kidney development and drug testing, both for research purposes and pre-clinical applications.

Funding Source: NWO

Keywords: Organoids, Kidney, Organ-on-chip

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ENHANCED KIDNEY ORGANOIDS MODEL SARS-COV-2 INFECTIVITY AND REVEAL VIRAL ENTRY PATHWAYS IN RENAL CELLS

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With a high incidence of acute kidney injury among hospitalised COVID-19 patients, considerable attention has been focused on whether SARS-CoV-2 specifically targets kidney cells and directly impacts renal function, or whether indirect effects are largely attributed to renal damage. Kidney organoid models represent a valuable tool in disease research, enabling in-depth interrogation of disease mechanisms within in a controlled environment. To date, several studies have utilised kidney organoids to understand the pathogenesis of COVID-19, revealing the ability for SARS-CoV-2 to predominantly, but not exclusively, infect cells of the proximal tubule, with reduced infectivity following administration of soluble ACE2 and evidence of cellular injury. However, the potential utilisation of alternate viral receptors, the preferred SARS-CoV-2 processing pathway, and the role of common hypertensive medications, such as ACE inhibitors, on tissue infectivity remain incompletely understood. The immaturity of kidney organoids, particularly in the proximal tubule segment which is a target of SARS-CoV-2, represents one of the barriers to the application of kidney organoids in this field. Recently, we developed kidney organoids with enhanced proximal tubule maturity, improved functionality, appropriate injury response following proximal tubule damage, and higher viral infectivity compared to standard kidney organoids. Here, exploiting this proximal tubule-enhanced (PT-enhanced) organoid model, in combination with genetic and drug-mediated inhibition of viral entry/processing factors, we revealed that while viral infection critically relies upon the presence of ACE2, redundancy exists in the utilisation of CTSL- and TMPRSS2-mediated endocytic and non-endosomal viral processing pathways. Furthermore, the kidney organoid infectivity was equivalent irrespective of viral strain and unchanged following ACE-inhibitor exposure, previously suspected to influence ACE2 expression. Together, these data provide deeper insight into the renal implications of not only the ongoing COVID-19 pandemic, but also the potential mechanisms of other diseases targeting the kidney.

Funding Source: This research was supported by the NHMRC (GNT1156440), the Victorian State Government, and The Novo Nordisk Foundation (NNF21CC0073729). MHL is an NHMRC Senior Principal Research Fellow (GNT1136085).

Keywords: Kidney organoids, stem cells, COVID-19

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ESTABLISHMENT OF VANCOMYCIN INDUCED ACUTE KINDEY INJURY MODEL USING HUMAN URINE TUBULOIDS

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The glycopeptide antibiotic vancomycin is the most frequently prescribed for gram-positive infection, accounting for up to 35% of infected hospitalized patients. Vancomycin can cause tubular injury, and it is well recognized that its monotherapy can result in acute renal injury (AKI). However, the detailed pathogenic mechanisms associated to antibiotics including vancomycin with patient factors are not understood. In this study, we established the kidney tubular epithelial organoids (tubuloids) derived from urine of vancomycin-induced AKI individuals. Among patients referred for vancomycin therapeutic drug monitoring consultation, the serum vancomycin concentrations were analyzed concentrations and pharmacokinetic curves were simulated. The area under the curve (AUC) of the estimated vancomycin concentrations was calculated in chronological order. Patients who presented trough level higher than 30 μg/mL and AUC24 higher than 600 were reviewed for AKI diagnosis according to KDIGO guideline by nephrologists. Clean mid-voided urines were collected from a total of 5 patients and 3 samples were succeeded in tubuloid culture. When compared to healthy tubuloids, AKI tubuloid invariably appears as a cystic epithelial structure, while the expression of renal injury markers including KIM1 were higher in patients than in healthy controls. We evaluated the cytotoxicity of tubuloids in healthy controls and AKI individuals at various vancomycin concentrations and observed that sensitivity differed between individuals. Individual patient-derived tubuloid mimics vancomycin-induced renal injury, and it is potentially useful for screening therapeutic candidates with integrated analysis with clinical outcomes.

Keywords: vancomycin, kidney tubular epithelial organoids (tubuloids), acute renal injury (AKI)

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RECAPITULATION OF THE CHOLESTATIC LIVER INJURY USING LIVER-ON-A-CHIP WITH HUMAN IPS CELL-DERIVED HEPATOCYTES AND CHOLANGIOCYTES

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Progressive familial intrahepatic cholestasis type 1 (PFIC1) is an inherited cholestatic liver disease caused by the mutations in the ATPase phospholipid transporting 8B1 (ATP8B1) gene. In PFIC1 patients, severe cholestasis is developed rapidly after birth and usually leads to end-stage liver disease. Therefore, it is needed to generate a model which recapitulates PFIC1 pathophysiology and to develop new drugs to treat PFIC1 patients. In this study, we attempted to generate the liver model which recapitulates the liver pathophysiology of PFIC1. First, we generated the liver model which recapitulates the bile acid homeostasis in the liver. In this study, human hepatocytes and cholangiocytes were cultured in the membrane-integrated microfluidic device to form physiologically relevant three dimensional liver- and bile duct-like structures (Liver-on-a-chip). In the liver-on-a-chip, bile acid transport, conjugation, and response activities could be confirmed. These results indicated that bile acid homeostasis could be recapitulated using liver-on-a-chip. Next, we established iPS cells from peripheral blood mononuclear cells obtained from PFIC1 patients. The genome sequencing analysis showed that ATP8B1 gene mutations in PFIC1 patients were preserved in the PFIC1 patient-derived iPS cells. We differentiate PFIC1-derived iPS cells into hepatocyte- and cholangiocyte-like cells (PFIC1-HLCs and CLCs), and cultured them in the microfluidic device (PFIC1-liver-on-a-chip). The amount of bile acids transported into the bile duct-like structure of the PFIC1-liver-on-a-chip was lower than that of the liver-on-a-chip with healthy donor (wild type; WT)-derived HLCs and CLCs (WT-liver-on-a-chip). Additionally, the release of cytotoxicity marker, lactate dehydrogenase (LDH), and the expressions of fibrosis markers in the PFIC1-liver-on-a-chip were higher than those in the WT-liver-on-a-chip. These results suggested that cholestatic liver diseases in PFIC1 patients could be recapitulated using liver-on-a-chip and patient-derived iPS cells. In conclusion, we succeeded in generating PFIC1-liver-on-a-chip and recapitulating the liver pathophysiology of PFIC1 patients.

Funding Source: This research was supported by the iPS Cell Research Fund, the Japan Agency for Medical Research and Development (AMED) (JP21gm1610005), and Japan Science and Technology Agency (JST) (JPMJAX222A).

Keywords: human iPS cells, liver-on-a-chip, cholestasis

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COMPARISON OF IPSC-DERIVED LIVER ORGANOIDS AND OTHER LIVER CELL MODEL USING PROTEOMIC ANALYSIS AND CYP450 ASSAY

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Hepatotoxicity induced by drugs is the major reason for drug removal from the market. A high-throughput screening method employing an in vitro liver model is useful for early-stage liver toxicity assessment. Historically, monolayer human hepatocytes or immortalized liver cell lines (e.g., HepG2, HepaRG) have been employed to assess the toxicity of compounds to the liver. However, monolayer-cultured liver cells are routinely employed for short-term toxicological testing despite their inability to mimic an in vivo situation. They may not be adequate for diagnosing drug-induced chronic and recurrent liver damage. Multiple three-dimensional (3D) liver models have been constructed recently. In this work, we compared iPSC-derived human liver organoids and 3D cultures of various human liver cell lines (human primary hepatocyte, hepaRG, and hepG2) utilized in toxicological testing of drugs with their 2D cultures. Our proteomics results indicated that the iPSC-derived liver organoids matured with the increased differentiation time, and the global protein expression pattern showed closer to functional liver cells at a later stage. The 3D cultures of hepaRG cells displayed more hepatic drug metabolic enzyme expression than their monolayer cultures, such as the CYP450 enzyme family (e.g., CYP3A4), indicating that the 3D culture had more physiological function than their 2D culture. In an in vitro cytochrome P450 (CYP) activity assay, human primary hepatocytes initially had the highest CYP3A4 activity. However, this activity rapidly decreased as the culture days increased. The iPSC-derived human liver organoids exhibited sustained CYP3A4 activity and exhibited similar IC50s (0.3–0.4 μM) as hepaRG cells when treated with ketoconazole, demonstrating that liver organoids had similar CYP activity as hepaRG in an in vitro culture condition. Our research implies that iPSC-derived liver organoids will be a promising in vitro liver model for toxicity testing for both drug development and possible environmental hazard chemical identification.

Keywords: iPSC-derived liver organoids, proteomics, 3D culture

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MODELING OF HUMAN NON-ALCOHOLIC FATTY LIVER DISEASE USING LIVER-SPECIFIC MICROENVIRONMENT-INTEGRATED ORGANOIDS

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Liver organoids derived from adult stem cells and pluripotent stem cells have attracted attention for their potential applications in disease modeling, drug screening, and tissue regeneration. However, conventional liver organoids often lack cellular and extracellular components essential for constituting the human liver microenvironments. Therefore, for more precise disease modeling and drug screening, human liver-mimicking organoid model should be developed in vitro. Herein, we established primary adult stem cell- and pluripotent stem cell-derived liver organoid models through co-culture with endothelial cells, Kupffer cells, and hepatic stellate cells in liver extracellular environments to increase complexity and functionality. Organoids incorporating liver microenvironmental factors showed improved hepatic metabolic functions, such as urea synthesis and albumin secretion, compared to conventional organoids. Expression of hepatic differentiation markers was also significantly upregulated in liver microenvironment-integrated organoids. Finally, we established a non-alcoholic fatty liver organoid model by treating free fatty acid to the liver microenvironment-integrated organoids. Our study demonstrates that organoids with liver-specific cellular and extracellular microenvironments enable efficient in vitro 3D modeling of various liver diseases and screening of candidate drugs.

Funding Source: This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation of Korea(NRF) funded by the Korean government, the Ministry of Science and ICT(MSIT) (2022M3A9B6082675).

Keywords: Liver organoids, Non-alcoholic fatty liver disease, Disease modeling

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EFFICIENT GENERATION OF FUNCTIONALLY RELEVANT HPSC-DERIVED HEPATOCYTES AND LIVER ORGANOIDS FOR HEPATOTOXICITY AND LIVER BIOLOGY MODELING

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Functionally relevant human hepatocyte culture systems are critical for studying diseases such as nonalcoholic fatty liver disease and nonalcoholic steatohepatitis, drug safety and efficacy screening, as well as cell therapy applications. Human pluripotent stem cell (hPSC)-derived hepatocytes represent a convenient and scalable alternative to current culture systems, including primary human hepatocytes and immortalized cell lines, which often rapidly de-differentiate in culture or lack metabolic maturity. STEMdiff™ Hepatocyte Kit supports efficient and reproducible generation of hepatocyte-like cells (HLCs) over 21 days. hPSCs are first patterned to definitive endoderm cells using STEMdiff™ Definitive Endoderm Medium (Hepatic), then differentiated to hepatic progenitors (HPs) using STEMdiff™ Hepatic Progenitor Medium, and finally matured to HLCs using STEMdiff™ Hepatocyte Medium. HLCs exhibited hepatic marker expression, ≥ 60% ALB+A1AT+ cells as assessed by flow cytometry (n = 8 cell lines), and mature functionality, including CYP3A4 enzymatic activity and albumin secretion (n = 11 - 15 experiments). Additionally, HepatiCult™ Organoid Growth Medium (Human) was used to generate HP- and HLC-derived liver organoids in Matrigel® domes, that could be expanded for at least 10 passages using split ratios between 1:2 and 1:6 (n = 4 organoid lines). These organoids were amenable to cryopreservation and could be further matured in 3D using HepatiCult™ Organoid Differentiation Medium (Human), resulting in significant downregulation of the fetal hepatocyte gene, AFP (5.7-fold decrease; n = 2 donors over 10 organoid passages), and upregulation of mature hepatic genes, ALB and CYP3A4 (18- and 6.9 x 10^3-fold increase, respectively; n = 2 donors over 10 organoid passages). Both HLCs and organoids derived from HPs and HLCs exhibited sensitivity to ketoconazole-, rifampicin-, and troglitazone-induced hepatotoxicity (average 2D HLC IC50 = 206 µM, 22 µM, and 36 µM, respectively; average organoid IC50 = 316 µM, 57 µM, and 36 µM, respectively; n = 2 donors), demonstrating the utility of STEMdiff™ Hepatocyte Kit for use in hPSC-derived liver modeling and drug screening applications.

Keywords: Hepatocyte, Organoid, Liver

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PATIENT-SPECIFIC HEPATOBILIARY ORGANOIDS ELUCIDATE FUNCTIONAL ALTERATIONS IN INTRAHEPATIC CHOLESTASIS

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Patient-specific induced pluripotent stem cell (iPSC)-based models are a versatile resource to study pathological changes of given genetic mutations in vitro. This is of particular relevance for progressive familial intrahepatic cholestasis (PFIC), which is attributed to mutations in several genes, such as ATP8B1 (FIC1), ABCB11 (BSEP), ABCB4 (MDR3), FXR (NR1H4), MYO5B, TP2, KIF12, and where a number of variants of unknown significance (VUS) were described and where in some conditions also heterozygous variants are associated with cholestatic phenotypes. We applied a Sendai virus based reprogramming system to generate iPSCs from patients' peripheral blood-derived cells covering compound heterozygous mutations in MYO5B, compound heterozygous mutations in ABCB11 or heterozygous mutations in ABCB11, ABCB4, and MYO5B. iPSC-derived hepatobiliary organoids from MYO5B-deficient iPSCs exhibited a reduced organoid size indicating a disturbed hepatic function and mirrored the diffuse mislocalization of the bilirubin transporter MRP2. Functional characterization of MRP2-mediated Cholyl-Lysyl-Fluorescein (CLF) and BSEP-mediated Tauro-nor-THCA-24-DBD transport demonstrat-

ed a marked reduction of transport in MYO5Bmut organoids, in comparison to unaffected control organoids. Interestingly, iPSC-based organoids derived from the patient carrying three heterozygous mutations in ABCB11, ABCB4, and MYO5B exhibited a significantly reduced BSEP-mediated Tauro-nor-THCA-24-DBD transport, but unaltered MRP2-mediated CLF-transport. In conclusion, iPSC-based organoid models allow functional characterization of mutations in PFIC-associated genes by assessing the hepatobiliary transport of fluorescent substrates for BSEP and MRP2, and thus, are valuable tools to functionally characterize hepatobiliary transport alterations.

Funding Source: HIChol consortium (rare disease porgam of BMBF): 01GM1904B & 01GM2204C

Keywords: iPSC -liver organoids, hepatobiliary transport, intrahepatic cholestasis

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EFFECT OF CO-CULTURED ADIPOSE MESENCHYMAL STEM CELLS ON GENERATION OF HEPATOCYTE ORGANOIDS

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Liver organoids cultured in a three-dimensional (3D) environment are often used as models for evaluating liver disease and drug toxicity. However, liver organoids generated from pluripotent stem cells or hepatocellular carcinoma cells are known to have significantly lower liver function compared to livers. In the present study, we attempted to improve the efficiency of liver organoid generation through the co-culture of GFP-expressing adipose mesenchymal stem cells (A-MSCs) and hepatocytes using a pig model. Hepatocytes alone (control, 2 × 10^4 cells) or hepatocytes and adipose mesenchymal stem cells (A-MSCs) (5:1 ratio) were cultured in 50 µl Matrigel dome (3D) for 14 days (passage 0; P0). All hepatocyte organoids were sub-cultured at a ratio of 1:2 per well. Analysis of the number and diameter of organoids in each group revealed that from day 5 of P0, the group co-cultured with A-MSCs had a significantly higher number and diameter of organoids than the hepatocytes-only group. Real-time polymerase chain reaction showed that the levels of ALB, a major functional gene in the liver, and KRT19, a liver stem cell factor, did not differ between the two groups. Furthermore, organoids were treated for 3 days with 25 µM rifampicin, a CYP3A29 inducer, and the expression of CYP3A29, which is involved in drug-metabolizing enzyme synthesis, was analyzed and compared with that in untreated groups. CYP3A29 showed higher upregulation in hepatocyte organoids co-cultured with A-MSCs (7.4-fold) than in hepatocyte-only organoids (5.3-fold). Histochemical analysis and absorbance measurements using Oil red O staining showed that lipid accumulation was higher in the group cultured with A-MSCs than in the hepatocyte-only group. In conclusion, we revealed that A-MSCs promote hepatocyte organoid generation and are helpful in inducing functional maturation of hepatocyte organoids.

Funding Source: This work was supported by the grant number PJ 015872.

Keywords: Porcine, Hepatocyte organoid, Adipose mesenchymal stem cells

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MULTICELLULAR LIVER ORGANOIDS RECAPUTILATE MURINE LIVER PERI-PORTAL ARCHITECTURE AND CELLULAR INTERACTIONS IN VITRO

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Tissue-derived organoid models are reductionist and easy to manipulate cellular systems, which provide basic biological understanding of the specific organ they derive from. We pioneered the development of mouse and human organoids from adult liver tissue, which are known as liver cholangiocyte organoids. Hepatocyte organoids from adult mouse hepatocytes have also been successfully generated by others. However, these liver organoids are only formed by one epithelial cell type, either cholangiocytes or hepatocytes, and lack any of the supporting stromal cells present in the tissue. We recently described an organoid co-culture method where primary murine liver mesenchyme and cholangiocyte cells develop as organoid structures. That allowed us to investigate regeneration dynamics in vitro by controlling respective cell numbers in culture. Here, we have increased the complexity of this co-culture and present a next generation organoid model composed of adult murine hepatocytes, cholangiocytes and primary liver mesenchymal cells, which we name peri-portal liver organoid. This model reconstructs some of the cell-cell interactions of the liver portal tract region in culture. First, we improved the previously published hepatocyte organoid models, to allow the formation of a bile canaliculi network that recapitulates in vivo murine bile canaliculi size and architecture. Then, by combining the three cell types we obtained multicellular organoid structures, where bile duct cells are surrounded by portal mesenchymal cells and are embedded within the hepatocyte parenchyma, thus recapitulating in vitro the in vivo architecture of the peri-portal region of the liver lobule. The organoid structures present functional bile duct-bile canaliculi connection, where bile canaliculi formed by hepatocytes drain bile acid into the lumen of bile ducts. Finally, by manipulating the number of portal mesenchymal cells we describe that non-physiological mesenchymal cell numbers result in hepatocyte cell death, suggesting that tissue fibrosis is actively driven by aberrant mesenchyme-hepatocyte interactions. This proof-of-concept study opens an avenue for more complex liver tissue-derived organoid models, which better recapitulate liver

complexity, as well as physiological and pathological liver features in vitro.

Keywords: organoid, liver, regeneration and fibrosis

TOPIC: MESENCHYMAL STROMAL CELLS, ADIPOSE, AND CONNECTIVE TISSUE

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SYSTEMIC AND INTRANASAL ADMINISTRATION OF MESENCHYMAL STEM CELL-DERIVED SECRETOME REDUCES WITHDRAWAL SYNDROME CAUSED BY OPIOID ADMINISTRATION IN TWO PRE-CLINICAL ANIMAL MODELS

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Opioid addiction affects millions of individuals worldwide and is characterized by a severe withdrawal syndrome upon opioid discontinuation, which leads to an urgent need to readminister the drug. Therefore, reducing opioid withdrawal severity is a key target to lessen morbimortality of opioid addicted patients. Based on recent evidence that associate opioid withdrawal with brain oxidative stress and neuroinflammation we evaluated whether the administration of the potent antioxidant and anti-inflammatory secretome derived from human mesenchymal stem cells (hMSC) could reduce opioid withdrawal. For this, we used two animal models of morphine administration. In the first model, Wistar rats were implanted subcutaneously with a mini-pump that continuously delivered morphine 20 mg/mL at 5 µl/hr, while control animals were implanted with a mini-pump delivering vehicle. Three days after mini-pump implantation, rats underwent simultaneous intranasal and intravenous administration of secretome derived from 1x106 hMSC or saline. After seven days of continuous morphine (~10 mg/kg/day) or vehicle administration, the withdrawal syndrome was triggered by the administration of the opioid antagonist naloxone. In the second animal model we used the two-bottle choice morphine self-administration paradigm. After four weeks of voluntary morphine consumption (~15 mg/kg/day) Wistar rats underwent simultaneous intranasal and intravenous administration of hMSC-secretome or saline and two days later the withdrawal syndrome was triggered by the administration of naloxone. In both animal models the severity of naloxone-elicited signs was measured by behavioural observation for 30 minutes. In both animal models the administration of hMSC-secretome significantly reduced withdrawal syndrome severity, evidenced by a marked decrease in the frequency of behavioural signs commonly associated to opioid withdrawal, compared to rats that received saline. Secretome administration also reduced opioid-induced neuroinflammation (astrocyte activation and microglial density) and brain oxidative stress (GSSG/GSH ratio). Thus, hMSC-secretome could be envisioned as a new biodrug to reduce the severity of the withdrawal syndrome induced by opioid use, helping to decrease the relapse rate of this devastating disease.

Funding Source: Proyecto FONDECYT 1200287

Keywords: Addiction, Opioid, Withdrawal Syndrome

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FUNCTIONAL ENGINEERING OF MESENCHYMAL STROMAL CELL BY REVITALIZING CULTURE CONDITION

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As one of the most promising cell therapy sources, the therapeutic applications of mesenchymal stem cells (MSCs) are currently being actively explored due to their broad regenerative efficacy. But strategies to control batch heterogeneity, stemness, and senescence of MSCs during ex vivo culture are limited, suggesting that the development of standardized culture methods to engineer MSC function is an important breakthrough in clinical intervention. Here, we extensively analyzed the functional properties of human dental pulp-derived MSCs (DPSCs) expanded in conventional FBS-containing media (control), or serum/xeno-free new culture conditions (S-J-01 and 02). Both S-J-MSCs showed similar proliferation during culture; however, they showed higher maintenance of self-renewal capacity with higher colony-forming unit-fibroblast (CFU-F) activity than the control. The S-J-MSCs showed enhanced differentiation potency into multiple dentin-related cell types and other non-mesenchymal cell lineages (endothelial, neuronal, and skeletal muscle cells). Furthermore, we investigated the vascular remodeling activity of MSCs in different culture conditions to evaluate the therapeutic capacity in a 3D microfluidic chip and examined the possible underlying mechanisms. Mechanical analysis revealed a significant increase in neural crest stem cell-like CD271+ subsets, which are rare in controls, suggesting that transcriptional rewiring by new culture conditions may contribute to this reprogrammed potential of S-J-MSCs. Interestingly, the effects of conditions S-J-01 and 02 were generally conserved in MSCs derived from other tissue sources, such as bone marrow and umbilical cord Wharton jelly, synchronizing distinct transcriptomic identities of MSCs according to tissue origin. Overall, we identified a culture condition-based engineering strategy for MSCs and described a promising therapeutic intervention for many diseases associated with MSCs.

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Keywords: mesenchymal stem cells (MSCs), stemness, culture condition-based engineering

TOPIC: MUSCULOSKELETAL

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PROGRESSIVE ROTATOR CUFF DEGENERATION ALTERS PROGENITOR FIBRO-ADIPOGENIC DIFFERENTIATION IN RESPONSE TO DRUG TREATMENTS

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Small animal models of massive tears of the rotator cuff (RC) have been extensively used to study the pathophysiology of chronically injured RC. Transection of murine RC tendon and suprascapular nerve results in progressive muscle atrophy and fibro-adipogenic progenitor (FAP)-derived fibrosis and fat accumulation within 7 weeks post induction of injury. However, it is still unclear if FAP degenerative potential changes with time and if adipogenesis is positively regulated at the expense of fibrogenesis in the presence of anti-fibrotic drugs. We therefore analyzed the adipogenic differentiation potential of non-activated FAP (naFAP), isolated from non-injured RC, and that of injury activated FAP (iaFAP) at 5 days and 7 weeks post injury, which represents early and late stages of RC degeneration, respectively. Additionally, FAP adipogenic differentiation was tested in the presence of two different anti-fibrotic compounds: CWHM-12, an inhibitor of TGF-β1 activation and 423F, a modulator of gp130 signaling. FAP adipogenic potential markedly changed at early and late stages of RC degeneration, peaking at 5 days post-injury and decreasing at 7 weeks post-injury. Both early and late stage iaFAP exhibited increased adipogenic differentiation compared to naFAP. While CWHM-12 and 423F only slightly increased adipogenesis of naFAP, both drugs markedly increased adipogenesis of 5 days iaFAP and significantly diminished adipogenesis of 7 weeks iaFAP in a dose-dependent manner. Additionally, both drugs mediated greater dose-dependent inhibition of collagen production by naFAP in comparison to iaFAP. Notably, there continued to be profound myogenesis in the presence of varying concentrations of CWHM-12 and 423F at 5 days post injury, which is a myogenic post-injury stage. Altogether, our findings demonstrate that both drugs affect fibro-adipogenic differentiation in a cell-state dependent manner, which is impacted by the kinetics of muscle degradation.

Keywords: muscle progenitors, fibro-adipogenesis, anti-fibrotic drugs

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SIX1+PAX3+ IDENTIFY A PROGENITOR FOR MYOGENIC LINEAGE COMMITMENT FROM HPSCS

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The earliest skeletal muscle progenitor cells (SMPCs) derived from human pluripotent stem cells (hPSCs) are often identified by transcription factors that are also expressed by a diverse number of progenitors. An early transcriptional checkpoint that defines myogenic commitment of hPSCs could improve lineage specific differentiation to skeletal muscle, which we find is confounded by genetic variability. We performed analysis of several myogenic factors in human embryos and from early hPSC differentiations and found several similarities and differences between the in vivo and in vitro environments. Of the markers tested, co-expression of SIX1 and PAX3 was most indictive of myogenesis, whereas PAX3 alone marked non-myogenic lineages. Using a single derivation protocol, we evaluated myogenic differentiation across five hPSC lines and found that the emergence of SIX1+PAX3+ precursor cells can be improved by manipulating seeding density, metabolism, and concentration of the mesoderm inducing small molecule CHIR. These modifications also resulted in the co-emergence of hPSC-derived sclerotome, cardiac, and neural crest cells that we hypothesized either enhanced or restricted hPSCs from adopting a myogenic cell fate. Using a dCas9-KRAB hPSC line, we inhibited transcription of master regulators of these non-myogenic lineages and found that blocking cardiac lineages improved SIX1+PAX3+ myogenic induction. Using these transcription factors and morphologic landmarks as a guide, we show that early expression of SIX1+PAX3+ is indicative of efficient myogenic differentiation of PAX7+ progenitors and myotubes. We completed five separate directed differentiations and compared these to fetal progenitor and adult satellite cells by RNA-seq and immunostaining. Overtime, hPSC and fetal SMPCs lost expression of PAX3, but SIX1 continued to be expressed by both SMPCs and myotubes to serve as a general myogenic lineage marker. RNA-Seq showed that while hPSC SMPCs are more like fetal than adult stem cells, they still express hundreds of unique genes involved with neuromuscular and extracellular matrix associated pathways. Here we provide a resource for generation of skeletal muscle from hPSCs to enable more efficient protocols to derive skeletal muscle progenitors that follow human development.

Funding Source: CIRM Training Fellowship (EDUC4-12822)

Keywords: hPSCs, Skeletal muscle progenitors, myogenic differentiation

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PROMOTING THE MATURATION OF SKELETAL MUSCLE PROGENITOR CELLS TO SATELLITE CELLS THROUGH ACTIVATION OF NUCLEAR RECEPTORS

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Skeletal muscle progenitor cells (SMPCs) contribute to developmental myogenesis and satellite cells (SCs) contribute to postnatal muscle homeostasis and regeneration. Differentiating human pluripotent stem cells (hPSCs) into SCs is valuable for developing cell replacement therapies for muscular dystrophies. However, the maturation process of SMPCs to SCs remains unclear, and current hPSC directed myogenic differentiation protocols result in immature SMPCs that are less regenerative than SCs. Several nuclear receptors (NRs) were identified as candidates that promote the maturation of embryonic SMPCs. We hypothesize that activating these NRs through the addition of their respective ligands in the culture media will promote a shift in maturation of hPSC-derived SMPCs. Induced pluripotent stem cells (IPSCs) and embryonic stem cells (ESCs) were differentiated into myogenic cultures and treated with ligands during the last seven days of differentiation. SMPCs were isolated via FACS and continued to be cultured with ligand addition. SMPC maturation is being analyzed through immunocytochemical staining of developmental myosin isoforms. From this analysis, we aim to further understand the effects of how nuclear receptors regulate the maturation process of SMPCs to SCs. We expect these findings contribute to the development of cell therapies that enhance the myogenic regenerative abilities in muscle diseases.

Keywords: Nuclear Receptors, Skeletal Muscle, Myogenic Culture

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DECONSTRUCTING HUMAN SOMITOGENESIS WITH STEM CELLS

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The vertebrate body displays a segmental organization which is most conspicuous in the periodic organization of the vertebral column and peripheral nerves. This metameric organization is first implemented when somites, which contain the precursors of skeletal muscles and vertebrae, are rhythmically generated. Somites then become subdivided into anterior and posterior compartments essential for vertebral formation and segmental patterning of the peripheral nervous system. How this key somitic subdivision is established remains poorly understood. Here we introduce novel tridimensional culture systems of human pluripotent stem cells (PSCs), called Somitoids and Segmentoids, which recapitulate the formation of somite-like structures with antero-posterior (AP) identity. Using these in vitro models, we found that somite AP compartments is established by cell sorting, rather than gene expression remodeling as classically postulated. We show that cells are pre-fated in a salt-and-pepper pattern in the newly formed segment and AP fated cells are sorted apart through differential cytoskeletal properties. We further identify a key function of the segmentation clock in organizing cell sorting timing to convert temporal rhythmicity into the spatial regularity of anterior and posterior somitic compartments. Together we define a novel framework for the symmetry breaking process initiating somite polarity patterning. Our work exemplifies how the resolution offered by PSC-derived in vitro systems can be used to answer long-standing developmental biology questions and advance knowledge of human development.

Keywords: Somite, Pattern formation, Organoids

TOPIC: NEURAL

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PRE-CLINICAL INVESTIGATIONS ON SULFORAPHANE USING SENSORY NEURONS DERIVED FROM FRIEDREICH'S ATAXIA PATIENT-INDUCED PLURIPOTENT STEM CELLS

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Friedreich's Ataxia (FA) is caused by inheriting an excessive number of GAA repeats within Intron 1 of the frataxin (FXN) gene which regulates mitochondrial iron transport and respiration. Consequently, epigenetic mechanisms promote the transcriptional suppression of the FXN gene, resulting in iron accumulation in tissues, oxidative stress, inflammation and cell death. Patients often become wheelchair bound 10 years post-diagnosis and 59% of patients die early (< 50 years) due to heart issues. There is no cure and current treatments cannot stop the worsening of symptoms. New drugs in clinical trials can only target one of many disease processes or cannot penetrate the blood brain barrier (BBB). The broccoli-derived compound, sulforaphane (SF), has epigenetic, anti-inflammatory and antioxidant effects in various cell types and the ability to cross the BBB. We propose the use of SF to rescue dorsal root ganglion sensory neurons, the dominant cell type that degenerates in early FA, from death. Few in vitro studies have reported SF's antioxidant effects in FA patient fibroblasts, murine FXN-deficient motor neurons or neural stem cells but these models lack the full GAA expansion and exhibit different characteristics to human sensory neurons. Such studies also do not reveal the epigenetic and anti-inflammatory effects of SF. Our study used FA patient induced pluripotent stem-cells (iPSC) to generate sensory neurons that express the full GAA expansion. Isogenic control lines were created by CRISPR-deletion of GAA repeats from the patient line. The identity of these neurons was validated by positive immunostaining for NF200, peripherin, parvalbumin and TrkC. Cell viability assays showed that 24-hour exposure to SF, at doses tested in healthy people and those with

chronic disease (e.g. asthma), was not toxic to both isogenic control and patient iPSC-derived sensory neurons but cell viability of the latter improved by up to 40% ($p < 0.001$). Taqman gene expression assays demonstrated that this protective effect was accompanied by decreased levels of genes encoding epigenetic enzymes (DNMT1/3A and HDAC1/3/6) and inflammatory cytokines (IL-6), and increased levels of genes encoding antioxidant proteins (NRF2, HO-1, NQ01 and GCLM) ($p < 0.05$). Our findings enhance the evidence needed to support clinical trials of SF in FA.

Funding Source: This study was supported by funding from Swinburne University of Technology, The University of Melbourne, Friedreich's ataxia Research Alliance/Association and Medical Research Future Fund Stem Cell Therapies Mission.

Keywords: Friedreich's ataxia, Pluripotent stem cell derived sensory neurons, Sulforaphane

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OPTIMIZED PRIME EDITING EFFICIENTLY GENERATES MUTATIONS IN HIPSCS TO MODEL INHERITED RETINAL DEGENERATION

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Prime editing is a versatile tool that can introduce point mutations as well as small insertions and deletions into the genome. Nevertheless, low efficiency can be an obstacle for use of prime editing in human induced pluripotent stem cells (hiPSC). This is especially true when the genetic context precludes the use of multiple prime editing guide RNAs (pegRNAs) and other strategies must be employed to achieve the desired edit. Such is the case of the c.25G>A (p.V9M) mutation in the NMNAT1 gene, causative of severe early onset inherited retinal degeneration (IRD). In this study, we efficiently generated an isogenic model of the p.V9M mutation in the NMNAT1 gene, optimizing the prime editing workflow without manipulating key prime editing components. Electroporated clones were identified with a transient GFP reporter in the nicking gRNA plasmid and analyzed after 48h using fluorescence microscopy and FACS. Single clones were manually selected, expanded, and analyzed with Sanger sequencing to confirm prime editing. After an in silico analysis of the genomic region of the c.25G>A NMNAT1 mutation, two pegRNAs were selected for molecular cloning based on the distance to the mutation and sequence length. Initial co-electroporation of PEmax, pegRNAs and nicking gRNA in hiPSCs showed transfection in few clones and low (< 1%) editing efficiency. We modified the workflow of hiPSC prime editing, including plasmid concentrations and prime editing components ratios and several conditions of the delivery method and we demonstrated that our optimized workflow enhanced editing efficiency. Fluorescent microscopy and flow cytometry of the electroporated hiPSC showed 60% transfection efficiency. Through NGS we identified 10% correctly edited alleles for the c.25G>A NMNAT1 mutation. After clonal expansion we identified up to 5.38% homozygous clones and 20.43% heterozygous clones. These findings show that our optimized workflow enhanced the prime editing efficiency in hiPSC and allowed for the generation of clonal isogenic cell lines with the heritable p.V9M mutation causative of NMNAT1-associated IRD. Our approach has proven to be useful in generating other isogenic models of IRDs.

Funding Source: Career Starter Research Grant Award in Pediatric Ophthalmology of the Knights Templar Eye Foundation

Keywords: prime editing, cone degeneration, NMNAT1

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HUMAN EMBRYONIC STEM CELL-DERIVED DOPAMINERGIC NEURON MODEL FOR NEURODEVELOPMENTAL TOXICOLOGY SCREENING

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There is a major push in the field of developmental neurotoxicity (DNT) to move away from traditional rodent guideline studies to in vitro new approach methodologies (NAMs). Such in vitro NAMs have been developed for a variety of endpoints critical for neurodevelopment, such as proliferation, migration, and synaptogenesis. One key endpoint that is currently lacking, however, is development of neuronal subtypes. Moreover, while most of these assays employ cell lines or rodent-derived primary cells, hESCs more closely mimic human embryonic development in vivo, which may provide critical insight into vulnerabilities of human-specific neurodevelopment. We are thus developing a DNT screening approach for differentiation of hESCs into particular neuronal subtypes. Specifically, we are differentiating hESCs into dopaminergic (DA) neurons, as their neurodevelopmental trajectory is relatively well-characterized, they are particularly sensitive to environmental factors, and they have been implicated in a variety of neurodevelopmental and neurodegenerative disorders. We are utilizing CRISPR-mediated genome editing technology to construct a hESC triple-reporter system to monitor stages of DA neuron differentiation using fluorescent imaging. The current reporter line includes nestin-EGFP to monitor differentiation down the neural lineage, and tyrosine hydroxylase-mScarlet to monitor maturation into DA neurons. Our third reporter will be targeted to the midbrain floorplate marker CORIN to monitor specification of neural stem cells towards DA neurons. We are developing our DA neuron differentiation protocol based on the dual inhibition approach, which includes separate stages of specification, expansion, and maturation. This toxicological screening approach will allow for carefully assessing specific windows of susceptibility to chemical exposures for DA neuron development. Screening will be performed using fluorescent high-content live-cell imaging and gene expression analyses. Hit compounds can subsequently be pursued for insight into mechanisms of action. We aim to incorporate this reporter-based model into the battery of DNT NAMs to identify environmental toxicants of DA neuron development in a human in vivo-relevant context.

Keywords: developmental neurotoxicology, dopaminergic neurons, risk assessment

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HIPSC-DERIVED DOPAMINERGIC AND GLUTAMATERGIC NEURONS OF SCHIZOPHRENIA PATIENTS SHOW NEURONAL ABERRATIONS IN A CO-CULTURE MODEL COMPARED TO DOPAMINERGIC MONOCULTURES

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Schizophrenia (SCZ) is characterized by aberrant development of the central nervous system and thereby results in impairing multiple aspects in synaptic transmission, neuronal connectivity, and activity patterns. In SCZ, the involvement of different neurotransmitter systems, especially aberrations in dopaminergic pathways, were already postulated several decades ago. One of the affected dopaminergic pathways is the mesocortical pathway, that connects the ventral tegmentum to the prefrontal cortex, proposing a blunting of cortical dopamine release. However, the mechanistic, underlying biology of aberrant dopamine release in SCZ remains elusive. Here, we present the establishment of an in vitro co-culture model comprising dopaminergic and glutamatergic neurons, both of human origin, to mimic the mesocortical pathway and to study underlying disease mechanisms in SCZ. Our hiPSC-derived dopaminergic and glutamatergic neurons express subtype-specific synaptic markers, release neurotransmitter and show robust single-cell activity in calcium imaging. In our dopaminergic monoculture, in which the neurons were cultivated separately, we cannot observe changes in presynaptic terminals, neither do single cell calcium traces exhibit differences in peak frequency or dopamine release when compared to healthy controls. Interestingly, when combining both neuronal cell types into co-cultures, dopaminergic neurons derived from SCZ patients show a reduction of presynaptic terminals, while glutamatergic SCZ neurons depict a slight increase. Subtype-specific examinations via single-cell calcium imaging of these co-cultures revealed significantly altered calcium peak frequency for both types of neurons individually when comparing schizophrenia patient neurons to healthy controls. In conclusion, with this co-culture model we generated a humanized 2D in vitro model system to study the reciprocal interaction of different neuronal cell types affected in SCZ. Moreover, we demonstrate the importance of getting as close as possible to the in vivo situation by combining appropriate cell types into disease model systems. These systems can be further employed for the study of molecular mechanisms underlying neuropsychiatric diseases and may be helpful for future drug development.

Keywords: Schizophrenia, Disease Modelling, co-culture models

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DERIVING ENTERIC NEURAL PROGENITORS FROM IPSCS FOR THE TREATMENT OF DIGESTIVE DISORDERS

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The enteric nervous system (ENS) provides unique microcircuits that orchestrate digestive function independently of central nervous system input. While ENS dysfunction often results in severe gastrointestinal disorders including Hirschsprung's disease (HSCR), no cell-based treatment has yet been developed to fully restore its function. Here we aimed to utilize an induced pluripotent stem cell (iPSC)-based technology to develop a novel therapeutic option for HSCR. We derived human enteric neural progenitors via neural crest lineage from iPSCs by recapitulating the normal progression of stepwise narrowing fate decisions in mammalian embryos. The iPSC-derived enteric neural progenitors (iENPs) expressed key genes such as SOX10 and PHOX2B and showed high differentiation potential into subtypes of enteric neurons and glial cells in vitro. We also used RNA sequencing to analyze the developmental trajectory of iENPs. To test the ability of iENP to form ENS in vivo, we injected iENPs into the distal rectum of immuno-deficient mice by perineotomy and confirmed that iENPs engrafted and expressed neural markers several weeks after transplantation. These data suggest that iENPs could provide a therapeutic option for HSCR by restoring absent ENS.

Keywords: enteric nervous system, cell therapy, induced pluripotent stem cell

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THE EFFICIENT INDUCTION OF HUMAN RETINAL GANGLION-LIKE CELLS PROVIDES A PLATFORM FOR STUDYING OPTIC NEUROPATHIES

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Retinal ganglion cells (RGC) are the critical types of neurons for visual perception by connecting the eyes to the brain via their long axons. Optic neuropathies, caused by damage to RGCs and their axons, can result in irreversible vision loss. To study the mechanisms of neuropathy and develop treatment, methods for generating functional RGCs from human induced pluripotent stem cells (hiPSC) have been developed. However, most currently available protocols are based on embryoid bodies and thus are usually complicated, time-consuming and with low efficiency. Here we demonstrate a rapid and high-yield protocol for directly inducing RGC differentiation from hiPSC using a strategy of overexpressing 3 pro-RGC genes. These hiPSC-derived RGC-like cells (iRGC) showed robust expression of various RGC-specific markers, such as ATOH7, BRN3B, BRN3A, EBF1, and ISL1. Transcriptomic analysis also revealed the similarity between our iRGC and other publicly available RGC/retinal transcriptome data. A functional assessment demonstrated that these iRGC displayed both spontaneous and stimulus-induced neuronal activity. To address the capability to model optic neuropathies, we applied ethambutol (EMB), a first-line anti-tuberculosis drug but known to cause RGC degeneration and visual impairment, to the iRGC culture. In our iRGC model, EMB was found to induce significant dose-dependent and time-dependent increases in cell death and neurite degeneration. Western blot analysis revealed that the expression levels of p62 and LC3-II were upregulated, and a tag RFP-eGFP-LC3 tandem fluorescent probe revealed that EMB caused a blockade of lysosome-autophagosome fusion; this indicates that impairment of autophagic flux is one of the adverse effects of that EMB has on iRGCs. In addition, EMB was found to elevate intracellular reactive oxygen species (ROS) levels increasing apoptotic cell death and could be partially rescued by the co-treatment with the ROS scavenger N-acetyl cysteine. Taken together, our findings suggest that this iRGC model, which achieves both high yield and high purity, is suitable for investigating optic neuropathies, as well as being useful when searching for potential drugs for therapeutic treatment and/or disease prevention.

Keywords: retinal ganglion cells, direct conversion, ethambutol

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THE RNA BINDING PROTEIN STAU2 REGULATES STRESS RESPONSE IN C9ORF72 CEREBRAL CORTICAL NEURONS

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A hexanucleotide (GGGGCC)n repeat expansion in the noncoding region of C9ORF72 is the most common pathogenic mutation associated with both frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). In C9ORF72 FTD patient cerebral cortical tissue, toxic dipeptide repeat proteins (DPR), polyGR and polyPR, colocalized with the RNA-binding protein STAU2 in large granular structures. This indicates a specific role for STAU2 in C9ORF72 pathogenesis. STAU2 has roles in RNA localization, transport, stability, and translation. We used C9ORF72 ALS/FTD iPSCs to examine the potential role of STAU2 in ALS/FTD disease mechanism. C9ORF72 iPSC-derived spinal motor neurons show several disease-related characteristics, including impaired mitochondrial and proteasomal function, autophagy, and nucleocytoplasmic transport, increased ER stress, and susceptibility to glutamate toxicity. In spinal motor neurons from C9ORF72 ALS/FTD iPSCs, we found that STAU2 colocalizes with polyGR and polyPR aggregates in nuclear and cytoplasmic granules in patient but not WT lines. To model FTD and ALS, we generated cerebral cortical neurons from multiple WT and C9ORF72 ALS/FTD patient iPSCs. We observed reduced C9ORF72 expression in C9ORF72 ALS/FTD patient cortical neurons, and increased stress granule formation in response to oxidative stress mediated by sodium arsenite. In WT cortical neurons, shRNA-mediated knockdown of STAU2 led to increased stress granules in response to arsenite. In contrast, patient neurons deficient of STAU2 showed higher number of stress granules that was not increased by arsenite treatment. Hence, patient cortical neurons show increased stress granule formation in response to arsenite, but unlike WT neurons, this is dependent on STAU2. RNA-binding protein immunoprecipitation (RIP) followed by sequencing under normal and arsenite stress conditions revealed genes differentially bound by STAU2 in patient neurons vs WT, including those involved in vesicle transport, synaptic transmission, lipid metabolism, and programmed cell death. Proteomics analysis of WT and patient neurons with STAU2 knockdown further implicates STAU2 in several disease-related processes. Taken together, our data reveal key cellular processes and novel roles for STAU2 in ALS/FTD.

Funding Source: R35NS097277

Keywords: STAU2, RNA-binding proteins, C9orf72

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TOWARD A BETTER UNDERSTANDING OF ITM2B PATHOGENICITY IN A SPECIFIC RETINAL DYSTROPHY

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Our team identified a missense variant in ITM2B in the C-ter cleaved peptide BRI23, underlying a novel autosomal dominant retinal dystrophy with ganglion cell (RGC) loss, inner retinal and photoreceptor (PhRs) dysfunction. The function of ITM2B in the retina and its physiopathological mechanisms remain poorly understood. Our recent work suggested that ITM2B may interact with proteins, implicated in oxidative stress as well as in synaptic transmission in the human retina (Wohlschlegel et al., 2021). This project aims to model the disease using patient-derived induced pluripotent stem cells (IPSCs) and retinal organoids (ROs) in order to investigate ITM2B function and associated pathogenic mechanisms. Two induced IPSC derived from an affected subject (Mut) and his unaffected brother (NA) were established and differentiated into ROs. Immunostaining showed that ITM2B is mainly localized in PhRs and RGCs and that the synaptic layer is structurally modified in ROs. Preliminary data were acquired on these ROs to document the cellular phenotype, protein interactions and mitochondrial function. In order to generate a relevant isogenic control, CRISPR/Cas9 technology was applied to the mutant IPSC. The CRISPR/Cas9 edited cells (Cor) were characterized for their pluripotency and the absence of genetic alterations prior to being used for further analysis. This new model will be used for further functional assays to investigate ITM2B function in the retina and its pathogenicity. Phenotypic (imaging) and functional (electrophysiology) studies of NA, Mut and Cor ROs are currently underway. Furthermore, transcriptomic and proteomic analyses will be performed in the 3 RO types to identify differential profiles. BRI23-NA and BRI23-Mut protein interaction analysis in the human retina are also currently ongoing. Altogether, this data will help determine the function of ITM2B in the retina and elucidate its implication in pathology.

Keywords: Retina, iPSC, CRISPR/Cas9

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HUMAN INDUCED PLURIPOTENT STEM CELL MODEL TO STUDY INTERNEURON DEFECTS IN DRAVET SYNDROME

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Dravet Syndrome (DS) is a developmental and epileptic encephalopathy (DEE) characterized by pharmacoresistant seizures, intellectual disability, and increased risk of sudden unexpected death in epilepsy (SUDEP). Most cases of DS are caused by variants in the SCN1A gene that encodes for the voltage-gated sodium channel Nav1.1 alpha subunit. These SCN1A gene variants result in delayed neuronal development and altered neuronal excitability, leading to seizures and severe cognitive impairment. Our goal is to understand interneuron defects in DS to develop precision therapies. To this end, we generated CRISPR-edited SCN1A+/- and isogenic control SCN1A+/+ human induced pluripotent stem cell (iPSC) lines as a model system to study DS. To generate a homogeneous population of GABAergic interneurons from iPSC lines, we used an induced differentiation approach based on the expression of the doxycycline (dox)-inducible transcription factors ASCL1 and DLX2. Stable dox-inducible cell lines were generated using the PiggyBac transposon system. Immunofluorescence analysis indicated the generation of multiple GABAergic interneuron subtypes, including somatostatin, calbindin-, and nitric oxide synthase-positive interneurons. Calcium imaging analysis of cells from days 10 to 50 of culture revealed that SCN1A+/- interneurons showed decreased network activity compared to SCN1A+/+ control interneurons. Multielectrode array analysis performed during days 15-60 of culture indicated that SCN1A+/- interneurons exhibited lower mean firing rate and burst frequency than control interneurons. Consistently, whole-cell current-clamp recordings of action potentials (APs) during days 34-52 showed that SCN1A+/- interneurons displayed significantly higher sensitivity to depolarization-induced block and lower action potential firing frequency than control interneurons. Overall, the data suggest that multiple SCN1A+/- interneuron subtypes are hypoexcitable, which may contribute to impaired excitatory/inhibitory balance and network hyperexcitability in DS. Our work offers the potential to dissect neuron subtype-specific mechanisms for the future development of precision therapies to treat DS.

Funding Source: NIH/NINDS NS088571

Keywords: Interneuron, Dravet Syndrome, Epilepsy

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ESTABLISHMENT OF A DUAL REPORTER SYSTEM FOR SUBTYPE-SPECIFIC ANALYSIS OF GABAERGIC INTERNEURONS FROM HUMAN IPS CELLS BY CRISPR/CAS9 ENGINEERING

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Cortical interneurons (cINs) are classified into various subtypes, including Parvalbumin-positive (PV) and Somatostatin-positive (SST) neurons, based on wide differences in morphological and electrophysiological properties and neuronal circuit formation in the mammalian neocortex. While the defects of cINs in neuropsychiatry disorders are extensively studied using patient-derived iPSCs, the neuronal vulnerability can be different among the subtypes, indicating the necessity of precisely characterizing each subtype using human iPSCs. Particularly, PV and SST neurons are both derived from common progenitor cells in medial ganglionic eminence (MGE), but it is poorly understood how the distinct subtypes emerge and separate from the same origin. Therefore, our study is aimed to distinguish cIN-subtypes derived from human iPSCs and analyze them individually for developmental research. First, we established a hiPSC line harboring PV and SST dual reporters for subtype-specific visualization. The mNeonGreen and tdTomato fluorescent reporter genes were respectively inserted into the direct downstream of PV and SST coding regions by CRISPR/Cas9-mediated genome editing. Next, we developed a regional patterning method to generate cINs using the reporter hiPSC line by modulating neuronal identity-inducing signals. Immunocytochemistry revealed co-localization of fluorescent protein expression with subtype marker gene expression in induced neurons. Moreover, we detected SAG (sonic hedgehog agonist) addition and co-culture with feeder cells significantly elevated the proportion of SST neurons while previous methods could not induce enough subtype marker positive neurons to analyze individually. Finally, Fluorescence-activated cell sorting based on fluorescence expression was performed for subtype-specific transcriptome analysis. In summary, we established a dual reporter hiPSC line and generated cINs with evident fluorescence expression along with differentiation. We anticipate that the reporter hiPSC line would serve as a valuable in vitro model to recapitulate various molecular features of cINs in the developing human brain, and subsequently contribute to the development of in vitro differentiation techniques for applications in disease analyses.

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Keywords: GABAergic interneurons, iPS cells, CRISPR/Cas

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NOVEL STRATEGIES FOR STUDYING PROTEOSTASIS IN THE AGING BRAIN

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Aging is the largest risk factor for the majority of neurodegenerative disorders, including Alzheimer's disease (AD) and related dementias. Despite this however, there are currently no therapeutics capable of reversing the progression of the aging-related cognitive decline seen in disease and healthy aging. Through the use of heterochronic parabiosis, or the surgical joining of circulation between young and old mice, our group and others have demonstrated that blood-borne factors from young animals can reverse many deleterious phenotypes seen in the aged brain. Here, we utilized tandem mass tag (TMT)-based mass spectrometry (MS) to profile the cortex and hippocampus of the murine brain during aging and after parabiosis to understand both processes' effects on protein homeostasis (proteostasis), the regulation of protein folding and production. In doing so, we identified a subset of RIPA soluble and insoluble (i.e. urea soluble) putative protein aggregates whose levels increase during aging and decrease post-parabiosis. Interestingly, many of these proteins are pathologically linked to protein folding disorders in humans and contain structural motifs prone to destabilization. In one instance, Musashi1 (Msi1) decreased in the soluble and aggregate-containing protein fractions of the aged hippocampus post-parabiosis. Interestingly, Msi1 has been shown to physically interact with amyloid-ß protein linked to AD pathology and form oligomers in vitro. To determine the effects of modulating levels of Msi1 on cells in the brain, we synergized our MS data with our group's recently published scRNAseq atlas of the aging mouse brain to identify astrocytes as the major cell type expressing Msi1. We then differentiated isogenic human iPSCs -/+ AD-associated APP Swedish mutation (KM670/671NL) into astrocytes and subsequently overexpressed GFP labeled Msi1. In doing so, we found Msi1 aggregated upon proteosome inhibition via addition of MG132 and affected AD-associated transcriptional signatures including: RNA processing, ER stress, and oxidative stress. Together, these data highlight proteomic-level changes in the hippocampus post-parabiosis and establish a pipeline for assessing the effects of aging-associated, destabilized proteins in iPSC-derived cell types.

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Keywords: aging, proteomics, parabiosis

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INNER EAR ORGANOIDS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS ENRICHED WITH VESTIBULAR-LIKE HAIR CELLS AND OTOCONIA

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Human hearing and balance are two of the most poorly understood senses at a molecular level, largely because the inner ear cannot be biopsied without invasive and damaging skull surgery. Therefore, human pluripotent stem cell (hPSC) derived inner ear organoids are a needed tool for studying the development, maturation, and apoptosis of human inner ear cells in vitro. We have developed a protocol that uses the dynamic 3D Rotary Cell Culture System (RCCS) to generate inner ear organoids from hPSCs. These organoids recapitulate crucial phases of inner ear development, including the generation of an enriched population of sensory mechanoreceptor hair cells, the sound and balance receptors in the ear. The addition of Matrigel at 14 and 21 DIV was found to significantly increase the expression of hair cell progenitor transcription factors Sox2 and ATOH1 at 21 and 28 DIV respectively ($p < 0.01$). Organoid-derived hair cells predominantly display the morphological and physiological phenotypes of developing human foetal balance receptors (vestibular type II hair cells), as demonstrated by micro-computed tomography indicating the development of accessory structures such as otoconia. These data were further supported by helium ion microscopy and patch clamp electrophysiology. In conclusion, our 3D in vitro system provides a novel platform for modelling human inner ear development and investigating the effects of novel therapeutics for hair cell protection and regeneration.

Funding Source: The University of Melbourne, The Australian Research Council, The Garnett Passe and Rodney William Memorial Foundation, and The National Health and Medical Research Council of Australia.

Keywords: hearing, balance, organoid

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THE ROLE OF PERINEURONAL NETS IN AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that is currently incurable. Perineuronal nets (PNNs) are one type of central nervous system extracellular matrix (ECM). Loss of PNNs has been associated with worsening ALS disease progression. Polyethylene glycol – norbornene (PEG-NB) hydrogels have been previously used for disease modelling. Specifically, PEG-NB gels functionalised with matrix metalloproteinase cleavable peptide sequences have been used to assess hydrogel encapsulated cell deposited ECM. Here, we aim to investigate if a causal relationship between PNN loss and ALS exists. We used human induced pluripotent stem cell (iPSC) with ALS associated mutations in TARDBP and C9ORF72 genes and their isogenic controls to create 2D and 3D models of PNNs. To assess ability of human iPSC-derived MNs and mouse ESC-derived astrocytes 2D co-cultures to deposit PNNs at 3, 6 and 9 week of culture we utilised immunocytochemistry. Using aggrecan, a protein that is specific to PNNs in the CNS, hyaluronan and tenascin-R we show formation of PNN-like structures after 6 weeks in culture in all cell types. As ALS has an associated excitotoxicity phenotype, we have engineered our iPSC to express channelrhodopsin. We use optogenetics as a method to investigate the effects of electrical activity on PNN formation and degradation. We show that at early (2 week) timepoints, optogenetic entrainment of iPSC-derived MN co-cultures has a negligible effect on PNN formation relative to non-entrained controls across cell types. We also demonstrate that iPSC-derived MNs can be encapsulated in 3D PEG-NB hydrogels but are not compatible with PEG vinyl sulfone hydrogels. PEG-NB gels are supportive of iPSC-derived MN viability as indicated by neurite outgrowth. PEG-NB hydrogels are also susceptible to degradation by iPSC-derived MNs. In summation, we demonstrate that PNN-like structures can be manufactured in 2D co-cultures of iPSC-derived MNs and astrocytes. We also show that PEG-NB hydrogels are compatible with iPSC-derived MNs. By providing a 3D scaffold, we propose that the encapsulated cell deposited matrix will reveal any differences in PNNs between patient ALS lines and their isogenic controls. We believe that future experimentation will reveal these PEG-NB hydrogels to be utile models of ALS.

Funding Source: Wellcome Trust

Keywords: Amyotrophic lateral sclerosis, Hydrogels, Perineuronal nets

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USING THE IPSC PARADIGM AS A PERSONALIZED MEDICINE APPROACH TO LATE-ONSET ALZHEIMER DISEASE

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The pathology of late-onset Alzheimer's disease (LOAD) is still poorly understood, but it is multifactorial and closely related to changes with age. We developed a cellular platform for LOAD collecting skin fibroblasts or blood cells from LOAD patients and healthy control individuals that are used in the induced pluripotent stem cell (iPSC) paradigm to produce brain cells for determining LOAD pathogenic processes in context of age, disease, genetic background, cell development, and cell type. This model has provided evidence for an innate inefficient cellular energy management in LOAD that is associated with alterations of the cellular transcriptomes and lipid compositions, and interconnected cause-and-effect linkages, such as impaired insulin/IGF-1 signaling, bioenergetic substrate deficiencies, diminished glucose metabolism, disruption of the autophagic flux, and others. In addition, testing of metabolic compounds revealed some restoration

of the altered bioenergetic and metabolic processes in LOAD cells. Altogether, using the iPSC paradigm, we have identified an inherent LOAD-associated cellular metabolic phenotype as a potential risk factor to develop neurodegenerative disease with age. We propose that this cellular model allows for patient-oriented examination of numerous mechanisms and interactions in LOAD pathogenesis, as a basis for a personalized medicine approach to predict altered aging and risk for development of dementia, and to test or implement (customized) therapeutic or disease-preventive intervention strategies.

Funding Source: Program for Neuropsychiatric Research at McLean Hospital

Keywords: Alzheimer disease, induced pluripotent stem cells iPSC, metabolism and bioenergetics

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TRANSCRIPTIONAL SAFEGUARDING OF NEURONS BY REPRESSION OF UNWANTED CELL FATES PREVENTS BRAIN DISORDERS

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Reprogramming technologies have challenged the notion that differentiated cells are in an irreversible cellular state and have enabled us to study cell types that were previously inaccessible, such as human neurons. The transcription factor (TF) MYT1L has been found to be a strong reprogramming factor that enhances neuronal identity by repressing progenitor and non-neuronal programs in neurons, acting as neuronal safeguard. MYT1L mutations are strongly associated with several neurodevelopmental disorders (NDDs), including ASD, suggesting that deficient repression might contribute to mental disease. In this work, we used human embryonic stem cell-derived neurons and mice to study how MYT1L regulates brain development, whether failure to repress unwanted genes causes disease phenotypes, and to test potential therapeutic interventions. We found that MYT1L deficiency caused upregulation of non-neuronal programs and ASD-associated phenotypes, including neurodevelopmental delays and behavioural deficits. Unexpectedly, we also found striking neuronal network hyperactivity upon MYT1L depletion. Genetic and pharmacological intervention targeting upregulated non-neuronal factors, such as cardiac sodium channels, normalised electrophysiological abnormalities in vitro and behavioural deficits in vivo. This shows that failure to repress unwanted genes upon MYT1L depletion causes neuronal phenotypes, which can be treated even after neurodevelopment is complete. Overall, we present the first evidence that MYT1L mutations destabilise neuronal cell fate and function, and are sufficient to cause ASD-associated phenotypes in human and mouse models. Hence, failure to silence non-neuronal gene expression in neurons represents a novel mechanism that, at least in part, could contribute to NDD aetiology. Since MYT1L is specifically expressed in virtually all neurons throughout life it is tempting to speculate that active lifelong repression of non-neuronal programs is an evolutionarily-conserved safeguarding mechanism that is critical for the prevention of brain disorders. Furthermore, TFs with similar safeguarding functions might exist in other cell types, and this concept of silencing unwanted cell fate signatures might be a general mechanism often disrupted in disease.

Keywords: Neurodevelopmental disorders, induced neurons, transcriptional repression

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EFFECTS OF LITHIUM AND VALPROATE ON TRANSCRIPTOME AND CHROMATIN ACCESSIBILITY IN HUMAN IPSC-DERIVED CORTICAL NEURONS

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Lithium and valproic acid (VPA) are first-line treatments for bipolar disorder. Extensive research has been done on the molecular effects of lithium and VPA but studies on the molecular mechanisms of action that mediate mood regulation remain elusive. This study could help shed light on a link between upstream and downstream gene regulatory drug effects. Historically, pharmacological studies used animal models, established cell lines, or tissue from deceased subjects. Our study used renewable human iPSC-derived neuronal cells to determine the impact of mood stabilizers on gene regulation, by analyzing accessible chromatin regions and the transcriptome. iPSCs of six unaffected individuals were differentiated into neural progenitor cells (NPC) and then into cortical neurons. Neurons grown post differentiation up to 6 weeks were divided into three treatment groups: a) untreated, b) treated with 1mM LiCl for one week, and c) treated with 1 mM VPA for 72 hours. Accessible chromatin was analyzed using ATAC-seq and analysis is ongoing. Transcriptome was determined through RNA sequencing. Differential gene expression analysis of untreated neurons versus lithium treated neurons did not yield significant genes. Alternatively, we found ~300 significantly differentially expressed genes (DEGs) when we compared untreated neurons to VPA treated neurons (p adj< 0.05). Gene ontology pathway analysis of these genes show enrichment of cholesterol biosynthesis and lipid metabolism pathways (p adj< 0.05). VPA treatment revealed some upregulated genes such as NRGN, a schizophrenia risk gene involved in synaptic development and remodeling; CLDN10, involved in cellular adhesion and ion transport; GNL3, a bipolar disorder GWAS hit involved in neural stem cell proliferation; and HDAC1, involved in chromatin remodeling, consistent with the known inhibition of HDAC1 by VPA. Profiling accessible chromatin could reveal regions that are primed for transcription but not yet reflected in downstream DEGs. This study illustrates

the potential for iPSC-derived neurons to investigate drug mechanisms and potentially develop novel therapeutics.

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Keywords: RNAseq, ATACseq, mood stabilizers

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THE ROLE OF PIEZO1 IN HUMAN BRAIN ORGANOID DEVELOPMENT

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As the brain develops, there are a series of dynamic events which produce local mechanical forces which can influence cell behavior and development. However, it is still relatively unknown how cells are able to detect and transduce these mechanical cues during neural development. The PIEZO1 mechanically-gated ion channel is an attractive candidate as it opens in response to a wide range of mechanical cues and is responsible for widespread physiological functions. Our lab has determined that PIEZO1 activity can regulate the fate of human neural stem progenitor cells and revealed neurodevelopmental abnormalities in Piezo1 knockout mice. However, the role of PIEZO1 in human neural development remains unknown. To fill this gap, we are using 3D brain organoids generated from induced pluripotent stem cells (iPSCs) which can model many aspects of early human neural development. To investigate how PIEZO1 affects human neural development, we generated brain organoids from WT and Piezo1 KO iPSCs. Consistently, we observed altered morphology in the Piezo1 KO organoids. Brain organoids display rosettes – lumens surrounded by neural progenitor cells – that are reminiscent of brain ventricles. Notably, we observe increased formation of rosette structures in Piezo1 KO brain organoids. To determine transcriptomic changes underlying this altered morphology, we performed bulk RNA sequencing on WT and Piezo1 KO organoids. Pathway analysis revealed an upregulation of neural development pathways and an enrichment of developmental signaling pathways in the Piezo1 KO samples, leading to our hypothesis that dysregulated signaling in Piezo1 KO may contribute to the altered organoid morphology and development. Ongoing experiments are further probing the Piezo1 KO phenotype in brain organoids and establishing which signaling pathways are implicated to cause morphological differences. In future, we aim to connect PIEZO1 channel activity to signaling regulation. Our work shows that mechanotransduction through PIEZO1 can influence human neural development and may reveal new connections between cellular mechanics and developmental signaling pathways.

Funding Source: NIH DP2 AT010376

Keywords: Mechanotransduction, Neural Development, Brain Organoid

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EXPLORING DIFFERENT STEM CELL MODELING PLATFORMS TO UNCOVER PHYSIOLOGICALLY RELEVANT ALS DISEASE PHENOTYPES

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Amyotrophic Lateral Sclerosis (ALS) is a heterogeneous neurodegenerative disorder characterised by the loss of upper and lower motor neurons in the central nervous system. The origin of motor neuron degeneration is unknown and may involve various pathogenic pathways. Patient-derived stem cells have become increasingly recognised as tools for the investigation of ALS. This project aims to model the pathogenic hallmarks of familial forms of ALS across various iPSC modelling platforms to identify and compare morphological and molecular phenotypes of ALS pathology in different stem cell settings. This study has characterised phenotypes of motor neurons generated from TDP and C9orf72 patient iPSC lines in 2D adherent cultures, organoids, and in the living central nervous system via surgical transplantation. Early results support our hypothesis that the physiological relevance of the neuronal environment correlates with the health of the motor neurons and the capacity to study disease progression over extended time frames. Neurons in 2D cultures displayed the highest degree of cellular stress, which was exacerbated in the disease lines when compared to controls. Organoids and transplanted neurons were healthier and could be investigated long-term, with transplanted neurons displaying complex patterns of anatomical integration in the host spinal cord. We hypothesise that physiologically relevant environments that broadly favour neuronal health may be imperative in revealing specific disease pathways linked to disease-associated mutations. This may ultimately assist in developing patient-specific therapies, including high-throughput drug discovery targeted at specific, and potentially subtle, early disease profiles.

Keywords: Amyotrophic Lateral Sclerosis (ALS), Organoid, Transplantation

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NMDA RECEPTOR ANTAGONISM MITIGATES GLIOBLASTOMA CELL INVASION AND GROWTH IN CORTICAL ORGANOIDS

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Glioblastoma (glioma grade IV) is the most frequent and the deadliest primary brain cancer. Glutamate, a neurotransmitter underlying normal neurological function, is also a factor that regulates proliferation and survival of glioblastoma cells. NMDA receptors are a class of glutamate receptors present at the membrane of different types of neural cells as well as some glioblastoma cells. How signaling through NMDA receptors regulates glioblastoma cell behaviour in the nervous system however remains unclear. We sought to validate whether inhibiting NMDA receptor signaling can prevent glioblastoma invasion of neural tissue. To test the effects of NMDA receptor antagonists on glioblastoma cell status and growth in a neural microenvironment, we used human cortical organoids, 3D cellular models that recapitulate important features of the cerebral cortex. Following a previously established protocol for directed differentiation of human embryonic stem cells, we generated cortical organoids that were subsequently cultured in the presence of human glioblastoma cells. Here, we report that NMDA receptor blockade is associated with decreased glioblastoma cell abundance in cortical organoids. We find, furthermore, that this decrease correlates with downregulation of SOX2, a pioneer transcription factor critical for cellular pluripotency widely used as a marker of stem cells. These results indicate that inhibition of NMDA receptor signaling impedes glioblastoma cell invasion or growth in the neural microenvironment and suggest that it may decrease the amount of glioblastoma stem cells, cells considered to play an important role in treatment resistance and recurrence. Our findings support the therapeutic potential of NMDA receptor antagonism as an adjuvant strategy to mitigate glioblastoma recurrence.

Keywords: Glioblastoma, Cortical organoids, NMDA receptor

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GENERATION AND CHARACTERISATION OF CRYOPRESERVED HUMAN IPSC-DERIVED MICROGLIA

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Microglia play a key role in neurodegeneration and inflammation in the brain, thus studying these cells is essential for the development of novel therapies. Current animal and primary cell models are not physiologically relevant or provide a finite amount of material, making it difficult to reliably produce a model that recapitulates the disease in a human background. Therefore, induced pluripotent stem cells (iPSC), alongside robust differentiation methodologies, can provide a human model with the large-scale production required for drug discovery. This study aimed to produce iPSC-derived microglia using an established protocol that could be cryopreserved at scale. After a short maturation, iPSC-derived microglia cells were quality control tested and functionally characterised. Across multiple batches, the cryopreserved cells showed expression of common microglial markers, IBA1, P2RY12 and TMEM119 through immunocytochemistry. Consistent and repeatable functional activity of the microglia was observed post thaw. A key functional role of microglia is the phagocytosis of toxic species, here we demonstrated the uptake of pHrodo-labelled bait by different batches of iPSC-derived microglia using an IncuCyte S3. In addition to phagocytosis, chemotaxis towards inflammatory signals was observed, with the iPSC-derived microglia demonstrating cell movement towards C5A and ADP on an IncuCyte S3. Transcriptomics using TempoSeq demonstrated the presence of microglia markers in matured thawed cells. Cryopreserved cells demonstrated an IL-6 inflammatory profile in response to lipopolysaccharide stimulation. Here we show the initial characterization of cryopreserved iPSC-derived microglial cells and demonstrated their use for studying functional activity. These iPSC-derived microglial cells could be an invaluable tool for modelling neurological disease in drug discovery at scale.

Keywords: Differentiation, Neurodegeneration, Microglia

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DISSECTING MUTANT P53-DRIVEN ASTROCYTOMA WITH A LI-FRAUMENI SYNDROME IPSC DISEASE MODEL

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N6-methyladenosine (m6A), one of the most prevalent mRNA modifications in eukaryotes, plays a critical role in modulating both biological and pathological processes. Dysregulation of m6A modification and m6A-associated regulators plays a critical role in tumor initiation and progression. However, it is unknown whether mutant p53 neomorphic oncogenic functions exploit the dysregulation of m6A epitranscriptomic networks. Patient-derived iPSCs present a powerful cancer platform to assess the cellular signaling, transcriptional, and chromatin landscapes resulting from well-defined genetic alterations and provide potential therapeutic insights into the early events of tumor initiation. Li-Fraumeni syndrome (LFS) is an autosomal dominant familial cancer syndrome caused by germline TP53 mutations and characterized by multiple primary neoplasms with early onset. Here, we investigated LFS-driven neoplastic transformation by mutant p53 in iPSC-derived astrocytes, the cell-of-origin of gliomas. We found that mutant p53 but not wild-type p53 physically interacts

with SVIL to recruit the H3K4me3 methyltransferase MLL1 to activate the expression of m6A reader YTHDF2, culminating in an oncogenic phenotype. Aberrant YTHDF2 upregulation markedly hampers expression of multiple m6A-marked tumor-suppressing transcripts, downregulates CDKN2B and SPOCK2 expression, and induces oncogenic reprogramming. Mutant p53 neoplastic behaviors are significantly impaired by genetic depletion of YTHDF2 or by pharmacological inhibition using MLL1 complex inhibitors. Our study reveals how mutant p53 hijacks epigenetic and epitranscriptomic machinery to initiate gliomagenesis and suggests potential treatment strategies for LFS gliomas.

Funding Source: A.X. was a CPRIT Postdoctoral Fellow in the Biomedical Informatics, Genomics and Translational Cancer Research Training Program (BIG-TCR, CPRIT grant RP210045).

Keywords: Li-Fraumeni syndrome, mutant p53, YTHDF2

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BRAIN AVATARS: MEASURING PATIENT-SPECIFIC NEUROINFLAMMATORY AND ELECTROPHYSIOLOGICAL PHENOTYPES IN IPSC-DERIVED NEURAL ORGANOIDS TO TAILOR THERAPEUTIC APPROACHES IN DRUG-RESISTANT EPILEPSY

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Approximately 30% of patients with epilepsy have drug-resistant seizures, defined as the failure to control seizures using two or more well-tolerated and appropriately chosen anti-seizure drugs. These drugs target electrophysiological processes and therefore the failure to respond suggests parallel or alternative mechanisms driving seizures in patients with drug-resistant epilepsy (DRE). Of particular concern are children with DRE, both in the form of explosive new onset refractory status epilepticus and in the manifold forms of DRE such as Lennox-Gastaut syndrome or Landau-Kleffner syndrome. DRE in children is associated with profound morbidity, increased mortality, and lifelong impairment. The ability to stop seizures in these patients is a critical unmet human health need. Based on animal model and patient findings, the concept of inflammation – both peripheral and in the central nervous system – as a key pathogenic driver in DRE has emerged. Based on our experience, we work from the overarching hypothesis that dysregulated innate immune responses induce, propagate, and/or amplify seizures within the context of an altered neural network threshold to ictogenesis. To test this hypothesis and to identify novel approaches to modulating neuroinflammation to attenuate seizures, we have developed a platform for measuring neural network properties in patient-derived induced pluripotent stem cell-derived neural organoids that contain neurons, astrocytes, oligodendrocytes, and microglia. Neural stem cell-derived and hematopoietic stem cell-derived cells are mixed and cultured to promote dense networking between neural clusters. These networks are grown on multielectrode arrays for direct electrophysiological profiling and electrical stimulation. Networks are also transduced with reporters like AAV.CaMKII.GCaMPf for calcium flux assessment. Patient brain avatars are stimulated with inflammatory drivers and changes in network bursting and spiking are measured to characterize the impact of neuroinflammation on “seizure threshold in a dish”. Our goal is to establish a robust platform for identifying patient-specific neuroinflammatory and electrophysiological response profiles and for screening therapeutic efficacy that will ultimately resolve drug-resistant seizures in these patients.

Keywords: Drug-Resistant Epilepsy, iPSC-Derived Neural Organoids, Therapeutics

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CHARACTERIZATION OF ROBUST AND CONSISTENT HUMAN IPSC-DERIVED INDUCED EXCITATORY NEURONS FOR DISEASE MODELING AND DRUG DISCOVERY

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Human induced pluripotent stem cell (iPSC)-derived neurons are integral for elucidating mechanisms and therapeutic targets underlying neurodevelopmental and neurodegenerative disease. Directed differentiation protocols have largely been employed for generating iPSC-derived neural models. While directed differentiated cells are highly predictive for drug screening, this differentiation method is challenged to meet the economies of scale required for high-throughput compound testing within discovery research and preclinical drug development pipelines. Induction of neural differentiation using NGN2 forward reprogramming of iPSCs offers a robust method for generating scalable quantities of excitatory neurons with low lot-to-lot variability. In this study we utilize NGN2 overexpression, under a doxycycline (DOX) promoter, to generate highly pure excitatory glutamatergic neurons from iPSC lines with an apparently healthy normal (AHN) background or a heterozygous pathogenic R493X nonsense mutation in the progranulin gene (GRN) to model frontotemporal dementia (FTD). We demonstrate that induced excitatory AHN and GRN (493X) neurons display gene expression profiles similar to neurons from published NGN2 protocols. We further show the presence of highly pure neuronal populations (> 90% Tuj-1+) that can be recovered post cryopreservation to form consistent neural cultures without continued use of DOX. We next show induced neuron monocultures are suitable for neurite outgrowth, survival, and toxicity assays. When co-cultured with isogenic iPSC-derived astrocytes, both AHN and GRN (493X) induced excitatory neurons produced robust neural networks on multielectrode array platforms. Induced excitatory neurons were co-cultured with iPSC-derived

astrocytes in ultra-low adhesion 96-well round-bottom plates to form neurospheres. AHN and GRN (493X)-containing neurospheres developed dynamic calcium oscillations, whose patterns were altered by addition of known neuromodulatory compounds (DNQX, AP5). These data show a robust and scalable method using NGN2 forward reprogramming to consistently produce fully differentiated induced excitatory neurons. These cells function across a variety of applications that support high-throughput preclinical drug discovery and compound screening.

Keywords: NGN2 Induced Neurons, Progranulin, Frontal Temporal Dementia (FTD)

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IDENTIFYING THE UNDERLYING MECHANISMS OF PPP2R5D-RELATED NEURODEVELOPMENTAL DISORDER IN PATIENT DERIVED NEURONS

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Neurodevelopmental disorders (NDDs) are common and affect more than 3% of children worldwide yet remain a notoriously difficult therapeutic area despite the identification of mutations underlying NDDs. A significant barrier to treating NDDs has been understanding the etiology of pathological mechanisms leading to disease. Mutations in PPP2R5D cause the rare neurodevelopmental disorder Jordan's Syndrome, which is associated with megalencephaly, developmental delay, intellectual disability, seizures, autism spectrum disorder, and early onset Parkinson's disease. PPP2R5D encodes a subunit of the major PP2A phosphatase which regulates substrate target specificity for Ser/Thr dephosphorylation. To determine the underlying mechanisms contributing to Jordan's Syndrome, we have derived induced pluripotent stem cells (iPSCs) from several patients carrying unique PPP2R5D mutations and differentiated patient derived iPSCs along with CRISPR-corrected isogenic controls into neural progenitors and neurons. We found that neural progenitors harboring PPP2R5D pathogenic variants are over proliferative compared to isogenic controls. Additionally, neurite outgrowth studies revealed that neuronal development is altered, and neurite length is significantly increased in patient derived neurons. Our findings suggests that PPP2R5D pathogenic mutations lead to increased neural progenitor proliferation and accelerated neurite extension, which is consistent with brain overgrowth and megalencephaly observed in patients. We hypothesize that PPP2R5D pathogenic variants in Jordan's Syndrome patients disrupt the specificity of the PPP2R5D/PP2A phosphatase by mis-regulating critical signaling pathways important for neuronal development, contributing to brain overgrowth and neurodevelopmental disorder observed in patients. To determine whether knockdown of pathogenic variants will abrogate dysfunctional phosphatase activity, we aim to develop preclinical therapeutic strategies for PPP2R5D neurodevelopmental disorder to rescue phenotypes in patient derived neurons. Uncovering the molecular mechanisms underlying pathogenic PPP2R5D variants will be a significant step toward treating these patients and will provide an framework for the treatment of other neurogenetic disorders.

Funding Source: This work was supported by the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant Number TL1TR001875.

Keywords: Neurodevelopmental disorder, Patient derived stem cells, Personalized medicine

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TOWARD UNDERSTANDING THE MOLECULAR BASIS OF RNA EXOSOME-LINKED PONTOCEREBELLAR HYPOPLASIA TYPE 1B: A CEREBELLAR ORGANOID MODEL

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Pontocerebellar Hypoplasia Type 1b (PCH1b) is a devastating autosomal recessive neurodevelopmental disorder characterized by significant atrophy of the cerebellum and pons. The cerebellum and pons integrate information from sensory systems, the spinal cord, and other parts of the brain to regulate motor movements, breathing, and learning motor behavior. Currently, there is no cure for PCH1b and treatment in purely palliative. Mutations that cause PCH1b occur in the EXOSC3 gene, encoding a structural cap subunit of an evolutionarily conserved RNA processing complex, the RNA exosome. The RNA exosome complex is a ubiquitously expressed ribonuclease composed of structural and catalytic subunits that play a critical role in the post-transcriptional regulation of RNA. The RNA exosome is required for 3'-5' processing and degradation of a variety of cellular RNAs. Cerebellar pathology in RNA exosome-linked PCH1b is challenging to understand based on current models of PCH1b. The goal of this study is to begin to define requirements for RNA exosome subunit EXOSC3 during cerebellar development through the study of EXOSC3-linked PCH1b mutations. To determine requirements for the RNA exosome during cerebellar development, we are developing a 3D human induced pluripotent stem cell (hiPSC)-derived cerebellar organoid platform to recapitulate neuronal dysfunction and degeneration known to characterize PCH1b. We have engineered an allelic series of missense mutations via CRISPR/Cas9-editing technology encoding PCH1b-linked amino acid changes (EXOSC3-G31A and -G191C) in hiPSCs to generate a cerebellar organoid model of PCH1b. We will combine single-cell transcriptomics and novel molecular tools to investigate the transcriptomic dynamics of generated cells during cerebellar differentiation as well as define key post-transcriptional regulatory events mediated by the RNA exosome. Results from this work will provide a framework to study how the RNA exosome functions in early cerebellar development and has the potential to transform our understanding of PCH1b pathogenesis.

Keywords: Cerebellar Organoid, RNA Exosome, Neurodevelopmental Disease

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HUMAN CORTICAL BRAIN ORGANOIDS TO STUDY ADAPTIVE CHANGES IN ALCOHOL ADDICTION

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Alcohol use disorder (AUD) is a global problem causing 2.5 million deaths per year and accounts to the world's third largest risk factor for premature mortality and disability. Although poses major challenges to public health care systems, the medical needs of the patients are largely unmet and underlying neurobiological causes only poorly understood. Most of our understanding of the molecular changes in the brain evoked by alcohol addiction is based on post mortem human tissue or animal models. Induced pluripotent stem (iPS) cell-derived brain organoids represent an attractive and innovative tool to decipher adaptive changes during disease onset caused by genetic or environmental challenges (including noxious substances) in a human setting and an unbiased forward approach. We set out to analyze how acute and chronic alcohol exposure changes transcriptional and epigenetic programs in the human cortex. We generated forebrain-type organoids applying iPS cells from healthy controls and alcohol addicts. We developed protocols allowing us to generate organoid slices cultured for >100 days, which develop a cell composition and histoarchitecture mimicking late stages of corticogenesis of the human brain. We performed a combinatorial single nuclei sequencing approach for the simultaneous detection of the cells' transcriptome and chromatin accessibility. Acutely and chronically treated organoids showed an increased expression of inflammatory, metabolic and ROS genes as well as alcohol-induced immune system activation biomarkers as SIM2. Differential expression of epigenetic modifiers have been observed in the treated conditions, whose among the targets are genes implicated in neuronal migration, synapses formation, neuroglia interactions and genes involved in AUD predisposition and behaviors regulation, such as CNR1, PSD3 and PDE4B. Particularly the acute condition shows an upregulated expression of acetyltransferases and demethylases and an increased accessibility of the chromatin compared to the chronic condition, suggesting that epigenetic adaptations in forebrain-type organoids could be induced by long-term EtOH treatment. We expect that this project will help to define critical contributors in the pathogenesis of alcohol addiction, eventually leading to new therapeutic paradigms.

Keywords: Cerebral Organoids, Alcohol Addiction, Multiome Sequencing

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INVESTIGATING THE SUBTYPE-SPECIFIC PATHOGENIC MECHANISMS OF PEDIATRIC BRAIN TUMOR USING A HUMAN EMBRYONIC STEM CELL MODEL

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Brain tumor is the leading cause of cancer-related death in children. Recent studies have identified multiple new subtypes of pediatric brain tumors defined by specific driver mutations in transcription and epigenetic factors, including histone H3, BCOR, and FOXR2. FOXR2-activated CNS neuroblastoma is a newly recognized brain tumor subtype that harbors genomic rearrangements at the FOXR2 locus and is characterized by elevated FOXR2 expression. However, the precise mechanisms underlying the subtype-specific tumorigenesis, including the cell type of origin, are not fully understood. Here, we developed a human embryonic stem cell (hESC) model to identify the cell type of origin and elucidate the pathogenic mechanisms. The expression profile analysis of the patient tumors showed an elevated expression of interneuronal marker genes, such as DLX1, NKX2.1, and SST (Somatostatin). Given that NKX2.1 and SST are the markers for interneuronal cells derived from the Medial Ganglionic Eminence (MGE) of the ventral forebrain, we hypothesized that MGE-derived interneuronal progenitor cells are the cell type of origin. To test this hypothesis, we differentiated hESC to the MGE-type interneuronal progenitor cells by a chemically defined protocol as well as cortical excitatory neural progenitor cells as a control cell type and then over-expressed FOXR2. In a low-density culture, FOXR2 overexpression in the MGE-type interneuronal progenitor cells resulted in a significant increase in the total cell number (3.75-fold; $p = 0.022$), while not in the cortical progenitor cells, indicating a cell-type-specific effect of FOXR2. Our new model has a promising potential to elucidate the molecular mechanisms underlying the formation of FOXR2-activated CNS neuroblastoma and pave the way for the discovery of therapeutic targets and effective treatments for patients suffering from this devastating disease.

Keywords: Brain tumor, Stem cell model, FOXR2

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HUMAN VENTRAL TELENCEPHALIC ORGANOIDS REPRODUCE MOLECULAR PATHWAYS DRIVING GABAERGIC NEURONAL DIVERSIFICATION

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GABAergic transmission finely tunes the signal flow by inhibiting action potentials in the receiving neuronal network. The modulation exerted by this class of neurons is essential for homeostasis and the precise processing of information in the nervous system. Growing evidence associates alterations in GABA signaling with complex brain conditions such as schizophrenia and autism spectrum disorder (ASD). In this study, we developed an organoid model of the embryonic ventral telencephalon that recapitulates GABAergic neuron development. Using single-cell RNA sequencing, we profiled over 215,000 cells from three different timepoints of organoid differentiation and demonstrate the presence of lineages corresponding to fetal LGE, CGE and MGE progenies. Through the reconstruction of developmental trajectories, we identified molecular drivers that define divergent pathways during cell fate commitment across multiple lineages, such as macroglia, and cortical and basal ganglia GABAergic neurons. Finally, we applied this model to understand the impact of haploinsufficiency of the ASD risk gene CHD8 to cellular commitment and cell states of the ventral telencephalon. In the mutant organoids, we observed a shift from CGE lineages towards MGE derivatives. We anticipate that this organoid system will aid in the investigation of human GABAergic neuron development and enable understanding of mechanisms underpinning neurodevelopmental disorders.

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Keywords: interneuron, subpallium organoid, ASD

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ACCURATE DRUG SCREENING BY DESIGN OF ANTI-FOULING CHANNEL WALL PDMS-BASED MICROFLUIDIC PLATFORM

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After the concept of the 3D cell culturing technique was introduced, various attempts have been made for accurate drug screening. Among the materials used for microfluidic devices in the laboratory, PDMS has been the most popular because of advantages such as flexibility, biocompatibility, and gas permeability. However, the permeability of PDMS promotes the non-specific adsorption of small hydrophobic molecules which is a major issue in drug screening. Thus, we developed Microfluidics Fabricated with APTES-perFluoro Infused Application (MAFIA) device. This device consists of two big parts. First, ECM coated membrane for cell culture. Second, perfluoropolymer for lubricant swell. This hydrophobic lubricant barrier possesses intrinsic advantages in resistance to absorption of organic solvent adsorption and also fully repels the adsorption of small molecules, including drugs. In this study, we evaluated cell culture in the MAFIA device with caco-2 and human iPSC-derived BBB cells and observed organ-like structures. A proper gas supply is crucial for cell survival. Gas permeability is one of the important characteristics of PDMS-based microfluidics. MAFIA device O2 and CO2 permeability dropped to almost 65% compared to the control group. We are concerned about inducing hypoxia through decreased gas permeability by PDMS. However, decreased gas permeability caused no adverse effect on cell culture which was evaluated by Real-time RT-PCR and fluorescence staining imaging. Quantitative PCR(qPCR) to evaluate BBB cell gene expression level changes such as CDH5, CLD5, OCLN, PECAM1, SLC1A1, SLC38A5, TJP1, ABCG2, ISNR, LRP1, MRP1, MRP1, SLC7A5, TFRC were no significant difference between the conventional microfluidic device and the MAFIA device. Drug compound quantification without cells was performed via HPLC. Compared to the MAFIA device group, the conventional microfluidic group drops the amount of drug by about 27% in various flow rate conditions. We envision that MAFIA device provides an environment that blocks drug loss to the PDMS and cell culture at the same time which leads to accurate drug screening.

Funding Source: This research was supported by Nano·Material Technology Development Program through the National Research Foundation of Korea (NRF) funded by Ministry of Science and ICT (2022R1A2C4001652)

Keywords: Organ on a chip, Accurate drug screening, human iPSC-derived BBB cell

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OVERCOMING PLURIPOTENT STEM CELLS VARIABILITY IN 3D NEURAL DIFFERENTIATION

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Stem cell derived models present unprecedented opportunity to study human development, especially with recently developed organoid methods. The variability between pluripotent stem cell variability constitutes a major hurdle in reproducible generation of good quality tissues but is also a learning opportunity to discovered how fate decisions are taken and adjusted in the embryo. Here we explore the source of differences in cell lines undergoing unguided neural differentiation and ways to mitigate them.We selected a panel of human PSC lines that either produce good quality cerebral organoids or differentiate spontaneously towards different tissue types. These cell lines were then characterised by proteomics, RNAseq and ATACseq to identify major pathways determining their likeliness of success in brain organoid generation. We identified a number of changes in metabolism, chromatin accessibility and activation of pathways involved in cell adhesion and spreading that lead to incompatibility with brain differentiation trajectory. Bad differentiators had higher levels of Wnt signalling, which led to acquisition of more neural crest/mesoderm phenotypes. In some cells lines these changes could be mitigated by culture on optimised coating matrix, inhibitors of cell adhesion kinases (SRC) or with canonical and non-canonical Wnt signalling inhibition. Other lines required more complex treatment, similar to chemical reprogramming of somatic cells to induced pluripotent stem cells. These reprogrammed bad differentiators could reproducibly produce good brain organoids if maintained with Wnt inhibition. In conclusion we postulate that due to intrinsic variability, the PSCs acquire a range of developmental states under identical culture conditions, some of which are incompatible with acquisition of brain fate. Wnt inhibition therefore maintains the most pluripotent state of primed stem cells. In a broader sense this work sheds light on how Wnt signalling determines the fate of the epiblast to become brain or body.

Keywords: stem cell differences, neural differentiation competency, differentiation bias

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ORGANOID COMPLEMENTATION PROVIDES INSIGHT INTO THE DEVELOPMENT OF FUCHS CORNEAL DYSTROPHY

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Modeling the human system to better understand disease progression and treatment utilizing animals is standard. In addition to the multitude of ethical complications that come with animal models, there are vast differences between animal and human systems. With the continuing progression of organoid technology, the possibility of forgoing animal disease modeling is becoming more feasible. The goal of this work is to investigate the potential for organoid complementation to study genetic diseases. Organoid complementation entails first creating a diseased organoid via deletion of a particular gene from an iPSC line resulting in development of a diseased phenotype niche. These diseased cells are then cocultured with cells from non diseased iPSC cells that complement the niche and reverse the diseased phenotype resulting in a healthy organoid. In this work we have chosen to study Fuch's Corneal Endothelial Dystrophy (FCED), a genetic disease that has clinical relevance, limited research, and no reasonably effective treatment. In order to gain more insight about the genes and cell types that are involved in the development of the disease, I have complemented a corneal dystrophy cell line, created previously by our lab, along with a GFP expressing non-diseased iPSC line. Upon development and demonstration of a non-diseased phenotype, the organoid is RNAsequenced to compare the gene expression of the healthy complemented cells and analyzed for differential gene expression. We are using this data to test the hypothesis that the lack of expression of the knocked out genes in the disease cell type will cause a compensatory differential expression of certain genes in the healthy GFP labeled cells. This analysis may provide insight into previously overlooked gene's involvement into the development of the disease, with the eventual goal of applying these findings to the clinic.

Keywords: Complementation, Organoid, Genetic Disease

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MODELING THE SPECTRAL DIFFERENCES IN RETT SYNDROME USING PATIENT-DERIVED BRAIN ORGANOIDS

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Many neurodevelopmental disorders are linked to neural network hyperexcitability related to imbalances in the establishment of excitatory vs inhibitory (E/I) neural connections. However, the mechanisms that regulate this process during normal development are not well understood. Rett syndrome (RTT) is a neurodevelopmental disorder primarily caused by a mutation in the X-chromosome linked MECP2 gene. It is recognized that the loss of E/I balance is a potential cause of many RTT neurological symptoms. Depending on the type of mutation RTT patients have, the disorder can present with varying degrees of severity. In our previous studies, we found that it was possible to model several aspects of neural network dysfunction associated with RTT using patient-derived brain organoids. However, it is not known whether this system can accurately report differences in network activities that mirror the spectrum of clinical severity seen in RTT patients. Here, we will present findings comparing the cytoarchitecture and electrophysiological profile of brain organoids generated from patients harboring mutations associated with mild and severe forms of RTT. Through these studies we seek to gain insights into how different MECP2 mutations impact human brain development, and more generally test the capacity of organoids to faithfully recapitulate salient features of neurodevelopmental disorders. De

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Keywords: Neurodevelopmental disease, Brain organoids, MECP2

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CORTICAL AND SPINAL MOTOR NEURONS DIFFER IN THEIR RESPONSE TO AUTOPHAGY INHIBITION

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Autophagy is one of the two major protein degradation systems in the cell and basal autophagy is considered being important for protein homeostasis (proteostasis). Here we set out to probe the hypothesis that inhibition of autophagy in neurons results in different - possibly neurodegenerative - phenotypes depending on the specific neuronal subtype. In detail, excitatory cortical (CTX) and spinal motor neurons (SMNs) were investigated via morphology, proteomics and transcriptomics upon autophagy inhibition in a system where autophagy is completely under external control through doxycycline. Strikingly and in line with the initial hypothesis, CTX and SMNs differed in their response to autophagy inhibition. SMNs acquired considerable degeneration phenotypes while CTX show a more subtle neurite retraction phenotype, and this difference in phenotypes was reflected in the proteomics and transcription data, where only SMNs showed a strong enrichment in OFF vs ON condition for proteins associated with neurodegeneration (Alzheimer's, Parkinson's disease, and ALS) and activation of cell death pathways (senescence, p53). The proteomics data further indicated that CTX mainly accumulate ER-related proteins upon autophagy inhibition and transcriptomics data pointed into the direction of induced ER stress response.To our surprise, neuronal differentiation was very sensitive to autophagy levels, questioning the view on autophagy as a mere bystander and homeostatic actor in cellular physiology and neural development. In addition, the covalently bound ATG5-ATG12 complex proved to have a slow degradation rate in neurons without complete exhaustion even after months of doxycycline withdrawal. Therefore, we generated a variety of different degron systems for the inhibition of autophagy to circumvent this limitation and enable intrinsic physiological autophagy levels during neuronal development as well as remove complete residual autophagy activity. Furthermore, the newly generated systems will be utilized to generate additional data for CTX and SMNs to verify the already acquired findings and could transferred more easily to additional areas of research, presenting a valuable technical and biological resource for the field.

Funding Source: Research Fellowship from the German Research Foundation (DFG) (PNo 401380638); NIH Exploratory/ Developmental Research Grant Award from the National Institute Of Neurological Disorders And Stroke of the NIH (R21NS116545)

Keywords: Autophagy, Neurodegeneration, Neuronal subtype specificity

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CHARACTERIZATION OF THE FUNCTIONAL AND MORPHOLOGICAL DISEASE PHENOTYPES OF THE KCNQ2 VARIANT HUMAN IPSC DERIVED GLUTAMATERGIC NEURONS

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KCNQ2 variant causes neonatal seizures and epileptic encephalopathy that can lead into developmental and cognitive disabilities. The aim of this project was to generate human-specific induced pluripotent stem cells (iPSCs) from the study participants with KCNQ2 variants and differentiate these cells into glutamatergic neurons to study neurodevelopmental disease phenotypes in vitro. To generate isogenic control lines from these iPSCs, we used CRISPR-cas9 method to correct the KCNQ2 variants in the cells and then compared them to the cells with KCNQ2 variants to establish a phenotypic battery which included transcriptional analysis, morphological measurements, and functional neural network analysis. The three KCNQ2 variants that we examined in this project were: 1) KCNQ2 c.875_877delTCCinCCT, p.L292_L293delinsPF; 2) KCNQ2 c.821C>T, p.T274M; 3) KCNQ2 c.766G>Y, p.G256W. In addition, we aimed to establish between two different laboratories repeatable characterization methods and quality control experiments for the neuronal differentiation experiments. Our preliminary data revealed longer neurite outgrowth and increased weighted mean firing rate and burst duration in two KCNQ2 variant neuron lines compared to their isogenic control neuron lines. Next, we will characterize the transcriptional gene expression profiles of these neurons to identify the key pathways involved in these molecular disease phenotypes. In the

future, the findings of these disease phenotyping experiments may be usable for identification of new molecular targets for drug screening studies. Importantly, all lines and data will be released as a community resource to serve as a baseline for the field.

Funding Source: Autism Speaks foundation, The Rosamund Zander Translational Neuroscience Center Boston Children's Hospital.

Keywords: Human iPSC-derived glutamatergic neurons, KCNQ2 in epilepsy and autism, multi-electrode arrays

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ORGANOIDS DERIVED FROM L-MYC IMMORTALIZED HUMAN NEURAL STEM CELLS (LM-NSC008): A REPRODUCIBLE IN VITRO MODEL OF CEREBRAL DEVELOPMENT

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The availability of platforms for ex vivo studies of human brain development and for devising therapeutic strategies for a variety of diseases has been hampered by limited access to human tissue as well as by the lack of rodent models that closely recapitulate human brain physiology. Here, we present a unique organoid platform developed using human fetal neural stem cells (NSCs) immortalized by expression of L-myc. These LM-NSC008 cells are well-characterized for NSC lineage differentiation, chromosomal stability, and lack of tumorigenicity. LM-NSC008-derived organoids, over 100 days in culture, displayed reproducible cell proliferation, morphological changes and expression of cerebral neural and glial markers (by immunofluorescence). Transcriptome analysis by NanoString showed time dependent changes in RNA expression patterns for genes involved in neuronal cytoskeleton, neuron-glia interaction, neural connectivity, neurotransmission, metabolism, axon and dendrite structure, tissue integrity, angiogenesis, and myelination. We propose that by avoiding challenges intrinsic to iPSC reprogramming and neural induction, the reproducibility of LM-NSC008-derived organoids position them as alternative to human embryonic stem cell (hESC)- and induced pluripotent stem cell (iPSC)-derived organoids. Thus LM-NSC008-derived brain cerebral organoids represent a robust model of human brain tissue useful for multiple purposes including high-content drug screening and disease model development.

Keywords: Organoids, Neural Stem Cells, Model of Development

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ESTABLISHING A NEW HUMAN IPSC-DERIVED CEREBELLAR ORGANOID MODEL FOR FRIEDREICH'S ATAXIA

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Friedreich's ataxia (FRDA), an inherited neurodegenerative disorder, is the most common form of hereditary ataxia in the United States affecting about 1 in every 50,000 people with no cure or effective treatment. It is caused by a GAA repeat expansion mutation in the mitochondrial FXN gene and leads to degenerative changes in the cerebellum, a particular brain region that controls movement and motor coordination. Most of our current understanding of the human cerebellum is derived from postmortem tissues and animal models. Over the past decade, human induced pluripotent stem cells (iPSCs) have emerged as a powerful platform for disease modeling. Here, we developed a novel 60-day organoid differentiation strategy that recapitulates the hallmarks of human cerebellar development. We confirmed the generation of two primordial regions, the rhombic lip and cerebellar plate ventricular zone, from iPSC lines derived from healthy individuals and FRDA patients. Molecular and cellular analyses confirmed that these specified regions can give rise to the main neuronal cell types of the cerebellum such as granule cells, interneurons, Purkinje cells, and Bergmann glia-like cells that guide migratory neuroblasts to their destination. In comparison to healthy controls, FRDA cerebellar organoids show disease-specific signatures such as a reduction of the mitochondrial enzyme ACO2 and an increase of apoptotic cells. Further analyses at the ultrastructural level indicate abnormal mitochondrial morphologies in FRDA cerebellar organoids. Moreover, elevated levels of reactive oxygen species were detected by using a flow cytometry method. Notably, cerebellar organoids with long GAA repeats, which correlate with clinical disease severity, also show a higher degree of mitochondrial abnormality in vitro. Currently, drug testing and gene editing experiments using CRISPR-Cas9 are underway to correct FRDA mutations and determine if disease phenotypes can be reversed. In summary, the newly established cerebellar organoid model provides new opportunities to investigate the fundamental pathophysiology of FRDA in vitro and examine innovative therapeutic approaches.

Keywords: Organoid, Cerebellum, Friedreich's Ataxia

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A HUMAN ORGANOID-DERIVED CHOLANGIOCYTE MODEL IN A PERFUSABLE MICROFLUIDIC PLATFORM

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Challenges in attaining adult-like maturation of liver epithelial cells, lead researchers to look beyond differentiating cells from pluripotent stem cells or cell lines of tumor origin. The establishment of liver organoids from isolated adult hepatic epithelial cells is currently at the innovative forefront of in vitro liver modeling. Intra-hepatic cholangiocyte organoids, resulting from liver progenitor cells of biliary identity, have emerged as a powerful tool to study cholangiocyte biology, as well as providing an expandable cell source. These organoids typically consist in extracellular matrix-embedded cyst-like monolayers enclosing a hollow apical lumen. Solving the intrinsic heterogeneity of organoid cultures as well as the inaccessibility of the apical lumen are vital next steps for full adoption of organoid technology in the industry and clinics. Microfluidics offer the possibility to carefully pattern the cells in a confined space. Here we coupled organoid technology and microfluidics to establish a standardized, fully accessible and perfusable human cholangiocyte organoid model from three different donors. After growing as traditional hydrogel-embedded organoid cultures, the cells were extracted and seeded inside a microfluidic channel adjacent to a collagen-I interface. We observed rapid cell attachment, as well as proliferation, leading to the formation of complete tubular structures. These tubes presented an intact and stable barrier function, active and directional transport as well as mature cholangiocyte phenotype. Basolateral and apical accessibility of the system allowed us to challenge the cholangiocytes with a range of bile acids while simultaneously monitor the integrity of the barrier. Depending on the hydrophobicity and the administered location of the bile acids, we found profound differences in their biological effects, ranging from high toxicity and barrier disruption to increase of barrier function. In conclusion, we present a standardized, reproducible, mature and perfusable cholangiocyte organoid model that recapitulates the three-dimensional structure as well as the function of the human bile duct.

Keywords: human organoids, 3D organ-a-chip, liver model

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LARGE-SCALE DIFFERENTIATION OF IPSC-DERIVED MOTOR NEURONS FROM ALS AND CONTROL SUBJECTS

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Using induced pluripotent stem cells to understand mechanisms of neurological disease holds great promise; however, there is a lack of well-curated lines from a large array of participants. Answer ALS (www.answerals.org) has generated over 1000 iPSC lines from control and amyotrophic lateral sclerosis (ALS) subjects, along with clinical and whole genome sequencing data. In the cur-

rent study, over 400 of these iPSC lines have been differentiated into motor neuron cultures and profiled by immunocytochemistry and bulk RNA-seq. Exploratory analyses and modeling with subject covariates reveal patient sex and cell composition to be significant sources of gene expression variation in motor neuron cultures. Identification of additional confounding variables such as differentiation batch, somatic cell used for iPSC reprogramming, and other technical features highlight challenges associated with large-scale patient-derived iPSC studies. By carefully controlling for confounding variables, we can better identify subtle disease signals. Interestingly, male ALS-derived motor neuron cultures have increased motor neuron production and stress-related gene expression patterns compared to male control cultures. We also identified a correlation of disease progression rate, estimated by the slope of the Revised ALS Functional Rating Scale scores, with several genes that can be used to predict with borderline acceptable accuracy, the patient progression rates. This is the largest set of iPSC lines to be differentiated into motor neurons and bulk RNA-seq data suggests that cell heterogeneity, patient sex, and likely genetic effects need to be accounted for in order to identify robust disease signatures and pathways affected in ALS. To further address these issues, we performed single nucleus RNA-seq for a subset of ALS samples carrying C9orf72 hexanucleotide repeat expansion (n = 9) versus healthy controls (n = 9). This approach revealed perturbation of several gene networks in ALS samples across various cell types present in the heterogeneous motor neuron cultures, suggesting widespread effects of C9orf72 mutation. Collectively, the results shed light on under-appreciated technical and biological variables inherent in differentiating large numbers of iPSCs and provide novel insights into genes affected in ALS.

Keywords: Amyotrophic lateral sclerosis, Motor neurons, Disease modeling

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CYCLIN-DEPENDENT KINASE INHIBITOR DYSREGULATION INDUCES OLIGODENDROCYTE DIFFERENTIATION/MATURATION IMPAIRMENT IN MULTIPLE SCLEROSIS CEREBRAL ORGANOIDS

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Multiple sclerosis (MS) is an auto-immune neurological disorder characterized by inflammation, demyelination, and neural degeneration. It has been shown that environmental and genetic factors contribute to the development of MS, however, the etiology of this condition remains unknown due to the lack of animal models and relative inaccessibility to human brain tissue. Cerebral organoids represent an interesting tool to study neurological disorders as they recapitulate early human neurodevelopment, including the generation, proliferation and differentiation of neural progenitors into glial cells and neurons. Members of the Cip/Kip family of cyclin-dependent kinases inhibitors (CDKi), such as p21, p27 and p57, are well characterized for their role as negative regulators of the cell cycle. Recent studies demonstrated that they play additional roles unrelated to cell cycle regulation, such as control of oligodendrocyte differentiation and maturation and thus, myelination. However, their importance in MS has yet to be thoroughly described. We report here the derivation of cerebral organoids from iPSCs of healthy control subjects as well as from PPMS, SPMS and RRMS patients. We first analyzed p21, p27 and p57 expression in cerebral organoids derived from healthy controls and from patients with the different subtypes of MS. Only p21 expression was strongly decreased in MS organoid, particularly PPMS. Because p21 is required for oligodendrocyte differentiation, we studied oligodendrocyte marker expression by immunofluorescence and RT-qPCR in organoid at d42 and d120. Analysis revealed a decrease of oligodendrocyte markers Olig2 and O4 at d42, and a reduction of oligodendrocyte maturation marker O4 and Olig2+/APC+ at d120. We then focused on the regulation of the CDK inhibitor p21 gene by PAK1 and E2F1. Preliminary results showed that both regulator expressions are altered in MS organoids compared to control. In conclusion, this work is a proof of principle, showing that c-organoids derived from patients with MS can be used as an innovative tool to better understand the genetic basis for phenotypic differences seen in MS. Using this model, we identified a dysregulation of the p21 pathway through PAK1 and E2F1, which may lead to oligodendrocyte differentiation/maturation defect in progressive forms of MS.

Funding Source: This work was supported by Tisch Multiple Sclerosis Research Center of NY private funds.

Keywords: Multiple sclerosis, cerebral organoids, Human pluripotent stem cells

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THE USE OF 3D BIOPRINTED HUMAN NEURAL PROGENITOR CELLS AS A MODEL TO STUDY EARLY NEUROGENESIS

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Brain organoids are the main three-dimensional (3D) in vitro model to study early neurodevelopment. Essential genes and molecular mechanisms involved in normal and pathological brain development were described using brain organoids generated from human iPSCs. The main downside of organoids is batch-to-batch heterogeneity, and much work is in progress to solve or minimize it. Three-D bioprinting has the potential for developing in vitro models that show higher complexity when compared to a bi-dimensional (2D) culture and may overcome issues that arise from the self-organization of organoids. We present the generation of complex 3D structures formed by human neural progenitor cells (hNPCs) bioprinted in Geltrex® and Gelatin methacryloyl (GelMA) bioink. Bioprinted hiPSCs maintained in a neural expansion medium for two weeks formed large clusters of cells connected by several broad processes. When 3D bioprinted hNPCs were cultured in a neural maturation medium containing ascorbic acid, db-cAMP, BDNF, and GDNF, we observed smaller and more compact clusters with thinner and longer processes connecting the clusters. Cells with neuron-like processes scattered around the construct were also observed. Time-lapse microscopy revealed that cells migrated between clusters, using the connecting processes as a track. Immunofluorescence at day 28 post-printing showed expression of GFAP and tubulin beta III by the cells in the clusters, while neuron-like scattered cells were negative for GFAP

and positive for tubulin beta III. The processes that link clusters within the 3D construct, on top of which cells migrated, associated with the expression of GFAP by cells on clusters but not on isolated cells, suggest the formation of structures that resemble the use of radial glia as migration tracks for NPCs. 3D bioprinting of hNPCs may be an excellent model to study cellular and molecular mechanisms involved in neural proliferation, migration, and differentiation during early neurogenesis.

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Keywords: 3D bioprinting, human neurogenic niche, human neuro progenitor cells

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IMPROVED PROTOCOL AND QC TO GENERATE REPRODUCIBLE CEREBRAL ORGANOIDS

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Brain organoids derived from human pluripotent stem cells (hPSCs) are a highly promising tool to study neurodevelopment, explore disease mechanisms, and screen candidate drugs. However, these complex 3D constructs have yet to reach their full potential as an in vitro model system because current protocols are often low in efficiency, lack robustness across hPSC lines, and exhibit high variability between organoid technical and experimental replicates. To address these limitations, we developed a series of innovative modifications. First, forebrain organoids are produced in a unique 96-well format, leading to more consistent size and composition across hPSC lines, individual organoids and production batches. Second, we rescued poor-performing hPSC lines by improving starting material quality with sustained levels of FGF2. hPSC lines maintained using FGF2 DISCs (StemCultures) expressed pluripotency-associated genes at higher levels, exhibited less spontaneous differentiation and performed more consistently in the differentiation protocol. Third, we identified SB431542 concentration during the first 6 days of organoid culture as a tunable process parameter to achieve line-to-line consistency in cortical patterning at 2 months. When maintenance with FGF2 DISCs and cell line-optimized SB431542 concentrations were implemented together, organoid consistency across lines and batches was vastly improved. Importantly, we also identified early QC checkpoints to reduce investment in poorly performing batches. We used scRNA-seq to identify specific clusters and gene expression associated with well-patterned and mis-patterned organoids, providing both positive and negative QC markers. Well-patterned organoids generated using this protocol were comprised of approximately 16 neuronal and glial subtypes, with robust representation of deep cortical layer neurons at 2 months and upper layer cortical neurons at 4 months. These modifications enable scale-up and scale-out to produce thousands of high-quality forebrain organoids across many lines in each production run. As a result, we are able to analyze tauopathy patient-derived hPSC lines and isogenic controls in parallel with higher efficiency and reduced batch effects, toward a better understanding of tau mutation-mediated neurodegeneration.

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Keywords: cerebral brain organoids, reproducibility in 3D models, tauopathy

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INDUCTION OF NEURAL DIFFERENTIATION IN HYBRID ORGANOID FORMED BY CO-CULTURE SYSTEM OF HUMAN INDUCED PLURIPOTENT STEM CELLS

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Human cerebral organoids produced by induced pluripotent stem cells (iPS cells) are useful models for various neuronal diseases. Although cerebral organoids posess cortical structures, their physiological function is limited in the absence of vascular structures; hence, there is a need to develop methods of enhancing their neural function though vascularization to provide oxygen, glucose and neutrition. One such method was described by Wörsdorfer et al. (2019) attempted to induce angiogenesis by co-culturing iPS cells with mesenchymal stem cells. In this study, we co-cultured embryoid bodies (EBs) of iPS cells with spheroids of adipose-derived stem cells, a type of mesenchymal stem cell using original biodevice. Usually,EB was usually produced from

8000~9000 cells of iPS cells, however, we produced 2000 cells of EBs and co-cultured with spheroid made from 2000 cells of mesenchymal stem cells using a biodevice. Then we performed co-culture in different rate of EBs and spheroid of mesenchymal stem cells. Induction of these co-cultured spheroids to neural differentiation and growth to brain organoid. Mesenchymal stem cells were fluorescent labeled by 605 nm quantum dot, and these labeled cells were aggregated in central region of organoids after neural differentiation. Morphological characteristics of vessel-like structure was observed, and the results of RT-PCR showed the expression of angiogenic marker CD31 in mesenchymal cell-rich cerebral organoid. The electrophysiological characteristics by MEA of these co-cultured organoids showed different patterns of electrical current on each three organoids. These results indicated that the induction of angiogenesis in cerebral organoids by mesenchymal stem cells might change to electrophysiological characteristics.

Keywords: cerebral organoid, co-culture, biodevice

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FOREBRAIN ORGANOIDS DERIVED FROM FERRET IPSC TO MODEL THE DEVELOPING CORTEX

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Cerebral organoid models have facilitated the rapid expansion of our understanding into the key mechanisms that govern brain development. However, these models have several key limitations, including the lack of spontaneous vasculature and issues associated with the presence of a hypoxic and necrotic core. Importantly, organoids in their current state do not fully recapitulate some of the later stage features of corticogenesis, such as cortical gyrification. Ferrets are commonly used as a model of brain development due to their contracted corticogenesis timeline. Furthermore, the ferret undergoes gyrification shortly after birth, which is in stark contrast to other similarly sized mammals, whose cortex do not fold. While animal models remain necessary in biomedical research, attention is shifting to induced pluripotent stem cell (iPSC)-derived 3D in vitro models due to their unique experimental and ethical advantages. In this study, we aimed to create a cerebral forebrain organoid derived from ferret iPSC (FiPSC). We hypothesised that due to the ferrets contracted corticogenesis timeline, such an organoid would better able to recapitulate features associated with later stages of corticogenesis, in particular, gyrification. Our results thus far indicate that: a) it is possible to direct FiPSC to self-assemble into a forebrain organoid; b) there is evidence of cortical architectural organisation, with distinctive Pax6+ and Sox2+ germinal zones after 7 days in maturation media, along with Tbr2+, Ctip2+, Tuj1+ cells emerging, indicative of intermediate progenitors, early born and maturing neurons, respectively; c) the surface of these organoids appears distorted or buckled, however further analysis is underway to determine whether this is 'true' gyrification. Collectively this new species model represents an advancement in our ability to apply organoid technology to non-human mammals, while providing a novel way to study aspects of cortical development.

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Keywords: Brain Organoids Induced Pluripotent Stem Cells, Neurodevelopment Corticogenesis, Gyrification

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MOLECULAR RESPONSE TO GLUCOCORTICOID ACTIVATION IN NEURAL CELLS FROM HUMAN IPSC

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Exposure to trauma and chronic stress is one of the most important risk factors for psychiatric disorders. Therefore, it is important to decipher factors influencing response to stress or trauma at molecular and cellular levels that will enable us to identify stress-related pathological mechanisms of psychiatric disorders. In-utero dysregulation of glucocorticoid receptors (GR) or stress hormone receptors due to glucocorticoid exposure has been liked to long-lasting effects on brain development and a high risk for psychiatric disorders. Therefore, in this project, we study the neural cell type-specific effect of GR activation by synthetic glucocorticoids like dexamethasone (Dex) or hydrocortisone (H-Cort). Human iPSCs were used to derive in vitro neural cells such as neurons, and astrocytes. For GR activation neural cells derived from healthy individual iPS-cell lines will be exposed to different concentrations of Dex or H-Cort for the varied duration or vehicle control (ethanol). Further, we quantified the subcellular distribution of GR in neural cell types after Dex or H-Cort stimulation using immunocytochemistry and confocal microscopy. By intensity quantification of the confocal images, we observed higher expression of the GR in the nucleus than in the cytoplasm in the cortisone treated compared to the vehicle control. Further by quantitative real-time PCR, we observed differential expression of the known GR target genes such as FKBP5, ZBTB16, TSC22D3, and SGK1 after Dex or H-Cort treated compared to the vehicle control. These results exhibit that our iPSC-derived neural cell stress model system is robust and responds to the cortisone exposure shown by GR translocation to the nucleus and by the change in expression for the GR-responsive genes. Furthermore, we also

performed whole transcriptome RNA sequencing the analysis of which is awaited. These results will aid us to identify GR-responsive genes after cortisone exposure in a neural cell-specific manner.

Keywords: iPSC-derived neurons and astrocytes, glucocorticoid receptor, dexamethasone (Dex) / hydrocortisone (H Cort)

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EFFECTS OF INTERLEUKIN-17A IN DEVELOPING HUMAN BRAIN ORGANOIDS

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Epidemiological data strongly link viral infections during pregnancy with development of several neuropsychiatric disorders in the offspring. Maternal immune activation (MIA) has been hypothesized to mediate the increased risk for these disorders in babies born to women had viral infections during pregnancy. Animal studies have led to the seminal finding that interleukin-17a (IL-17a) plays a critical role in mediating behavioral and neuropathological effects of MIA in the offspring. However, it is not clear whether these pathways mediate MIA-associated increase in risk for neuropsychiatric disorders in humans. To investigate the translatability of these findings in the human context, we are dissecting the effects of IL-17a exposure during human neurodevelopment, using brain organoids differentiated from human induced pluripotent stem cells (iPSCs). We confirmed that iPSC-derived human neural progenitor cells and mature neurons express the IL-17 receptor, by quantifying mRNA and protein levels in these cells. In our preliminary experiments, we found that IL-17a exposure during the early stages of cortical organoid differentiation affected the size of the organoids, led to a decrease in the neural progenitor cell pool and resulted in aberrant laminar organization of upper-layer and deep-layer neurons. These data suggest that IL-17a-mediated signaling may have critical cellular and molecular consequences that alter the trajectory of neurodevelopment and increase the risk for neuropsychiatric disorders.

Keywords: Maternal immune activation, Interleukin-17a, Brain organoids

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GENERATION AND CHARACTERISATION OF IPSC LINES FROM PARKINSON'S DISEASE PATIENTS WITH MUTATIONS IN THE PRKN GENE

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Mutations in the gene encoding a protein ubiquitin ligase, PARKIN (PRKN), with complete or partial loss of its function, is one of the more common genetic risk factors for early-onset Parkinson's disease (PD). To provide greater mechanistic insights into the key determinants of disease aetiology and progression, we generated clonal induced pluripotent stem cell (iPSC) lines using fibroblasts from three patients carrying compound heterozygous PRKN mutations. The new lines displayed pluripotent stem cell characteristics including expression of the key PluriNet gene expression network components after reprogramming transgene loss on mRNA and protein levels, trilineage differentiation capacity in vitro, as well as their ability to readily form teratomas in athymic rodents. Using our customised protocols, we have differentiated the 3 PRKN lines into the ventral midbrain and dorsal forebrain-patterned neural cells (neurons and astrocytes). Differentiated cells have been transplanted into athymic rodents to assess disease phenotypes at prolonged time points (3+ months) – specifically to assess changes at the histological, biochemical and transcriptional levels. By comparing these PRKN-mutant lines with the isogenic controls (currently generated in our lab through CRISPR-aided correction of the original PRKN mutant), we hope to improve our understanding of the temporal, spatial and cellular components of PD pathobiology. These lines will provide a valuable resource for the PD research community (where the number of available fully-characterised PARKIN-mutant lines is limited) and will become the first PRKN mutant (and isogenic) lines to be deposited in the Parkinson's Progression Marker Initiative (PPMI) cell bank (an initiative of the MJ Fox Foundation aimed at sharing

datasets and biosamples to accelerate breakthroughs and treatments).

Funding Source: ASAP Aligning Science Across Parkinson's Michael J. Fox Foundation

Keywords: Parkinson's Disease, Reprogramming, Dopaminergic Neurons

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DISSECTING HUMAN MIDBRAIN SIGNALING DYNAMICS AND FATE PATTERNING USING MICROPATTERNED COLONIES

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The embryonic brain is segmented into the forebrain, midbrain and hindbrain. Defects in midbrain development have been associated with various neurological and psychiatric disorders. Its development is an intricate process, requiring the involvement of multiple morphogens and the coordination of diverse tissues. The midbrain develops along two axes: Anterior-Posterior (AP) and Dorsal-Ventral (DV). The signals involved in patterning the midbrain along these axes have been established, with FGF and WNT setting the AP coordinate and opposing gradients of Shh and BMP organizing the DV axis, however, the precise links between signaling and fate and how signals are coordinated across these two orthogonal axes remains incompletely understood. Midbrain progenitors express LMX1A, FOXA2, OTX2, and EN1, and deletion of any of these genes results in abnormal or failed midbrain development. To study signaling dynamics and morphogen patterning in the midbrain, we developed a micropattern protocol that generates colonies composed of the ventral midbrain cell fates (LMX1A+,FOXA2+,OTX2+). Cells within these micropatterns acquired mid-dorsal fates by default but could be fully ventralized through the addition of Shh. Moreover, by modulating WNT we generate colonies composed of anterior (OTX2+,LMX1A+,FOXA2+) and posterior ventral midbrain (EN1+, LMX1A+,FOXA2+) cell fates, showing that changing the AP coordinate did not affect DV patterning. However, inducing DV patterning with Shh at a later time-point did affect AP fates, revealing cross-talk between these two axes. Finally, using this protocol we found that endogenous WNT signaling was sufficient to suppress the most anterior fates and inhibiting WNT signaling allowed us to observe forebrain patterning (FOXG1+, OTX2+). Collectively, we believe these findings will enhance our understanding of brain development and contribute to potential clinical applications.

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Keywords: Embryonic Midbrain, micropatterning human stem cells, signaling dynamics and fate patterning

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GENOMIC AND FUNCTIONAL CHARACTERIZATION OF ASD AND ID-ASSOCIATED MYT1L MUTATION

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MYT1L is a zinc finger transcription factor highly expressed in the developing brain. Pathogenic variants in the MYT1L gene are associated with autism, intellectual disability, developmental delay, schizophrenia, and microcephaly. Overexpression of MYT1L in fibroblasts with two other neurogenic transcription factors promotes reprogramming into neurons by repressing non-neuronal lineages. MYT1L mutation in mouse models causes precocious differentiation of neurons, potentially contributing to phenotypes seen in individuals with pathogenic MYT1L variants. Here, we modeled the consequences of pathogenic MYT1L mutation by deriving pairs of isogenic human pluripotent stem cells (hPSCs) with versus without a MYT1L p.S707Q mutation found in our index patient. These isogenic pairs of hPSC lines were constructed both by correcting the mutation in patient-derived induced pluripotent stem cells (iPSCs) and by knock-in of the same variant into wild type hPSCs. We found that immature cortical interneurons (cIN) carrying this MYT1L variant exhibited reduced MYT1L mRNA and protein levels. Transcriptomic analysis revealed upregulated expression of genes associated with the generation of neurons, neuron development, and synaptic regulation, while downregulated genes were associated with more general cellular processes (e.g. cell adhesion and animal organ development), suggesting that neurons carrying this variant differentiated prematurely or more efficiently than wild type neurons. Consistent with this interpretation, neurons carrying the MYT1L variant had more complex morphology, with increased numbers of primary and secondary neurites. The upregulated genes included those involved in synapse formation and function, including GABA receptors, while MYT1L variant neurons exhibited altered sodium and potassium channel activity function in electrophysiological analyses. During neuronal maturation, variant neurons also exhibited impaired maintenance of normal cIN morphology and identity. Together, these findings suggest that pathogenic MYT1L variants disrupt both the neurodevelopment and subsequent functional characteristics of cortical neurons. These alterations may be a major contributor to the neurodevelopmental disorders seen in patients carrying pathogenic MYT1L variants.

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HD087011-S1) Children's Discovery Inst. (CDI-LI-2019-819) The Jakob Gene Fund PreMIER, ICTS, CRM at WUSTL

Keywords: MYT1L, Autism spectrum disorder, IDD, Immature cortical interneuron

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DEVELOPMENT OF PLURIPOTENT STEM CELL MODEL FOR THE STUDY OF EPILEPTIC ENCEPHALOPATHY CAUSED BY CYFIP2 R87C VARIANT

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Mutations in the CYFIP2 gene are associated with the neurological disorder epileptic encephalopathy. One of the variants associated to the disease is the R87C, which, compared to previously described variants, is associated with more severe symptoms. This disparity in disease presentation and molecular signatures hinders the development of efficient treatment to alleviate patients' symptoms. To date, there is a single animal model with the CYFIP2 R87C variant, however this model does not fully recapitulate disease pathogenesis. Thus, establishing a human model that can evaluate the impacts of the mutation in the neuronal context is crucial to understanding the molecular mechanisms of the variant, as well as enable new therapeutic discoveries. This study aims to develop a pluripotent stem cell (PSC) model to study the effect of CYFIP2 R87C variant in neural cells. Thus, we will differentiate PSCs into cortical neurons and cortical organoids and investigate the impact of CYFIP2 R87C to neuronal morphology and electrophysiological function. For that purpose, we initially reprogrammed urine cells from a patient with the R87C variant, and generated iPSC CYFIP2WT/R87C cells. The iPSCs were characterized for their pluripotent markers. Additionally, we edited ESCs using CRISPR/Cas9, to generated CYFIP2 knockout and CYFIP2R87C/R87C isogenic cell lines. These cells were then differentiated into neural progenitor cells (NPCs), and it was possible to observe that CYFIP2 was expressed as cytoplasmic granules in the CYFIP2WT/R87C cells compared to controls. These cells were also differentiated into cortical organoids and comparative molecular, morphological, and electrophysiological assessments are being performed. Lastly, these models will be used in a low-throughput drug screening with compounds previously predicted in silico to selectively inhibit the mutant CYFIP2 (R87C).

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Keywords: CYFIP2, induced pluripotent stem cell, disease modeling

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GENERATION OF FUNCTIONAL CEREBELLAR ORGANOIDS FROM HUMAN PLURIPOTENT STEM CELLS

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The development of the human cerebellum is quite more complex and protracted than most mammals leading to an increase in susceptibility to neurodevelopmental disease. The evolutionary expansion of the cerebellum began in parallel lineages of apes, but only rapidly increased in the great ape clade, suggesting a role in acquiring human-specific traits. Human pluripotent stem cell-derived brain organoids have emerged as an effective in vitro system to interrogate the development and disease of multiple brain regions; however, a protocol that models the developing human cerebellum with high fidelity is not yet available. Here we report the establishment of a robust protocol that can reproducibly generate the cellular diversity of the human cerebellum within and across multiple cell lines. The human cerebellar organoids exhibit an organized laminar layering with spatially segregated ventricular and rhombic lip progenitor zones that give rise to functional, inhibitory and excitatory cerebellar neuronal subtypes respectively. In addition, extending the time in culture increased the level of maturation of these organoids, which were identified through changes in transcriptomic profiles, increased neurite outgrowth, and increased coordinated network activity. This demonstrates that cerebellar organoids are suitable to model various aspects of human cerebellar development and disease, including disruptions in developmental trajectories and cerebellar circuit functionality.

Keywords: cerebellum, cerebellar, organoid

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GENERATION OF IN VITRO AND IN VIVO DISEASE MODELS TO STUDY THE ROLE OF RD3 MUTATION IN LCA12 PATHOGENESIS

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Retinal Degeneration 3 (RD3) gene encodes a 23 kDa protein that mediates the trafficking of retinal guanylyl cyclase (RetGCs) from the inner to outer segments of photoreceptors. The mutations in the gene cause Leber Congenital Amaurosis, Type 12 (LCA12), a severe form of congenital retinal dystrophy resulting in early onset vision loss in young children below five years of age. This study aims to decipher the role of RD3 on retinal development and functioning by generating iPSC-derived retinal organoids and rd3 knockout zebrafish as in vitro and in vivo disease models, respectively. The iPSC lines were derived from the normal and patient-specific dermal fibroblasts. Upon differentiation, both the healthy and patient-specific iPSCs formed normal-appearing eye fields and generated retinal cups at comparable timelines and efficiencies, suggesting the early retinal commitment remains unaffected in mutant cells; however, lamination defect was observed in the diseased tissue. The neuroretina organoids in the dish represent the fetal stage tissues and are limited by incomplete morphological maturation of photoreceptors cells. To further confirm the direct involvement of RD3 mutations in retinal defects, we generated rd3-/- knockout zebrafish models by targeting the exon 2 of the zebrafish rd3 gene using CRISPR/Cas9 gene editing tool. Immunohistological analysis of the rd3-/- mutants over a period of one year has shown the presence of underdeveloped cones at 3 months time point with missing outer limiting membrane and lamination defects in the outer nuclear layer. A major loss in all cone subtypes was observed at later time points (6 and 12 months), with a corresponding increase in rod nuclear layer, indicating defects in photoreceptor precursor differentiation. Together, the observations confirmed that the loss of RD3 expression in retinal precursors results in early retinal development and maturation defects.

Keywords: RD3, iPSC, retinal organoids, zebrafish, retina, disease model, rods, cones, photoreceptors

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FUNCTIONAL AND MORPHOLOGICAL CHARACTERIZATION OF HEALTHY AND EPILEPSY-RELATED 3D NEUROSPHERES ASSEMBLED USING IPSC-DERIVED NEURONS AND ASTROCYTES

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Neural 3D spheroids are a rapidly developing technology with great potential for understanding brain development and neuronal diseases. They provide a more advanced and biologically relevant system for basic research and high-throughput drug discovery, including compound profiling and toxicity testing. Here, we describe methods for assembling human iPSC-derived cell types, including glutamatergic neurons, GABAergic neurons, and astrocytes into 3D neurospheres. For disease modelling of epilepsy phenotypes, we used two different genetically modified GABAergic neurons (SCN1A KO or KCNT1 P924L mutation) and their isogenic pairs as matched controls. The SCN1A gene encodes the alpha subunit of the sodium channel NaV1.1 and it is the major gene implicated in Dravet Syndrome, a severe childhood epilepsy. The KCNT1 gene encodes a potassium channel and the P924L mutation is linked to an early-onset epileptic encephalopathy. We monitored the formation, morphology, and functional activity (Ca2+ oscillations) of the neurospheres after 3 weeks in culture. The microtissues were also analyzed by confocal fluorescence imaging for cell organization and expression of neuronal markers (TUJ1) and astrocytes (GFAP). Cellular and spheroid morphology was characterized by using high-content imaging. The calcium assay was performed on a FLIPR instrument capable of fast kinetic recordings using a calcium-sensitive dye and oscillation patterns were analyzed for peak frequency, amplitude, width, & spacing. Different baseline oscillation patterns were observed between control and disease neurospheres, however within each group calcium kinetics and patterns were highly consistent. For pharmacological characterization, we used a panel of 14 compounds, including selected molecules that affect GABA, AMPA, NMDA, sodium and potassium channels, dopamine receptors, also select neuroactive and neurotoxic substances. The functional responses demonstrated the predicted effects based on mode of action, consistent across control and disease model 3D neurospheres. Moderately increased excitability was observed for mutated phenotypes. This system of 3D neurospheres paired with high-content imaging and intuitive analysis of calcium oscillations demonstrates a promising tool for disease modeling and compound profiling.

Funding Source: none

Keywords: 3D neutrospheres, epilepsy disease models, calcium oscillations

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MODULAR APPROACH TO DISEASE MODELING USING DIFFERENTIATED HUMAN IPSC-DERIVED NEURONS AND ASTROCYTES

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3D neural spheroids are a rapidly developing technology with great potential for understanding neuronal diseases, including neurodegenerative disease (i.e., Parkinson's Disease; PD) and neurodevelopmental disorders (i.e., epilepsy). Differentiation into spheroids starting from neural progenitor cells or induced pluripotent stem cells (iPSC) is time-consuming, potentially variable, and limited for analyzing disease phenotypes within specific cell types. In this study, we demonstrate an efficient and modular approach for creating disease-specific neurospheres using defined combinations of fully differentiated iPSC-derived neural cells in tri-culture. We first show how neurospheres can be customized to assemble brain region-specific cultures by adjusting the composition of cell types (glutamatergic, GABAergic, dopaminergic, astrocytes), ratios of each cell type, and the total number of cells per spheroid. Using live cell imaging, we show that 384-well ULA plates containing neurospheres form quickly with consistent size and morphology, and these structures survive for extended periods of time in culture. The functional impact of varying cell ratios and composition was demonstrated using calcium oscillation assays. Analysis revealed that peak count, peak amplitude, and peak width varied by neurosphere cell composition. To model epilepsy we assembled neurospheres containing iPSC-derived GABAergic neurons with either a SCN1A knockout or KCNT1 P924L mutation. Epilepsy neurospheres exhibited an excitable calcium oscillation phenotype compared to isogenic controls, which could be modulated further using seizurogenic compounds. To model PD, we utilized either patient-derived LRRK2 G2019S and GBA N370S or gene-edited SNCA A53T dopaminergic neurons. PD neurospheres displayed altered calcium oscillation kinetics compared to controls. These data demonstrate that assembling iPSC-derived neurons and astrocytes into 3D neurospheres offers an efficient, flexible, and modular approach to interrogating cell-specific disease mechanisms within advanced culture models.

Keywords: 3D neural spheroids, Human iPSC-derived neurons and astrocytes, disease modeling in advanced culture models

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LONG-TERM FEEDER-FREE CULTURE OF HUMAN PANCREATIC PROGENITORS ON FIBRONECTIN POTENTIATES BETA-CELL DIFFERENTIATION

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The pancreas plays an essential role to control the metabolism of nutrients, and endocrine pancreas regulates blood glucose by secreting hormones, such as insulin and glucagon. Impaired function of insulin-producing beta-cells results in dysregulation of blood glucose and leads to diabetes mellitus. Aiming to produce beta-cells for replacement therapies to treat diabetes, several protocols have been established to differentiate human pluripotent stem cells to beta-cells via pancreatic progenitors. While in vivo pancreatic progenitors expand throughout development, the in vitro protocols have been designed to make these cells progress as fast as possible to beta-cells, without a possibility to expand the progenitors. This means differentiation needs to be started always from stem cells, which creates batch-to-batch variations in efficiency and takes longer time. To circumvent them, a small number of studies have developed conditions to expand pancreatic progenitors, but those conditions are not fully defined, requiring large numbers of feeder cells. Here, we report on a protocol enabling a long-term expansion of human pluripotent stem cell-derived pancreatic progenitors in a defined medium on fibronectin, in the absence of feeder layers. The expansion condition enables increased NKX6-1 expression, which is critical for beta-cell generation. Furthermore, compared to directly differentiated progenitors, the expanded progenitors differentiate more efficiently into glucose-responsive beta-cells and produce fewer glucagon-expressing cells. Additionally, these expanding progenitors can form pancreatic organoids in the condition that we previously reported. Currently we are in the process of fine-tuning the expansion conditions to accommodate the adaptation of multiple pancreatic progenitor lines derived from induced pluripotent stem cells as well as embryonic stem cells. The ability to expand progenitors under a defined condition and to cryopreserve them will provide flexibility in research and therapeutic production.

Keywords: human pancreas progenitor, Beta-cell, Long-term culture

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CA19-9 MODIFIED PROTEINS INDUCE TUMOR MICROENVIRONMENT REMODELING IN PANCREATIC DUCTAL ADENOCARCINOMA

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Pancreatic ductal adenocarcinoma (PDA) is a lethal common malignancy that is difficult to detect early, and with a 5 year survival rate of 10% is on track to become the second leading cause of cancer related deaths. With only 10-15% of patients eligible for surgical resection, the only cure for PDA, treatment is limited to combinatorial chemotherapy and immune checkpoint inhibitors, which are seldom effective. This barrier to drug delivery within the pancreatic tumor microenvironment (ME) is the result of the hypovascularized stroma consisting of cancer associated fibroblasts (CAFs) and tumor associated macrophages (TAMs) that contribue to desmoplasia and immunosuppression. Carbohydrate antigen 19-9 (CA19-9) is a glycan elevated in serum of 10-30% of pancreatitis and 75% of pancreatic cancer patients and is used as a biomarker for treatment response. Prior work demonstrated that CA19-9 directly causes pancreatic disease but the mechanism by which it remodels the pancreatic ME is largely unknown. CA19-9-modified proteins such as macrophage migration inhibitory factor (MIF) and insulin-like growth factor 2 receptor (IGFR2) were identified via immunoprecipitation and mass spectrometry from KRAS-mutant CA19-9 expressing mouse organoids and evaluated for changes in abundance, localization, and protein interactions. The direct role of CA19-9 modification of these effectors will be delineated using both in vitro and in vivo tools. To determine their role in ME remodeling in vitro, effectors will be CRISPR ablated in mouse organoids in a novel macrophage, organoid, and fibroblast (MOrF) co-culture platform to assess changes to gene expression programs and proliferation of each compartment. Furthermore, effectors will be inhibited pharmacologically in KRAS- mutant CA19-9-inducible mice to evaluate their role in vivo. Identifying the mechanisms by which CA19-9 modified proteins directly affect tumor ME remodeling will inform of effective therapies in the future.

Keywords: CA19-9, Pancreas, organoid

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TARGETING ZNT8 TO GENERATE MATURE AND METABOLIC STRESS RESISTANT SC-B CELL FOR CELL REPLACEMENT THERAPY OF DIABETES

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Application of human embryonic stem cell (hESC)-derived β cell (SC-β cell) is a promising therapeutic strategy for diabetes treatment. However, lack of functional maturation and long-term stability still restrict curative capacity of SC-β cells. ZnT8 (zinc transporter), encoded by SLC30A8 is predominantly expressed in β cell, loss-of-function (LOF) of which protects the carriers from diabetes. Herein, we introduced the ZnT8 LOF into the genome of hESCs and differentiated them into SC-β cells to explore whether ZnT8 LOF improves SC-β cells. The single-cell RNA sequencing analysis revealed that the genes regulating the function and maturation of SC-β cells, such as INS, NKX6.1, PDX1, SYT13 were upregulated in ZnT8 LOF SC-β cells compared with WT counterparts, suggesting that ZnT8 LOF accelerates functional maturation of SC-β cells. To verify that, we conducted the glucose-stimulated insulin secretion assay and found that the ZnT8 LOF SC-β cells were more sensitive to glucose stimulation. Moreover, it revealed that ZnT8 LOF SC-β cells were more resistant to the lipotoxicity and glucotoxicity induced cell death compared with WT SC-β cells. Of note, our results further demonstrated that the reduction of zinc releases the zinc inhibitory role on insulin secretion and attenuattes ER stress to enhance survival of SC-β cells under metabolic stress. To test in vivo efficacy for insulin-dependent diabetes mellitus (IDDM), the SC-β cells were transplanted in pre-existing diabetic SCID-Beige mice induced by streptozotocin. Notably, the mice implanted with ZnT8 LOF SC-β cells exhibited improved glycemic control and better glucose tolerance compared to those with WT SC-β cells. In summary, these results indicate that targeting ZnT8 for producing more robust SC-β cells is a promising strategy for the cell therapy of IDDM.

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Keywords: ZnT8, Human embryonic stem cell-derived β cell, Diabetes

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PAX4 LOSS OF FUNCTION INCREASES DIABETES RISK BY ALTERING HUMAN PANCREATIC ENDOCRINE CELL DEVELOPMENT

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Diabetes is a major chronic disease with an excessive healthcare burden on society. A coding variant (p.Arg192His) in the transcription factor PAX4 is uniquely and reproducibly associated with an altered risk for type 2 diabetes (T2D) in East Asian populations, whilst rare PAX4 alleles have been proposed to cause monogenic diabetes. In mice, Pax4 is essential for beta cell formation but neither the role of diabetes-associated variants in PAX4 nor PAX4 itself on human beta cell development and/or function are known. Here, we demonstrate that non-diabetic carriers of either

the PAX4 p.Arg192His or a newly identified p.Tyr186X allele exhibit decreased pancreatic beta cell function. In the human beta cell model, EndoC-βH1, PAX4 knockdown led to impaired insulin secretion, reduced total insulin content, and altered hormone gene expression. Deletion of PAX4 in isogenic human induced pluripotent stem cell (hiPSC)-derived beta-like cells resulted in derepression of alpha cell gene expression whilst in vitro differentiation of hiPSCs from carriers of PAX4 p.His192 and p.X186 alleles exhibited increased polyhormonal endocrine cell formation and reduced insulin content. In silico and in vitro studies showed that these PAX4 alleles cause either reduced PAX4 expression or function. Correction of the diabetes-associated PAX4 alleles reversed these phenotypic changes. Together, we demonstrate the role of PAX4 in human endocrine cell development, beta cell function, and its contribution to T2D-risk.

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Keywords: Diabetes, Pancreas, PAX4

TOPIC: NO TISSUE SPECIFICITY

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GENERATION OF CRISPR/CAS9 CORRECTED IPSC LINES FOR THE MODELING OF NGLY1 DEFICIENCY

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N-glycanase 1 (NGLY1) is an enzyme involved in the N-deglycosylation of misfolded protein during degradation. Mutations in the NGLY1 gene cause a rare recessive disorder called NGLY1 deficiency, which is characterized by movement disorders, seizures, and liver disease. To address the need for patient-derived models for this rare disease we generated two CRISPR/Cas9 gene corrected iPSC lines from a patient-derived iPSC line that carried a homozygous p.R401X mutation in the NGLY1 gene. These lines contain either one or two CRISPR/Cas9 corrected alleles of NGLY1. Isogenic iPSCs generated by CRISPR correction of the mutant gene in patient derived iPSCs is a useful control necessary to examine whether phenotypic variability in patient -derived cells is caused by the gene mutation or other nonpathogenic genetic variations of iPSC lines. Here we present data showing the characterization and confirmation of pluripotency for these cell lines, the evaluation of NGLY1 expression and their application to disease modeling.

Funding Source: This work was supported by the Intramural Research Program of the National Center for Advancing Translational Sciences, National Institutes of Health.

Keywords: Genome editing, Rare disease, Disease modeling

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ANTICANCER DRUG RESISTANCE IN HAPLOID HUMAN EMBRYONIC STEM CELLS

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Anticancer drugs are at the frontline of cancer therapy. However, innate resistance to these drugs occurs in one-third to one-half of patients, leaving them vulnerable to the side effects of these drugs with no meaningful benefit. To identify genes and pathways that confer resistance to such therapies, we performed a genome-wide screen in haploid human embryonic stem cells. These cells possess the advantage of having only one copy of each gene, harbor a normal karyotype, and lack any underlying point mutations. We initially show a close correlation between the potency of anticancer drugs in cancer cell lines and those in human embryonic stem cells. We then exposed a genome-wide loss-of-function library of mutations in all protein-coding genes to ten selected anticancer drugs, which represent five different mechanisms of action among drug therapies. The genetic screening enabled us to identify genes and pathways which can confer resistance to these drugs, demonstrating several common pathways. We validated several of the resistance-conferring genes, demonstrating a significant shift in the effective drug concentrations to indicate a drug-specific effect. Strikingly, the p53 signaling pathway seems to induce resistance to a large array of anticancer drugs. The data shows dramatic effects of loss of p53 on resistance to many but not all drugs, calling for clinical evaluation of mutations in this gene prior to anticancer therapy. This research has shown the potential to contribute to the efficiency of cancer treatment and to spare patients from needless discomfort, while shedding light on the cellular processes underlying drug response in this disease.

Keywords: Haploid human embryonic stem cells, Anticancer drug resistance, Genome-wide screen

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SARS-COV-2 INFECTION OF HUMAN EMBRYONIC STEM CELLS: EFFECTIVENESS OF POTENTIONALLY THERAPEUTIC DRUGS

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Little is known about SARS-CoV-2 infection of human embryos and fetuses in pregnant women with COVID-19. Our goal is to identify drugs that can inhibit SARS-CoV-2 infection during prenatal development. We established a “disease-in-a-dish model” to address this research problem with: (1) an appropriate cell line, (2) a SARS-CoV-2 pseudoparticle infection system, and (3) drugs for screening. We used H9 human embryonic stem cells (hESCs) and their derivatives to model critical stages in human prenatal development. H9 hESCs have the characteristics of epiblast cells, which are found in post-implantation human embryos at weeks 2 to 3 of development. Immunocytochemistry results showed that H9 cells and their germ layers expressed the SARS-CoV-2 infection machinery, the ACE2 receptor and the TMPRSS2 protease, indicating that these cells are susceptible to SARS-CoV-2. SARS-CoV-2 pseudoparticles infected H9 hESCs, and all three germ layers had higher infectability than the undifferentiated H9 hESCs. Ectoderm had the highest infectability, suggesting that SARS-CoV-2 could be a teratogen affecting neural development. To prevent infection, we screened small molecule inhibitors targeting protease- and endocytosis-based entry. Our results showed that infection in undifferentiated H9 cells, endoderm, and mesoderm can be blocked with endocytosis inhibitors (Dyngo4a, Pitstop2, nystatin). Infection in ectoderm was blocked with aprotinin (a TMPRSS2 inhibitor). Overall, these data suggest the conclusion that endocytosis may be the major infection pathway during early embryonic development. Our research uses a disease-in-a-dish model to study SARS-CoV-2 infection in human embryonic and fetal cells and identify drugs that can prevent infection. The results of our study will benefit pregnant women and their embryos and fetuses and may prevent loss or damage to the unborn. The drugs that we identify may also benefit men and non-pregnant women who experience breakthrough infection following vaccination. Our “COVID-19-disease-in-a-dish model” may have future applications that will help identify toxicants and teratogens that are harmful to the unborn.

Funding Source: TRDRP Award #R00RG2620, Bridges Fellowships from the California Institute for Regenerative Medicine (CIRM) (Grant #TB1-01185), a fellowship from the CIRM Research Training Program (#EDUC-12752)

Keywords: SARS-CoV-2 virus, infection assay, drug screening

815

GENERATION AND CHARACTERIZATION OF INDUCED PLURIPOTENT STEM CELLS DERIVED FROM A CYSTIC FIBROSIS PATIENT CARRYING THE I1234V MUTATION

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Cystic Fibrosis (CF) is an inherited autosomal recessive disorder, that causes chronic obstructive pulmonary disease. The etiology of CF disease pathogenicity is due to specific mutations within a gene that encodes a protein responsible for regulating chloride ions transportation in the epithelium. The most predominant mutation among Qatari CF patients is the I1234V. There is a deficiency in the presence of a reliable model that can be used to study CF pathogenesis and thus hinder the discovery of an effective treatment for the disease. In this study we successfully generated induced Pluripotent Stem Cell (iPSC) model from a Qatari CF patient carrying I1234V mutation. To the best of our knowledge, this is the first report to record the generation of such cell line. The formed (hiPSC) colonies were monitored daily until we observe the typical morphology of PSCs. These colonies were fully characterized by different molecular and cell biology techniques and showed similar characteristics of the commercial ones. Indeed, different known pluripotent protein markers were efficiently expressed as assessed by Western Blot analysis. The presence of these pluripotent markers was also confirmed using immunofluorescence assays and PCR using TaqMan hPSC scorecard panel. In addition, the generated cells markedly expressed alkaline phosphatase, which is a key marker of pluripotent stem cells. Finally, The clones maintained normal karyotype chromosomal integrity of 46, XY. These results indicate that we successfully generated for the first time a CF-I1234V-hiPSCs colonies that can be used as a cell model to tailor personalized treatment specific for Qatari patients carrying this mutation.

Funding Source: Qatar National Research Fund, UREP25-035-3-007

Keywords: Cystic Fibrosis, I1234V mutation, iPSC

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PRODUCTION OF LARGE, DEFINED GENOME MODIFICATIONS IN RATS BY GENE TARGETING IN RAT EMBRYONIC STEM CELLS

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For many decades, rats were the most widely used organism for human disease modeling. The mouse claimed this position with the advent of gene targeting technology using mouse Embryonic Stem Cells (mESCs). Rat Embryonic Stem Cells (rESCs) were first reported almost 15 years ago and generally display the same capabilities as mESCs; however, rESCs are not frequently used and have been largely overshadowed by CRISPR-based gene editing in rat zygotes. This approach is quite useful for making certain modifications such as simple loss-of-function mutations, but it is not practical for the production of precise insertion or replacement mutations, especially modifications that are larger than 5 - 10 kilobases. We have derived and validated germline competent rESC lines from several rat strains. These rESCs lines are efficiently targeted using large BAC-based targeting vectors (LTVECs), and they maintain their potential for germline transmission, including after serial targeting with LTVECs. Targeting efficiency can also be improved with the use CRISPR/Cas9-directed cutting within the locus of interest. Using these methods, we have successfully replaced entire rat genes as large as 101 kb with their human orthologs, and we have produced rat strains bearing multiple targeted mutations. rESC technology expands and advances the use of transgenic rats for human disease modeling, target discovery and therapeutic validation.

Keywords: genetically modified rats, embryonic stem cells, human disease modeling

819

THE STUDY OF MAGNETIC NANOPARTICLE TRANSFECTED CELLS APPLICATION

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Magnetic nanoparticles (MNPs) for biomedical applications has increased considerably over the past two decades. MNPs consist of a magnetic iron oxide core coated with a biocompatible material such as polysaccharide, lipid, protein, small silane linkers, etc. that these surfaces impart the nanoparticle with its multi-functional (primarily in magnetic resonance imaging, MRI), theranostic character (targetable drug carrier for therapy). We aim to evaluate and compare the assembling efficiencies of MNP transfected cells. MNPs obtained from Magnetospirillum sp. AMB-1 Strain which has a lipid bilayer membrane and ferromagnetic properties. MNPs were characterized forward Transmission Electron Microscope (TEM), Physical Property Measurement System (PPMS), and Inductively Coupled Plasma Atomic Emission Spectrometer (ICPS), then transfected with concentration gradient of MNPs into mouse myoblast cell (C2C12) and human umbilical vein endothelial cell (HUVEC). MNP transfected cells were evaluated by Prussian Blue staining, Cell counting (CCK8) assay, Live-dead staining, and Real-time polymerase chain reaction (RT-qPCR). From iron detection, transfected MNPs was located in cytosol that transfection efficiency was revealed rely on MNP's surface of composition. Because lipid membrane contained MNPs was transfected whereas detergent treated MNPs was not transfected into the cell line. Furthermore, MNPs of concentration gradient was no effect to cell toxicity from cell transfection to cell assembly. It was means MNPs was possible to application tools as a clinical trial. In MNPs cell assembly, cell to cell compaction was more interactive better then control cell assembloid but has no significant, which was compared the gene expression of Gab junction protein as like Connexin 40 (Cx40), Connexin 45 (Cx45), and hypoxia related gene, Hypoxia-induced factor 1-α (Hif1-α). In conclusion, MNPs application can be a promising novel strategy to enhance cell assemble efficacy and outcomes of current stem cell therapy.

Funding Source: This work was supported by Korea government grant funded by the Ministry of Science and ICT (MSIT, NRF-2020H1D3A1A04081286, NRF-2022M3A9B6082680), and by the Ministry of Trade, Industry and Energy (MOTIE, No.20016553).

Keywords: Magnetic nanoparticles, Assembloid, Transplantation

821

DIRECT REPROGRAMMING OF HUMAN FIBROBLASTS INTO RETINAL PROGENITOR CELLS BY SMALL MOLECULES TO TREAT PHOTORECEPTOR DEGENERATION

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According to WHO, globally there are 285 million people visually impaired, and 39 million blind. In a real-world survey, blindness is reported to be the most terrifying disease among all human illnesses. There is more than 10% of blindness is caused by photoreceptor degeneration, which considered untreatable once the degeneration progress to reach the end-stage. For example, primary retinal progenitor cells (RPCs) from the fetal retina suffer from ethical issues and low proliferative abilities, embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) derived retinal lineage cells have relatively low efficiency of differentiation and are high-cost and labor-consuming. RPCs are capable to rescue vision in late-stage photoreceptor degeneration. But the availability of primary RPCs was restricted to post-abortion fetal tissue. Here in, we directly reprogram human fibroblasts into RPC-like cells by small molecules, aiming to overcome the current bottleneck in translational medicine. In preliminary experiments, qRT-PCR, immunofluorescence assay, and Western Blot showed the induced cells expressed classical markers of RPCs. In bulk RNA-seq analysis, we compared the transcriptome between human fibroblasts and induced RPC-like cells, induced RPC-like cells demonstrated upregulated expression of transcripts related to extracellular matrix component, axon, dendrite, synaptic and postsynaptic membrane, and transport vesicle formation. By co-culture assay, the induced cells were found to be able to integrate with the host rat retina in vivo. By calcium imaging, intracellular calcium concentrations were found to elevate in the induced cells upon glutamate stimulation, thus confirming these cells' functionality. Last but not the least, we investigated the therapeutic effect in an animal model of photoreceptor degeneration. Induced RPC-like cells were found to rescue the visual function of transplanted rats without causing significant adverse events. In the future, surface marker screening by flow cytometry will be carried out to generate exogenous reporter DNA-free cells for better clinical safety. Finally, we will build a standardized cell manufacturing protocol following Good Manufacturing Practice requirements and aim for technology transfer with a biotechnology company.

Keywords: Retina, cell therapy, photoreceptor degeneration

Clinical Trial ID number: no

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A HUMAN IPSC-DERIVED VASCULAR SMOOTH MUSCLE CELLS MODEL TO INVESTIGATE THE HDAC9 LARGE-VESSEL STROKE RISK VARIANT AND SCREEN FOR HDAC INHIBITORS

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A common variant in the Histone Deacetylase 9 (HDAC9) gene has been identified in GWAS studies as the strongest genetic risk for large-vessel stroke to date and is also associated with promoting carotid atherosclerosis. The mechanism linking the HDAC9 variant with increased risk of stroke is still unknown due to the lack of suitable models. HDAC9 was found to be up-regulated in carotid plaques and we know that sodium valproate, a pan-HDAC inhibitor, is associated with lower risk of stroke in human. We suggested that the HDAC9 stroke-associated variant may affect gene expression, and this may promote vessel damage, which contributes to the pathophysiology of ischaemic stroke. To test this hypothesis, we generated a vascular smooth muscle cells (SMC) model from human induced pluripotent stem cells (iPSC) with the stroke-associated variant rs2107595 in the HDAC9 gene. The stroke risk SMC show up-regulation of HDAC9 gene expression and disease-relevant phenotypes, including increased apoptotic levels, higher inflammatory response and a reduced proliferative rate compared to healthy controls. Moreover, our iPSC-derived SMC has allowed us to identify new pathways/targets for therapeutic development through transcriptomic analysis and potential therapeutically effective HDAC9 inhibitors by high-throughput phenotypic screening of HDAC class II inhibitors.

Funding Source: British Heart Foundation and Medical Research Foundation mid-career fellowship

Keywords: Histone Deacetylase 9, stroke, vascular smooth muscle cells

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INSIGHTS INTO THE DEVELOPMENT OF BAP1 MUTANT UVEAL MELANOMA WITH A SELF-FORMED ECTODERMAL MULTI-ZONE DIFFERENTIATED FROM IPSCS

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Uveal melanoma (UM) arises from neural crest of the eye and has a mortality rate of 80%. Almost all UMs harbor oncogenic muta-

tions in GNAQ, GNA11 or CYSLTR2. However, these do not affect the metastatic competency of UM which is defined by a classic gene expression profile. Class 1 tumors (low risk) harbor mutations in SF3B1 or EIF1AX whereas Class 2 tumors (high risk) harbor inactivating mutations in BAP1. BRCA1-Associated Protein 1 (BAP1) is a ubiquitin carboxy-terminal hydrolase that regulates multiple cellular processes including chromatin modification, DNA repair and cell cycle control. BAP1 is also mutated in several other highly aggressive cancers. Cell based studies have shown that loss of BAP1 function in UM cells leads to a stem-like phenotype. However, the role of BAP1 in tumorigenesis and metastasis remains largely unknown. We hypothesized that understanding the consequences of BAP1 loss in development would provide insights into its role in the development of UM and other cancers. We used human iPSCs and induced them to differentiate to form a primordium of four concentric zones termed Self-formed Ectodermal Multi-Zone (SEAM). The SEAM mimics whole eye development and cells within each zone are indicative of lineage. SEAMs were stained for several lineage and ocular markers. BAP1 localized to Zone 2, which is the developmental analog of neural crest cells. Zone 2 also developed dense melanin pigmentation at around Day 20 of differentiation. Next, using the CRISPR-Cas9 based system, we successfully generated iPSCs with downregulated BAP1 which were also differentiated into ocular SEAMs. RNA-sequencing revealed significant alterations in various pathways as a consequence of BAP1 knockdown. These included melanogenesis, morphogenesis (WNT signaling), ECM assembly and degradation, chromatin modeling and DNA repair. BAP1 downregulation also led to markedly reduced melanin pigmentation in Zone 2, consistent with a role for BAP1 in melanogenesis. Hence, ocular SEAMs provide insights eye development, neural crest differentiation and potentially tumorigenesis and metastasis in UM.

Funding Source: NYSTEM Training Grant, National Cancer Institute, Waldman Fund

Keywords: iPSCs, melanoma, neural crest

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BIOLOGICAL AND EPIGENETIC EFFECTS OF ANTENATAL EXPOSURE TO BISPHENOLS AND PERFLUOROALKYLS POLLUTANTS ON HUMAN STEM CELL MODELS

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Endocrine-disruptors (EDs), such as Bisphenols (BPs) and Perfluoroalkyls (PFs), are a class of plastic pollutants widely used in industrial applications. They can enter the human body through the ingestion of contaminated food and water interfering with endogenous hormone signaling and consequently leading to a wide spectrum of diseases. Due to the extensive use of plastic in daily life, particular attention should be paid to the EDs exposure of pregnant women: fetal development is a very susceptible period and in utero exposure to plastic pollutants seems to increase the risk of arising chronic pathologies later in adulthood. It has been reported that BPs and PFs can cross the placental barrier accumulating in the fetal serum and amniotic fluid, but the detrimental consequences for human development remain to be clarified, especially when different classes of EDs are combined; indeed, humans are simultaneously daily exposed to different chemicals which may have additive, synergistic, or negative biologic effects. Here we analyzed the effects of Bisphenol A (BPA) and S (BPS), perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), alone or in combination, on two different models of human perinatal stem cells: the fetal membrane mesenchymal stromal cells (hFM-MSCs), that derive from the placental amniochorionic membrane, and amniotic fluid stem cells (hAFSCs), that are isolated by the amniotic fluid. Data from impedance analysis and Ki67 expression showed that both BPs and PFs, alone or combined, increased the proliferation rate of perinatal stem cells, except the combination BPS +PFOS that was highly toxic for both cell models. Mitochondrial membrane potential measured with specific fluorescent labeling was scarcely affected by the EDs exposure. On the other side, BPs and PFs had a great impact on many key pluripotent genes, whose expression was up- or down-regulated by the EDs. More interesting, also DNTM and TET family members, which have a pivotal role in chromatin remodeling and epigenetic reprogramming in the early stages of fetal development, were highly affected by the treatments. All these data suggest that antenatal exposure to BPs and PFs may modify the biological and epigenetic characteristics of perinatal stem cells, perturbing normal human development.

Funding Source: This work was supported by PRIN Italian Ministry of University and Research (MUR), grant number PRIN20203AMKTW and by European Union- Fondo Sociale Europeo - PON Ricerca e Innovazione 2014-2020

Keywords: Endocrine disruption, perinatal stem cells, antenatal exposure

TRACK: ETHICS, POLICY AND STANDARDS (EPS)

Session 1: Even

6:45 PM - 7:30 PM

TOPIC: NO TISSUE SPECIFICITY

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HPSCREG - A RESOURCE FOR FOR HUMAN PLURIPOTENT STEM CELL RESEARCH

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The human pluripotent stem cell registry (https://hPSCreg.eu) is a public database and knowledge hub for human pluripotent stem cells. It was first established in 2007 with funding from the European Commission as the human embryonic stem cell registry to deal transparently and informative with the ethical and scientific status of human embryonic stem cell research. Upon the discovery of induced pluripotent stem cells (iPSC) and the widespread uptake of this technology, the registry expanded its mission to include human iPSC - lines. The hPSCreg knowledgebase was further extended to include a database of clinical studies using hPSC-derived cells as source materials for clinical applications. Now in its 4th funding period from the European Commission, the registry continues its original function as a registry for hPSC lines, but now finds itself at a crossroads on a number of impending issues, spanning: 1) transparent tracking of cell lines from research to clinical application; 2) adoption of guidelines and standards for reporting of biological characteristics and using data to increase reproducibility of research; 3) linkage of cell lines to additional data (omics, PSC-derived cell types and organoids); 4) providing guidance to ethical provenance and of hPSC lines; 5) tracking ethical provenance of the cell lines and their unforeseen usage in potentially controversial applications; 6) adherence to FAIR data principles; 7) maintenance of a flexible and scalable IT infrastructure to manage the growing knowledgebase; In this presentation, we present the registry's origin, current status and future developments in the face of the dynamic field of pluripotent stem cell research, ethics and clinical application.

Funding Source: Horizon Europe GA 101074135

Keywords: Pluripotent stem cells,, data quality and ethics, research reproducibility

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REGULATING UNPROVEN STEM CELL TREATMENTS IN THE U.S.: REGULATORY CHALLENGES

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Despite comprehensive FDA regulations on cell-based interventions, marketplace expansion occurs. In the U.S., clinics offering stem cell therapies not formally approved by the Food and Drug Administration (FDA) increased 4-fold in 5 years, according to research conducted by the University of California, Irvine, led by a bioethicist, Professor Leigh Turner. These putative medical treatments lack solid evidence of safety and efficacy. There are enforcement steps taken by regulatory bodies like the FDA, the Federal Trade Commission (FTC), and others. However, there are regulatory challenges. For instance, in a legal suit brought by the FDA, a judge in California entered a judgment in favour of the defendant, California Stem Cell Treatment Center (CSCTC) et al. The judge's ruling conflicts with an earlier decision on a similar case in Florida and thus, his decision has caused regulatory uncertainty. Meanwhile, the FDA is appealing. This presentation will explore those regulatory challenges and possible recommendations to resolve or at least mitigate the problem of untested stem cell therapies in the country.

Keywords: Regulation, Legal cases, Unproven stem cell treatments

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LONG-TERM IMPACT OF SCIENCE POLICY ON STEM CELL RESEARCH

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This study aims to clarify the long-term impact of political interventions on scientists' behavior in stem cell research. It examines how policy change for supporting hESC research had affected the number of ESC research after 2009 in the US. The easing of federal funding restrictions on hESC research is assumed to have increased the number of relevant research. Articles were analyzed to investigate whether the number of ESC research for cardiac regeneration and diseases had increased since 2009. The sample data were compiled from articles on stem cell research on the heart published between 2009 and 2019 and registered in PubMed. Articles were identified if they had "heart" and "ESC," "iPSC," "MSC," or "BMC" in MeSH terms. Moreover, to investigate the characteristics of organizations influenced by policy change, this study examined the institutions where research was conducted based on authors' affiliations. The affiliations include educational, clinical, research, commercial, governmental, and other organizations. When authors belong to multiple organizations, all the relevant organizations were counted in respective categories to build the data. Additionally, medical schools and colleges of pharmacies were counted as both educational and clinical organizations because they have the two institutions. The data demonstrate that ESC research had not increased significantly since 2009. It increased somewhat after 2009 but started declining in the late 2010s. Instead, iPSC research had increased. Regarding types of affiliations, the analysis uses probit regression to measure their different responses to policy change. The dependent variable is whether the respective stem cell is chosen

for research. Results about ESC show that the coefficient of affiliations categorized in both educational and clinical organizations is statistically insignificant. In contrast, research and commercial organizations have a statistically significant positive impact on choosing ESC. This study concludes that policy change in 2009 had not provided a strong push to increase ESC research in educational and clinical organizations. Conclusions suggest that a temporary suspension of funding hESC research would make it difficult to revive the relevant research again even after the restrictions were removed.

Funding Source: This article was supported by Grants-in-Aid from the Japan Society for the Promotion of Science (Kiban-C-21K00247).

Keywords: Science Policy, Embryonic stem cells, Policy change

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INCREASING OPENNESS AND TRUST IN STEM CELL RESEARCH AND THERAPEUTICS: TOWARD A NEW MODEL FOR EFFECTIVE GOVERNANCE

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Practices associated with stem cell research and therapeutics are at a crossroads: there is increasing urgency for crafting replicable, open, and trustworthy science and fostering responsible innovation practices in the stem cell domain. This will require researchers and clinicians to be aware of the need to be accountable and actively engage with stakeholders, and to develop skills and strategies for doing so. However, many issues in the field are hotly contested, leaving regulators, funders, publics, and other stakeholders frustrated and unclear about how to find accurate and reliable information, and how to contribute to shaping the future of this field. Our project seeks to provide robust guidance based on stakeholder identification of the main factors that must be addressed to build trust in and to support potential acceptance and greater uptake of stem cell research and therapies, and processes for setting priorities. In this paper, we assess a policy model designed to foster transparency and trust in medical research/therapeutics known as a 'commons' utilised in genomics and other fields in medicine. We analyse our findings from conceptual and empirical research exploring the shared values, goals, and priorities of diverse stakeholders (including researchers and other experts, industry, patients, regulators, and publics) that might be supported through a commons-type model and required modifications to apply such a policy model to the stem cell field in the Australian context and beyond.

Funding Source: "Developing an Evidence–Based Model for Building Trust in Australian Stem Cell Research and Therapies," Australian National Health and Medical Research Council, Medical Research Future Fund, Stem Cell Therapies Mission (2021-24)

Keywords: Governance and policy, Stakeholder engagement, Trust in science

TRACK: CELLULAR IDENTITY (CI)

Session 1: Even

6:45 PM - 7:30 PM

TOPIC: CARDIAC

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METABOLIC AND EPIGENETIC BARRIERS IN THE DIRECT REPROGRAMMING OF FIBROBLASTS INTO INDUCED CARDIOMYOCYTES

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Heart disease is the leading cause of mortality in developed countries, therefore, novel regenerative procedures are warranted for improving patients well fare. The mammalian neonatal heart is capable of substantial regeneration following injury, but this capacity is lost at postnatal stages when cardiomyocytes become differentiated and shift from glycolysis to mitochondrial respiration. Besides energetics, metabolites impact chromatin marks with implications for cell fate conversion and regeneration. The use of direct reprogramming of resident fibroblasts by the expression of the cardiogenic factors Mef2c, Gata4, and Tbx5 (MGT) can create induced cardiomyocytes (iCMs). Besides holding great promise, it still lacks effectiveness especially in adult cells, and the impact of metabolic and age-associated epigenetic barriers remains elusive. We observed that retroviral-induced MGT transduction produced increase expression of cardiac troponin T in mouse embryonic (MEFs) compared to adult ear skin fibroblasts (AEFs). Moreover, transdifferentiated MEFs present increase expression of additional cardiac markers and repression of fibroblast genes whereas MGT-transduced AEFs failed to achieve this state. Mass spectrometry-based histone proteomics revealed epigenetic differences in transdifferentiated MEFs and AEFs, compatible with shutting down of the fibroblast program and an open chromatin state (with decreased histone methylation), resembling the epigenetic landscape of cardiomyocytes. Immunofluorescence

analysis revealed increase mitochondrial elongation and network connectivity in transdifferentiated MEFs, with modest changes in transdifferentiated AEFs. Mass-spectrometry based metabolomics and energetic analysis suggests significant mitochondria metabolism remodeling during transdifferentiation into iCMs. Importantly, 2-DG-mediated inhibition of glycolysis or modulation of energetic fuels can increase direct reprogramming into iCMs. Overall, our results suggest that extensive histone landscape and mitochondrial remodeling takes place during direct reprogramming of fibroblasts into iCMs and modulation of bioenergetic and mitochondrial metabolism may potentiate direct reprogramming and cardiac regeneration.

Funding Source: This work is supported by the transnational funding network ERA-CVD/0001/2018- INNOVATION, FCT national funds EXPL/BIA-CEL/0358/2021 and the iBiMED (UIDB/04501/2020 and UIDP/04501/2020).

Keywords: Direct reprogramming into Cardiomyocytes, Mitochondria Metabolism, Chromatin Marks

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GENOME-WIDE CRISPR SCREEN IDENTIFIES AN NF2-ADHERENS JUNCTION MECHANISTIC DEPENDENCY FOR CARDIAC LINEAGE

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Cardiac differentiation involves a stepwise clearance of repressors and fate-restricting regulators through the modulation of BMP/Wnt-signaling pathways. However, the mechanisms and how regulatory roadblocks are removed with specific developmental signaling pathways remain unclear. Here, we performed a genome-wide CRISPR screen to uncover essential regulators of cardiomyocyte specification in human embryonic stem cells (hESCs) to better delineate the molecular events that control the earliest step of cardiovascular specification. We identified NF2, a Moesin-Ezrin-Radixin Like (MERLIN) Tumor Suppressor, as an upstream driver of cardiomyocyte specification. Transcriptional regulation and trajectory inference from NF2 knockout cells reveal the loss of cardiomyocyte identity and redirected towards an epithelial-like cell identity. Sustained elevation of early mesoderm lineage repressor SOX2 and upregulation of late anti-cardiac regulators CDX2, and MSX1 in NF2 knockout cells reflect a necessary role for NF2 in removing regulatory roadblocks. Since YAP is a known repressor of mesendoderm genes, we found that NF2 and AMOT cooperatively bind to YAP during mesendoderm formation, thereby preventing YAP activation in a LATS1-independent manner. Mechanistically, we show that the seven amino acid blue-box region within the FERM domain of NF2 is critical for the formation of the AMOT-NF2-YAP scaffold complex at the cellular adherens junction, which is essential for mesodermal lineage specification. These results provide mechanistic insight into the essential role of NF2 for cardiomyocyte lineage specification by sequestering the repressive effect of YAP and relieving regulatory roadblocks en route to cardiomyocytes.

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Keywords: Cardiomyocyte Differentiation,, CRISPR Screening, Cell states

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UNRAVELING THE ROLE OF THE AP-1 TRANSCRIPTIONAL PATHWAY IN THE DEVELOPMENT OF PACEMAKER CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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The sinoatrial node (SAN) is the primary pacemaker of the heart which triggers each heartbeat. Pacemaker cells from sinoatrial node have a gene expression program that allows them to integrate input from the autonomic nervous system and fire automatically. Mechanisms underlying pacemaker development and function remain incompletely understood in humans due to the rarity of the cell type and the difficulty of isolating a large number of pacemaker cells for molecular analysis. Our lab has previously developed a high-yield, high-purity monolayer protocol to generate pacemaker-like cardiomyocytes from human induced pluripotent stem cells (hiPSC). This allows studying gene regulatory mechanisms and molecular and functional aspects of SAN development. Single-cell (sc) RNA sequencing of these pacemaker cardiomyocytes revealed that they have distinct cellular subtypes which recapitulated many aspects of SAN development in vivo. sc-ATAC-sequencing and time course analyses of cellular differentiation further showed growth factors and stress signaling pathways that could be important in pacemaker cell specification. Specifically, our sc-ATAC sequencing data showed AP-1 motif enrichment in pacemaker cardiomyocytes, but not in atrial cardiomyocytes during differentiation. Here we seek to uncover the role of the AP-1 transcriptional pathway and identify the members of the AP-1 family involved in pacemaker differentiation. Understanding the molecular process involved in pacemaker cell

development will broaden our knowledge of SAN development and function. It will open avenues for in vitro cardiac arrhythmia modeling and discovering new approaches related to the regeneration of SAN for cell therapies.

Keywords: Unraveling regulatory mechanisms involved in specification of pacemaker cells from Sinoatrial node (SAN), Differentiating human iPSCs into monolayer pacemaker cells, Modeling Sinoatrial node development in vitro

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LEFT VENTRICLE-LIKE CARDIOMYOCYTES GENERATED FROM HUMAN PLURIPOTENT STEM CELLS HAVE INCREASED HOLISTIC MATURITY

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Decreased left ventricle (LV) function caused by genetic mutations or injury often leads to debilitating and fatal cardiovascular disease. LV-cardiomyocytes are, therefore, a potentially valuable therapeutical target. Human pluripotent stem cell derived cardiomyocytes (hPSC-CMs) generated using current protocols are neither homogenous nor mature, which reduces their utility. Here we exploit our understanding of cardiac development to devise a new protocol to generate LV-cardiomyocytes from hPSCs. Correct mesoderm patterning (relying on an interplay between Activin, BMP4 and WNT signalling) and blocking of the retinoic acid pathway were essential to generate near-homogenous hPSC-LV-CM populations. As expected, hPSC-LV-CMs transit via first heart field progenitors expressing TBX5 and HAND1 and display typical ventricular action potentials. Unexpectedly, hPSC-LV-CMs display increased metabolism, reduced proliferation and improved cytoarchitecture (including sarcomere organisation and length) and functional maturity compared to age-matched cardiomyocytes generated using the standard WNT-ON/WNT-OFF protocol. Of note was the calcium response to sarcoplasmic reticulum specific inhibitors (ryanodine and thapsigargin), which showed that hPSC-LV-CMs have more appropriate drug-responses. This increase in maturity is also observed in engineered heart tissues (EHTs) made from hPSC-LV-CMs, which are better organised, produce higher force and have a slower beating rate but can be paced to physiological levels. This demonstrates the mechanical load induced maturity exerted by the EHTs was additive rather than transformative. Together, we show that hPSC-LV-CMs with mature properties can be rapidly obtained (in 20 days), even without exposure to reported maturation regimes. These cells are, therefore, a suitable model to study LV development and disease and will likely enable more faithful LV-specific cardiotoxicity screens. Moreover, this work opens the possibility of like-for-like cell replacement therapy becoming an accessible treatment for heart failure patients.

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Keywords: left ventricle cardiomyocytes, cardiomyocyte differentiation + maturation, engineered heart tissues

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PREDICTING AND ENHANCING CARDIAC POTENTIAL OF IPSC-DERIVED CARDIAC PROGENITOR CELLS

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Human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) exhibit marked potential for use in cell therapies, drug screening, and disease modeling applications. The efficiency of their production; however, remains quite variable resulting in poor process robustness. Thus, methods to monitor this differentiation, predict final CM yield and purity at earlier stages, and enhance these metrics will significantly increase process reliability and productivity. Cardiac progenitor cells (CPCs) are a key metastable intermediate in this process and thus present an opportune cell type to study. Herein, we have undertaken a multi-omics discovery approach to identify early differences in cell attributes between high- and low-potency CPCs batches (i.e. those giving rise to high- and low-purity populations of hPSC-CMs) to provide systems-level insights into underlying mechanisms which drive these divergent outcomes. Specifically, we have combined epigenomic (ATACseq), transcriptomic (RNAseq), proteomic, and lipidomic profiling methodologies. To date, we have identified differential molecular and functional signatures between high- and low- potency CPCs with high power to predict terminal hPSC-CM purities. Among these signatures, in addition to confirming some canonical cardiac developmental genes (NKX2-5, CRIP2) being enriched in high-potency CPCs, we have also identified marked

differences in WNT, MAPK, and EMT pathways, epigenetic priming of key sarcomeric-related genes, lipid storage, and oxidative metabolism (Seahorse assay). Furthermore, through inferential dynamics we have identified cell-intrinsic speed of differentiation to be a potential significant contributor to divergent terminal hPSC-CM outcomes. Finally, we are beginning to utilize these data to enhance hPSC-CM differentiation outcomes (yield and purity). Thus far, in addition to gaining fundamental insights into the underlying biology of CPCs, our findings are being used to 1) predict final hPSC-CM differentiation process outcomes early, 2) enhance these process outcomes through process optimization, and 3) establish potential novel feedback control schemes to further enhance process robustness.

Keywords: Multi-omics, Cardiomyocytes, Cardiac progenitor cells

TOPIC: EPITHELIAL INCLUDES SKIN GUT LUNG

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HYPOXIA AND HIF PATHWAYS PROMOTE AIRWAY DIFFERENTIATION OF HUMAN FETAL LUNG EPITHELIAL PROGENITOR CELLS

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The development of first-trimester human embryos happens in a highly hypoxic uterine environment before the placental-maternal circulation is completely established. This developmental stage also overlaps with active organogenesis. To explore the effects of hypoxia on human lung development, we isolated the epithelial progenitors from human fetal lung tips and cultured them as organoids in a self-renewal medium. In this medium under normoxia the lung progenitors self-renew (remain undifferentiated and proliferate rapidly). However, chronic hypoxia arrested proliferation and induced the differentiation of first-trimester lung progenitors towards multiple airway cell types, but not alveolar fates, even in the self-renewal medium. This induction of airway fates was enhanced by lower oxygen levels and longer durations of exposure. To delineate the underlying mechanisms, we focused on HIF (Hypoxia-Inducible Factor) pathways. The activity of HRE (Hypoxia-Response Element) reporters and expression of known HIF-pathway genes were induced by hypoxia. Chemical stabilization of HIF1a and HIF2a in normoxia mimicked the hypoxia effects. Knocking-down HIF1a efficiently rescued the phenotype by decreasing the airway differentiation caused by hypoxia. Transcriptomic analysis showed HIF1a regulated the expression of genes involved in cell proliferation, epithelial development, and cholesterol and surfactant biosynthesis which is important for alveolar cell formation. In contrast, inhibition of HIF2a couldn't prevent the hypoxia-induced airway differentiation. These results show that hypoxia and the HIF pathways play an important role in regulating lung epithelial progenitor fate during development. Moreover, preliminary data suggests that the HIF-induced transcriptional responses are conserved in human adult lung stem cells during hypoxia, such as that caused by severe injury.

Funding Source: Wellcome Trust. Cambridge Commonwealth, European & International Trust.

Keywords: Lung epithelial progenitors, Hypoxia and Hypoxia-Inducible Factors, Airway cell differentiation

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CENTRAL ROLE OF THE CDK1/TFCP2L1/ID2 CASCADE ON STEM-CELL PLURIPOTENCY AND BLADDER CARCINOGENESIS

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Accumulating evidence supports the notion that aberrant activation of pluripotency-associated genes is frequently observed in tumors, and stemness features have been shown to be associated with oncogenic dedifferentiation and tumor metastasis. Recently, we reported that overexpression of transcription factor CP2-like protein-1 (TFCP2L1) and its phosphorylation at Thr177 by cyclin-dependent kinase-1 (CDK1) are central mechanisms in embryonic stem cell (ESC) pluripotency as well as adult bladder carcinogenesis. In murine ESCs, this phosphorylation determined the binding of TFCP2L1 to targets related to cell cycle and differentiation processes. The physical and functional interaction between TFCP2L1 and CDK1 is conserved in human bladder cancer (BC) cells and modulate their proliferation and stemness features. However, the clinical relevance and therapeutic potential of this novel CDK1-TFCP2L1 molecular network remain elusive. Here, we demonstrated that the inhibitor of DNA binding-2 (ID2) functions as a crucial mediator by acting as a direct repressive target of TFCP2L1 for modulating the stemness features and cell survival of BC cells. Low ID2 and high CDK1 expression were significantly associated with unfavorable clinical characteristics. TFCP2L1 downregulated ID2 by directly binding to its promoter region. Consistently, ectopic expression of ID2 or treatment with apigenin, a chemical activator of ID2, triggered apoptosis and impaired cell proliferation, stemness features, and the invasive capacity of BC cells. Combination treatment with the specific CDK1 inhibitor RO-3306 and apigenin significantly suppressed tumor growth in an orthotopic BC xenograft animal model. This study demonstrates the biological role and clinical utility of ID2 as a direct target of the CDK1-TFCP2L1 pathway for modulating ESC function and bladder cancer progression.

Keywords: Bladder cancer, Transcription factor, CDK1/TFCP2L1/ID2 cascade

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EPITHELIAL TO MESENCHYMAL TRANSITION (EMT) IN HUMAN IPSCS CAN BE EXPLAINED BY A SMALL SUBSET OF GENES USING MACHINE LEARNING MODELS

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The Allen Institute for Cell Science aims to understand the principles by which human induced pluripotent stem cells (hiPSCs) establish and maintain robust dynamic localization of cellular structures, and how they transition between states during differentiation and disease. The epithelial to mesenchymal transition (EMT) is a cellular state change in which non-motile epithelial cells transition into motile mesenchymal cells. In addition to the switch in cell behavior, EMT is also characterized by changes in cellular identity (e.g., transcription, protein expression) and organization. To understand the interplay between these features, we use large-scale imaging and multimodal quantitative assays on EMT- directed hiPSCs from the Allen Cell Collection (allencell.org). Using a small molecule inhibitor of GSK-3β, we can direct hiPSCs to undergo EMT, characterized by cellular migration at ~20-25 hours post-treatment with a concomitant decrease in epithelial transcripts (SOX2, CDH1) and increase in mesenchymal transcripts (VIM, CDH2). To correlate transcription with this behavioral change, we collected GSK3-inhbited hiPSCs every three hours for 48 hours and performed scRNA-seq. We first confirmed that our ~43,000 cells largely clustered by timepoint, then ordered them into a latent time dimension via pseudotime analysis. Differential gene expression analysis identified that over 3000 genes undergo a statistically significant change associated with differentiation. To identify a small subset of genes that are both predictive of pseudotime and capable of being used in spatial transcriptomic assays (n < 500 genes) we implemented machine learning models that were able to accurately (MAE=1.05) predict cellular pseudotime using only 30 genes. Including more genes resulted in both minimal gain in accuracy as well as small importance scores suggesting that 30 genes is enough to predict hiPSC directed EMT. We will apply this set in spatial transcriptomic experiments that combine live imaging and cellular behavior annotations to integrate cell behavior with identified transcriptional states. Our study serves as a framework to understand the intersections between cellular organization, molecular identity, behavior, and environment to aid in understanding cell states and state transitions more generally.

Keywords: EMT, bioinformatics, hiPSC

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A NEW PATH TO REGENERATE CORNEAL ENDOTHELIUM

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Regeneration of the corneal endothelial tissue, fundamental for maintaining a clear vision, is prevented by human corneal endothelial cells (HCEnCs) poor proliferative capacity. The proliferative potential is partially retrieved in vitro, even though after a limited number of passages the cells undergo an endothelial-mesenchymal transition (EnMT) that alters cell morphology and protein expression, thus hampering any autologous regenerative treatment. EnMT is a process also occurring during normal stem cells expansion but needs to be finely regulated to be reversible. Although different strategies have been proposed to counteract the excessive EnMT in HCEnCs and prolong passages in culture, this process has still not been fully understood and successfully counteracted. In this perspective, we herein identified a single GSK-3 inhibitor, CHIR99021, able to revert and avoid EnMT in primary HCEnCs from old donors. HCEnCs can thus be maintained in culture until late passages in vitro (P8) without loosing their morphology, as shown from circularity assessment. In accordance, CHIR99021 reduced expression of a-SMA and CD44 EnMT markers, while restored endothelial markers such as ZO-1, Na+/K+ ATPase and N-cadherin. The CHIR99021 mediated EnMT reduction does not increase proliferation, as demonstrated by FACS, cell count and upregulation of the proliferation repressor p21, similarly to what previously found in neural stem cells. CHIR99021 reversed for the first time EnMT process at late passages in HCEnCs from old donors, an innovation in this field that allows using a greater number of discarded corneas deriving from old donors and opens the way for a more controlled regeneration of this tissue. These results represents therefore a novel and important advancement towards the development of corneal endothelial regenerative therapies.

Keywords: Corneal endothelial regeneration, Endothelial-mesenchymal transition, GSK-3 inhibitor

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CD99 MAINTAINS HOMEOSTASIS AND REGULATES DIFFERENTIATION IN HUMAN EPIDERMIS

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CD99 is a transmembrane glycoprotein encoded by the MIC2 gene. CD99 was reported to be expressed in the epidermis in 2016, but its function has not been elucidated. This study aimed to clarify the role of CD99 in the human epidermis. CD99 was particularly expressed in cells of the basal layer of the human epidermis. When normal human epidermal keratinocytes (NHEKs) were cultured on plastic dishes, they were strongly expressed in membranes in contact with other cells. NHEKs with high or low CD99 expression were separated by flow cytometry and analyzed for gene expression. NHEKs with high CD99 expression had significantly increased expression of differentiation-related genes such as K14 and HAS3 and genes related to barrier function such as CDH1 and CLDN1. We found that NHEKs with high CD99 expression decreased their colony-forming ability when cultured on culture dishes seeded with feeder cells. Epidermal cell sheets made by NHEKs with high CD99 expression did not show sufficient stratification. Furthermore, the knockdown of CD99 in NHEKs resulted in cell hypertrophy, fewer cells expressing Ki67, and decreased not only differentiation markers such as IVL, LOR, and FLG, but also stem cell markers such as p63, and K14. These findings suggest that a decrease in CD99 causes abnormalities in various markers of stem cells and differentiation markers required for normal differentiation of NHEKs. Therefore, CD99 appears to be involved in maintaining skin homeostasis and initiating early differentiation in the human epidermis.

Keywords: Human Epidermal Keratinocytes, Differentiation Marker, Tissue Engineering

TOPIC: GERMLINE AND EARLY EMBRYO

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ALTERING STEM CELL STATES BY CONTROLLING CELL SIGNALLING INFORMATION

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Cell fate determination induced by cell signaling is central to stem cell and developmental biology. Pluripotent stem cells such as embryonic stem cells (ESC) are an attractive model for understanding the relationship between cell signaling and cell fates. Cultured mouse ESCs can exist in multiple cell states resembling distinct stages of early embryogenesis, such as Totipotent, Pluripotent, Primed and Primitive Endoderm. The signaling mechanisms regulating the Totipotent state acquisition and coexistence of these multiple cell states are poorly understood. Here we identify BMP4 as an inducer of the Totipotent state. However, we discovered that BMP4-mediated induction of the Totipotent state is constrained by the cross-activation of FGF, TGF-β, and WNT pathways. We exploited this finding to enhance the proportion of Totipotent cells in ESCs by rationally inhibiting the cross-activated pathways using small molecules. Activation of the BMP4 signaling in combination with inhibition of the cross-activated pathways resulted in efficient reprogramming of ESCs to the Totipotent state. Next, we utilized single-cell mRNA-sequencing (scRNA-seq) to analyze the resulting impact on cellular heterogeneity. The scRNA-seq analysis revealed that induction of the Totipotent state is accompanied by the suppression of both the Primed and Primitive Endoderm states. Furthermore, the reprogrammed Totipotent cells generated in culture have a molecular and functional resemblance to the Totipotent cell stages of the preimplantation embryo. Our findings reveal a BMP4 signaling mechanism in ESCs to regulate multiple cell states, potentially significant for managing stem cell heterogeneity in differentiation and reprogramming.

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Keywords: Totipotency, Embryonic Stem Cell Heterogeneity, Cell Signalling in Stem Cells

TOPIC: GERMLINE AND EARLY EMBRYO

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INHIBITION OF BMP4-MEDIATED CROSS-ACTIVATION OF SIGNALING PATHWAYS ENHANCES TOTIPOTENT-LIKE STEM CELLS WITH BLASTOID FORMING POTENTIAL

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Embryonic stem cells (ESC) are defined by their inherent ability to self-renew and give rise to all cell types within the organism. Recent evidence suggests that cultured mouse ESCs co-exist in multiple phenotypic states such as unbiased pluripotent state (naïve), totipotent (2Cell-like), EpiSC (primed) and are driven by distinct transcriptional regulators. An important aspect of these specific cell states is that they have different developmental potency: while totipotent cells can give rise to both embryonic and

extraembryonic tissues, pluripotent cells give rise to only embryonic tissues and primed only to certain embryonic tissues. However, the signaling mechanisms initiating this remarkable developmental plasticity (co-existence of diverse cell fates) in genetically identical cells are unclear. Moreover, it remains unresolved which signaling pathway (if any) among those commonly active in ESC culture conditions is important to induce the Totipotent state. In this study, by examining various ESC culture conditions, we identified BMP4 signalling to promote Totipotent state. In addition, we also observed alternative cell states to be co-expressed and found BMP4 mediated signaling to curtail the induction of totipotent state through cross-activation of FGF, Nodal and WNT pathways associated in promoting pluripotent and primed cell states, respectively. Furthermore, perturbation of cross-activated pathways using putative small molecules enabled in identification of a reprogramming condition (namely LBPXRS) to enhance totipotent state in ESCs. Integrated single-cell RNA-seq analysis of pre- and post-implantation mouse embryo, various ESC culture conditions and LBPXRS confirm the molecular features of LBPXRS akin to totipotent cell stages of pre-implantation embryo. Lastly, generation of in-vitro blastocyst-like structures (Blastoids) of phenotypically reprogrammed totipotent cells exhibited functional and molecular features akin to totipotent cell stages of preimplantation embryos. Therefore, our findings reveal a novel BMP4-mediated signaling mechanism in ESCs to regulate diverse cell fates, and close association between BMP4 signaling cross-activation and totipotent-like reprogramming.

Keywords: Totipotent-like stem cells, Signalling cross-activation, Blastoid

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CRISPR-MEDIATED GENOME-WIDE SCREENING IN PRIMORDIAL GERM CELL SPECIFICATION

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The germ cell lineage ensures the transmission of heritable genetic and epigenetic information to the next generation. In mammals, the pluripotent epiblast cells in the post-implantation embryos give rise to both the somatic and germ cell lineages during subsequent development. Under inductive cell interactions, a small number of epiblast cells adopt the fate of the primordial germ cells (PGCs) and become the multipotent precursor of germ cells. However, the underlying mechanism is not fully understood. Fortunately, the epiblast to PGC specification can be recapitulated in vitro using embryonic stem cells (ESCs), albeit with low efficiency. The specification of PGCs is accompanied by the induction of key germ-cell genes, repression of the nascent somatic program, and widespread epigenetic remodeling. Here, we carried out a CRISPR/Cas9-mediated genome-wide genetic screen in PGC-like cells (PGCLCs) in vitro differentiation system and identified several key factors that are epigenetic barriers to PGCs specification. Phenotypically, when the knockout of these genes or the use of chemical inhibitors against them, it promoted PGCLCs differentiation significantly up to ~20% in vitro. Consistently, the transcriptional analysis showed that the germ cell maker genes are highly activated in the knockout cells. We are currently using genomic approaches including RNA-seq and Cut&Run to dissect the underlying mechanism. Further, we are generating knockout mouse models to test whether the deletion can promote PGC specification in vivo. Together, our study uncovered epigenetic barriers in PGC cell fate specification and illustrated the power of genetic screens in the study of cell fate transitions.

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Keywords: primordial germ cells (PGCs), CRISPR-mediated screening, epigenetic barriers

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AN RNAI SCREEN OF RNA HELICASES IDENTIFIES EIF4A3 AS A REGULATOR OF EMBRYONIC STEM CELL IDENTITY

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Embryonic stem cells (ESCs) can self-renew and differentiate into multiple cell lineages, the unique feature collectively known as pluripotency. Post-transcriptional and translational regulatory layers are being increasingly scrutinized for their roles in controlling pluripotency. In these regulatory layers, RNA helicases are heavily involved in RNA metabolism to direct their roles in gene expression, yet their functions in pluripotency control remain largely unexplored. To systematically explore the roles of RNA helicases in ESCs and dissect their mechanistic actions on stem cell pluripotency, we started from an RNA interference (RNAi) screen of RNA helicases and identified that eIF4A3 (aka Ddx48), a DEAD-box (Ddx) helicase component of the exon junction complex, is essential for the maintenance of ESCs. Mechanistically, we show that eIF4A3 depletion causes the loss of pluripotency via cell cycle dysregulation. eIF4A3 binds to mRNAs encoding the pluripotency-related cell cycle regulators and controls these transcripts in a post-transcriptional manner. Specifically, eIF4A3, particularly its helicase activity, is required for the efficient nuclear export of Ccnb1 mRNA, which encodes Cyclin B1, a key component of the pluripotency-promoting pathway during the cell cycle progression of ESCs. Our results reveal a previously unappreciated role for eIF4A3, emphasizing its function as an RNA helicase in maintaining stem cell pluripotency through post-transcriptional control of the cell cycle.

Keywords: RNA Helicase, eIF4A3, Pluripotency

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IDENTIFICATION OF ENPEP AS A CRUCIAL EPIGENETICALLY REGULATED MARKER DURING EARLY TROPHOBLAST DIFFERENTIATION

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Early trophoblast differentiation is crucial for embryo implantation, placentation and fetal development. Previous studies revealed that dynamic changes in DNA methylation occurred during pre-implantation embryo development and a few epigenetic gate-keepers were identified to safeguard the trophoblast fate. However, the underlying regulatory mechanism is poorly understood. Recently, we established human expanded potential stem cells (hEPSC-em) directly from preimplantation embryos, providing a powerful tool for studying early trophoblast differentiation. To investigate the roles of DNA methylation on trophoblast differentiation, trophoblastic spheroids derived from hEPSC-em were treated with Tet methylcytosine dioxygenase inhibitor (dimethyloxalylglycine, DMOG) and DNA methyltransferase (5-Azacytidine, 5-AzaC) inhibitor during differentiation. We found that DMOG treatment hindered trophoblast differentiation with reduced expression of trophoblast markers including KRT7, HLA-G, SDC1, ERVW-1 and CGB3, and reduced the attachment of trophoblastic spheroids onto receptive Ishikawa cells; while 5-AzaC promoted trophoblast differentiation. We further conducted integrative and bioinformatic analyses to identify new epigenetic determinants. We found that Aminopeptidase A (ENPEP) was hypermethylated in 8-cell and morula-stage embryos with promoter methylation levels of 57.5% and 70.8% respectively, but hypomethylated in trophectoderm (37.3%) and trophoblastic stem cells (TSC) (1.6%), which was negatively correlated with its expression. Concordantly, progressive demethylation of ENPEP was detected during trophoblast differentiation. Moreover, DMOG treatment delayed demethylation of ENPEP and suppressed its expression. We generated an ENPEP-/- hEPSC-em line using CRISPR/Cas9 approach and found that ENPEP deletion delayed the downregulation of pluripotent markers and decreased the expression of trophoblast markers in hEPSC-em-derived trophoblastic spheroids. In addition, ENPEP-/- hEPSC-em line exhibited lower efficiency of TSC derivation and impaired its competence in extra-villous trophoblast and syncytiotrophoblast differentiation. These results suggested that ENPEP was epigenetically regulated and played a vital role in trophoblast fate commitment.

Funding Source: National Key Research and Development Program of China (2022YFC2702503), Shenzhen Science and Technology Program (KQTD20190929172749226), Health@InnoHK from Innovation and Technology Commission.

Keywords: Trophoblast differentiation, Human expanded pluripotent stem cells, DNA methylation

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SINGLE-CELL CLUSTERING AND CLASSIFICATION OF EMBRYONIC STEM CELLS USING GENE REGULATORY NETWORKS

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Individual cells often have distinct morphological, functional, and molecular features. Interest in such cellular heterogeneity within a biological sample or tissue is increasing studies using single-cell technologies such as single-cell mRNA-sequencing (scRNA-seq). Cell fate decisions of individual cells shape the normal development of an embryo as well as the disease state such as cancer. Thus, it is crucial to precisely understand the molecular mechanisms that drive these decisions. We culture mouse ESCs in non-reprogramming conditions (BMP4-mediated) and reprogramming conditions (inhibition of BMP4-cross-activated signaling pathways) to characterize and quantify the alterations in cell state heterogeneity. Our single-cell analysis confirmed reprogramming of mouse ESCs to the totipotent state by inhibiting BMP4-cross-activated signaling pathways. Molecular mechanisms can be illustrated and quantified by mathematical models called Gene Regulatory Networks (GRNs). Specific GRNs often drive cell fate specification during development and disease. Progenitor cells such as stem cells can transition from one functional state to another, an ability known as cell plasticity. For instance, embryonic stem cells (ESCs) are known to stochastically transition between the totipotent and pluripotent states. By using GRN, RNA velocity and trajectory bioinformatic tools, we predict and describe the molecular networks involved in pluripotent-to-totipotent state transitions in ESCs. Our results show that circadian clock genes, such as Bhlhe40, Bhlhe41, and Nr1d1, are important regulators in pluripotent-to-totipotent stem cell state transitions. In scRNA-seq analysis, cell states are partitioned and annotated by observing the expression of biomarker genes. This manual annotation task is often subjective and labor-intensive. We question the accuracy of describing a cell fate using clustering methods and biomarker-based annotation practices in single-cell analysis. As a solution, we propose to use Artificial Intelligence and GRNs to build classification tools that accurately annotate cell states (or fates) in single-cell analysis.

Keywords: Single-cell RNA sequencing analysis, RNA velocity and trajectory analysis., Gene Regulatory Networks

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THE PLURIPOTENCY FACTOR TEX10 FINETUNES WNT SIGNALING FOR PGC AND MALE GERMLINE DEVELOPMENT

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Testis-specific transcript 10 (Tex10) is a critical factor for pluripotent stem cell maintenance and preimplantation development. Here, we dissect its late developmental roles in primordial germ cell (PGC) specification and spermatogenesis using cellular (degron) and animal (conditional knockout) models. We discover that Tex10 binds the Wnt negative regulator genes, marked by H3K4me3, at the PGC-like cell (PGCLC) stage in restraining Wnt signaling. Depletion and overexpression of Tex10 hyperactivate and attenuate the Wnt signaling, resulting in compromised and enhanced PGCLC specification efficiency, respectively. Using the Tex10 conditional knockout mouse models combined with single-cell RNA sequencing, we further uncover critical roles of Tex10 in spermatogenesis, with Tex10 loss causing reduced sperm number and motility associated with compromised round spermatid formation. Notably, defective spermatogenesis in Tex10 knockout mice correlates with aberrant Wnt signaling up-regulation. Therefore, our study establishes Tex10 as a previously unappreciated player in PGC specification and male germline development by fine-tuning Wnt signaling.

Keywords: pluripotency, Spermatogenesis, Wnt signaling

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NAKED MOLE-RATS IN A DISH - A NOVEL IPSC-BASED TOOL TO UNDERSTAND MECHANISMS OF HETEROCHRONY, LONGEVITY AND REPRODUCTIVE LIFESPAN

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Although the average lifespan has increased over the last century, regulation of aging still remains elusive. In many organisms, evolution has bound lifespan tightly to fecundity. In flies and worms, removal of the germline promotes longevity, whereas a functional ovary in humans is associated with better health. Understanding the mechanistic relationship between fertility and aging would potentially benefit both fields. A species that defies evolutionary constraints on reproduction and longevity is the naked mole-rat (NMR), a small eusocial rodent with a >30-year life expectancy and lifelong fecundity. Similar to ants, NMRs live in colonies with different castes: one queen reproduces while other females remain pre-pubertal through social suppression. Observations that queens outlive subordinates, together with the potential for reproductive activation of subordinates upon queen removal, suggest an epigenetic basis for puberty and lifespan. An in vitro system would facilitate the study of eusociality, reproduction and longevity. Previously, NMR iPSCs exhibited limited pluripotency and were derived from animals of unknown origin. We devised a robust, efficient and scalable platform to generate bona fide NMR iPSCs from males, females, subordinates and queens from birth to 12 years of age, representing an entire NMR “colony in a dish”. These NMR iPSCs meet stringent pluripotency criteria, including ICM-like molecular signature, genomic stability as well as in vitro differentiation potential and in vivo integration in E3.5 mouse embryos. The duration of NMR reprogramming is exceedingly long, reminiscent of the previously reported prolonged 2-week pre-implantation. Strikingly, NMR iPSCs form blastoids resembling rodent blastocysts. Finally, we observed discrete phenotypes of iPSCs derived from queens compared to similarly aged subordinates, including slower proliferation and higher frequency of transgene silencing. These phenotypes indicate a retention of epigenetic memory of the source animal based on reproductive status, and further support protracted developmental tempo and epigenetic resilience in queen iPSCs as strategies for longevity. This collection of NMR stem cells provides an accessible toolbox

for mechanistic studies of early development, aging and reproductive longevity.

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Keywords: naked mole-rat, iPSC, epigenetic memory, fertility, aging

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INTEGRATED SINGLE-CELL ROADMAP OF EMBRYOS ALLOW INTERROGATION OF BLASTOID AND STEM CELL MODELS

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Single-cell profiling has allowed us to identify the lineage specification and temporal dynamics during early development. Several studies have profiled different stages of early development, ranging from pre-implantation to post-implantation to gastrulating embryos. However, technical differences present a barrier to data interpretation and further incorporation of new single-cell data. Here, we integrated these datasets to construct a single-cell roadmap of early embryo development spanning embryos obtained from embryonic day 3 to day 19. Detailed analysis of this roadmap unravelled key regulators driving lineage specification during implantation & gastrulation as well as cell-cell communication between cell types of different lineages. More importantly, this single-cell roadmap is designed such that new single-cell datasets can be easily incorporated and compared. This allowed us to interrogate recent blastocyst organoid (blastoid) models, assessing their developmental maturity and elucidating unknown cell populations. We envision that our single-cell roadmap can be further used to understand inter-species differences during early development and evaluate the fidelity of other in vitro differentiation models. Overall, this single-cell roadmap provides a comprehensive description of the molecular changes during early development, which will be of use by stem cell biologists to probe various stem cell models.

Keywords: single-cell atlas, early embryo development, blastoid

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PROTEOME ASYMMETRY IN MOUSE AND HUMAN EMBRYOS BEFORE FATE SPECIFICATION

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Pre-patterning of the embryo, driven by spatially localized factors, is a common feature across several non-mammalian species. However, mammals display regulative development and thus it was thought that the blastomeres of the early embryo do not show such pre-patterning. Unexpectedly, the blastomeres of 2-cell mouse and human embryos have been reported to have distinct developmental fates, potential and heterogeneous abundance of certain transcripts. Nevertheless, the extent of the earliest intra-embryo differences remains unclear and controversial. Here, utilizing multiplexed and label-free single-cell proteomics by mass-spectrometry, we show that the 2-cell mouse embryo contains an alpha and beta cell as defined by differential abundance of hundreds of proteins exhibiting strong functional enrichment for protein synthesis, transport, and degradation. The 4-cell mouse embryo also comprises alpha and beta cells with increasingly diverging proteomes. Intriguingly, halved mouse zygotes display asymmetric protein abundance that resembles alpha and beta blastomeres, suggesting differential proteome localization already at the zygote stage. When the subsequent fate of alpha and beta cells was assessed, alpha cells were found to be more likely to give rise to a blastocyst competent to develop further. Proteins involved in protein transport and degradation, including Gps1 and Nedd8, are highly abundant and asymmetrically distributed in alpha and beta cells. Depletion of Gps1 and Nedd8 in one cell of the 2-cell mouse embryo impacts lineage segregation in blastocysts. Human 2-cell stage blastomeres also form two clusters that share strong concordance with clusters found in mouse, both in terms of differentially abundant proteins and functional

enrichment. To our knowledge, this is the first demonstration of intra-zygotic and inter-blastomere proteomic asymmetry in mammals.

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Keywords: Single-cell proteomics, Mammalian embryonic development, Cell fate specification

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ZFP281 COORDINATES DE NOVO DNA METHYLTRANSFERASES AND TET1 IN DRIVING PRIMED PLURIPOTENCY

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The continuum of pluripotency encompassing the naïve, formative, and primed pluripotent stem cell states recapitulates the in vivo embryonic epiblast development during the pre-to-post-implantation period. The progression and transition between these pluripotent states are accompanied by a sharp activation of the de novo DNA methyltransferases and the reorganization of transcriptional and epigenetic landscapes. Here we identified Zinc Finger Protein 281 (ZFP281) as an essential factor in the formative-to-primed pluripotent state transition. Using a knockout mouse model and a knockin degron cell system, we revealed that transcription of DNMT3A/3B depends on the activity of ZFP281 in embryonic stem cells, epiblast-like cells, and epiblast stem cells. Mechanically, chromatin-bound ZFP281 and DNA hydroxylase TET1 are decreased in the formative state but recovered in the primed state and increases in chromatin-bound ZFP281 and TET1 are important to compete with DNMT3A/3B for establishing the DNA methylation and gene expression programs in the primed state. In addition, ZFP281 and TET1 depend on the formation of the R-loop structures at the ZFP281 target gene promoters for their chromatin occupancy. Our study demonstrates a comprehensive role of ZFP281 in modulating DNA methylation- and demethylation-associated transcriptional programs driving the formative-to-primed transition and maintaining primed pluripotency.

Keywords: Pluripotent state transition, DNA methylation, DNMT3 and TET1

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IDENTIFYING MOLECULAR REGULATORS OF MAMMALIAN DEVELOPMENTAL PAUSING USING A CRISPR/CAS9 SCREEN APPROACH

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Developmental pausing, also called diapause, is a reproductive mechanism employed to separate fertilization from birth by temporarily arresting embryonic development in response to environmental perturbations. Characterized as a unique state of development, the molecular mechanisms controlling diapause within the mammalian embryo are largely unknown. Our lab previously identified the mTOR pathway as a central regulator of mammalian diapause. In 2016, we reported that inhibition of mTOR induces a paused pluripotent state in mouse embryos and embryonic stem cells (ESCs) that recapitulates diapause. In order to broadly identify pathways that regulate diapause within the embryo, I have employed a genome-wide CRISPR/Cas9 screening approach using our mouse ESC pausing model. Data from this CRISPR screen approach and follow-up experiments has uncovered a diverse array of pathways and factors involved in metabolism, DNA repair, and chromatin regulation that I am exploring further. I have validated the screen hits in a secondary screen and am dissecting the role of chromatin factors that appear to provide vulnerabilities specifically in the paused state, and not in other pluripotent ESC states. Understanding the role of the pathways that regulate diapause will have implications for research on reproduction, development, aging, and cancer. Our most recent progress and findings will be discussed in further detail during the poster session.

Keywords: Diapause, Embryonic Development, Chromatin Regulation

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PLURIPOTENCY IS REGULATED BY SET-ESRRB

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The multifunctional protein SET is a chaperone of linker histone H1 and is also involved in many cellular processes, including inhibition of histone acetylation and in oncogenesis. In a pluripotent state, SET exists mainly in one isoform, SETα, whereas its alternative isoform, SETβ, is predominantly expressed in differentiated cells. In our previous work (Harikumar, Lim et al., Stem Cell Reports, 2020) using SET-KO ESCs, we found that absence of SET is embryonic lethal and leads to premature differentiation and transition out of a naïve pluripotent transcriptomic state. To date, attempts to obtain genome-wide binding profiles of SET have failed, and further, the functions of the two SET isoforms in ESCs remain elusive. To this end, we generated SET-KO cell lines capable of doxycycline-inducible addback of either HA-epitope tagged SETα or SETβ, enabling us to perform ChIP-seq experiments for SETα-HA and SETβ-HA independently and to identify binding-sites common and unique to both isoforms. Genomic profiling with our ChIP-seq data revealed that SET accumulates at the boundaries of characterised ESC super enhancers and at

the binding sites of several pluripotency factors, including Oct4, Nanog, and Sox2. In addition, SET is enriched at the promoters of expressed genes. Many of these genes are themselves targets of core pluripotency factors and are implicated in embryonic development and lethality phenotypes. Further, we identified several thousand ChIP-seq peaks shared by both isoforms; and a subsequent HOMER de novo motif discovery analysis detected a high enrichment of binding-motifs associated with nuclear receptors, including the pluripotency marker ESRRB. Interestingly, co-immunoprecipitation experiments of SET followed by liquid chromatography mass spectrometry (LC-MS/MS) identified a direct interaction with ESRRB. RNA-seq experiments following isoform-specific addback in a SET-KO background reveal that SETα has a greater repressive effect than SETβ on genes with proximal ESRRB binding sites. Many of these genes are related to aberrant early developmental phenotypes. Taken together, our results suggest a co-regulatory role for SET and ESRRB in pluripotency.

Keywords: Pluripotency, ESRRB, Super Enhancers

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UNIQUE ROLES OF DIFFERENT CANONICAL WNT LIGANDS IN EARLY HUMAN DEVELOPMENT

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WNT ligands play crucial roles in mammalian gastrulation and the formation of the A-P axis. Previous studies in mice have demonstrated that Wnt3 is necessary for initiating gastrulation, while other WNTs are less critical at gastrulation stage and play roles in later development. In 2D micropatterned human gastrulation models, WNT3, WNT3A, WNT6, and WNT8A are the canonical WNT ligands with the highest RNA expression. We tested whether these WNTs could function interchangeably during early development as might be expected from their high sequence conservation. We generated tetracycline-inducible hESC lines to over-express each WNT ligand together with a nuclear marker to trace and quantify their expression levels. We subjected these cell lines to differentiation assays that probe the capacity of these ligands to induce mesoderm from pluripotent cells, to induce neural crest within the ectoderm, and to posteriorize neural cells. We observed that WNT3 and WNT3A can perform all these functions while WNT6 and WNT8A only induce a subset of these cellular behaviors. Whether these differences result purely from variations in the potency of the ligands or to other functional differences requires further investigation. Overall, our findings demonstrate that different Wnt ligands should not be viewed as redundant, and caution is required in interpreting their contributions to various cell signaling and cell fate behaviors.

Funding Source: NIH award R01GM126122

Keywords: Wnt ligands, morphogens, spatial patterning

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UNRAVELING SMALL NONCODING RNAS AND THEIR CHROMATIN STATE IN TRIPLE LINEAGE DIFFERENTIATION

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Small non-coding RNAs (sRNAs) have increasingly been found to have a wide range of functions in both healthy and pathophysiological conditions. However, little is known about their regulatory programs during early development and their role in establishing cell identity. To address this, we differentiated in vitro two isogenic induced-pluripotent stem cell(iPSC) lines, derived from the same patient but from different tissues, towards mesoderm, endoderm, and ectoderm. We measured changes in the transcriptome by small noncoding-RNA sequencing and total RNA sequencing, chromatin accessibility by ATAC sequencing and methylation patterns by Twist Targeted Methylation Sequencing at various time points during differentiation, which allowed us to identify novel sRNA and mRNA markers at different stages of each germ layer establishment. By integrating all datasets together, we found genetic and epigenetic features that do not change during differentiation, suggesting an epigenetic ‘memory’ due to tissue origin in the different regulatory scales. Finally, we identify three categories of microRNA which, depending on whether they sit in inter-genic or intra-genic regions, share, or do not share, their regulatory mechanisms with their host gene. This study provides a first insight into the presence or absence of specific groups of sRNAs in iPSCs, based on their origin and upon differentiation into each of the three germ-layers.

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Keywords: differentiation, sequencing, noncodingRNA

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THE REGULATION OF P-BODY ASSEMBLY BY O-GLCNACYLATION OF PSME3 MAINTAINS THE PLURIPOTENCY IN MOUSE EMBRYONIC STEM CELLS

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Glycosylation plays important roles, such as cell adhesion, signal regulation, endocytosis, protein folding and protein stability. O-linked β-N-acetylglucosamine (O-GlcNAc) is the only glycosylation occurred in cytoplasmic and nuclear. O-GlcNAc is transferred to the serine (Ser) or threonine (Thr) residues of proteins by O-GlcNAc transferase (OGT) and removed from proteins by O-GlcNAcase (OGA). Mouse embryonic stem cells (mESCs) were established from pre-implantation embryos and maintain pluripotency by the complicated mechanisms. We and some groups have been revealed that O-GlcNAc is required for maintenance of ESCs pluripotency. However, its mechanism is still largely unclear because there are divergent O-GlcNAcylated proteins. Here, we explored O-GlcNAcylated proteins in mESCs and mouse epiblast-like cells (mEpiLCs). We found that proteasome activator subunit 3 (Psme3) protein as a O-GlcNAcylation protein in both mESCs and mEpiLCs. It has been reported that Psme3 binds to the 20S proteasome and facilitates protein degradation. However, the relationship between regulation of mESCs pluripotency and that of proteasome regulation by O-GlcNAc is unknown. Psme3 was O-GlcNAcylated on Ser111 and interacted with DEAD box polypeptide 6 (Ddx6), which is necessary for processing bodied (P-bodied) assembly. P-bodies are cytoplasmic organelles that lack membranes and are formed by phase separation involved in the storage or decay of untranslated mRNAs. Loss of O-GlcNAcylation on Se111 of Psme3 caused inhibition of the interaction between Psme3 and Ddx6 and an increased in P-bodies, result in mESCs differentiated. Moreover, expressions of pluripotent core transcription factors such as Klf2 and Klf4 were decreased at the protein level and P-bodies were increased in mESCs by reduction in O-GlcNAcylation on Se111 of Psme3. Our results demonstrated that O-GlcNAcylation of Psme3 is required for maintenance of the pluripotency in mESCs through regulation of P-body assembly.

Funding Source: This work was supported by Japan Society for the Promotion of Science (JSPS) KAKENHI grant JP18K06139 (to S.N.) and Japan Science and Technology Agency (JST)-Mirai Program grant JPMJMI18GB (to S.N.).

Keywords: O-GlcNAc, pluripotency, P-body

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DIVERGENT ROLES OF OCIAD FAMILY PROTEINS IN MITOCHONDRIA GOVERN HUMAN PLURIPOTENT STEM CELL DIFFERENTIATION

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Human pluripotent stem cells (PSCs) exist in a continuum of developmental sub-states with diverging potencies. How these discrete cellular states arise, transition and ultimately impact differentiation outcomes is unclear. Regimens that can prime cells for selective differentiation, while simultaneously retaining attributes of pluripotency, can be exploited for controlled and efficient expansion strategies. Increased insights into mechanisms controlling stem cell sub-states is required to trap and expand such tunable states in vitro. We previously reported that the conserved OCIA domain containing protein, Asrij/OCIAD1 promotes pluripotency and is downregulated during differentiation. Notably, Asrij/OCIAD1 depletion in human embryonic stem cells (hESCs) generates a metabolic sub-state with increased oxidative phosphorylation and propensity for differentiation. Loss of Asrij/OCIAD1 enhances mesoderm specification through a shift in mitochondrial dynamics and energy metabolism. Here we investigated the role of its paralog, OCIAD2 in pluripotency and differentiation of hESCs. We observed that OCIAD2 expression increases as stem cells differentiate. Constitutive OCIAD2 overexpression or its depletion by CRISPR/Cas9-mediated gene targeting, perturbed mitochondrial morphology and increased ROS levels, but had no apparent effect on pluripotency. However, directed mesoderm differentiation analysis revealed that OCIAD2 facilitates differentiation of hESCs. Interestingly, pharmacological enhancement of mitochondrial respiration in OCIAD2 depleted hESCs restored their mesoderm differentiation potential. Thus, OCIAD proteins play distinct and opposing roles in pluripotent stem cells, which may reflect differences in mitochondrial function. Taken together, our study proposes how mitochondrial activity of pluripotent stem cells can be tuned to give distinct differentiation responses. This will help identify targets for modulating mitochondrial function to devise strategies for enhancing the differentiation potential of human stem cells.

Keywords: Human pluripotent stem cell differentiation, mitochondria, mesoderm differentiation

TOPIC: HEMATOPOIETIC, IMMUNE, AND ENDOTHELIAL

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REPROGRAMMING OF HUMAN PLURIPOTENT STEM CELLS-DERIVED MACROPHAGES TOWARDS KUPFFER-LIKE CELLS AND INHIBITION OF PATHOGEN INFECTION

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Many human pathogens target or primarily affect the liver due to the large volume of blood going through this organ. The myriad of hepatic sinusoids offers a nutrient-rich microenvironment for microorganisms to thrive. Kupffer cells are the largest population of tissue residential macrophages and have pivotal roles in the defense against pathogens in the liver. Kupffer cells are derived from yolk sac myeloid progenitors (YSMPs), formed early during embryogenesis, then migrate and reside in the liver. We have established a simple and highly efficient protocol to produce a large number of macrophages (iMACs) from human pluripotent stem cells. ScRNA-seq analysis revealed that cells in the induced differentiation culture mainly comprised macrophages and monocytes. These iMACs displayed robust phagocytotic activity but relatively weak inflammatory cytokine release activity compared to adult peripheral blood monocyte-derived macrophages. Upon co-culture with human hepatocytes, iMACs adapt to the tissue microenvironment, transform to Kupffer cell-like morphology, display corresponding gene expression change and reprogrammed their epigenome. Kupffer-like iMACs showed a strong inhibitory effect towards HCV and pathogenic bacteria S. Pneumoniae. The inhibition depends on iMAC in direct contact with pathogens and requires scavenger receptors. Upon engulfing different pathogens, iMACs turned on distinct sets of immune genes depending on the type of pathogens. Our results suggest that human pluripotent stem cells derived iMACs can be a useful model system to study the immunopathogenic response in liver infection.

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Keywords: Kupffer cells, human pluripotent stem cells, Infection

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ENHANCING THE IMMUNOTHERAPEUTIC EFFECT OF CYTOKINE INDUCED KILLER CELLS BY CHEMICAL INDUCTION TO TREAT PANCREATIC CANCER

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The development of immunotherapy using autologous or allogeneic immune cells, such as cytokine-induced killer cells (CIK) or natural killer cells, has had an enormous impact on the treatment of cancer in recent years. However, most of the immunotherapy clinical trials for pancreatic ductal adenocarcinoma (PDAC) patients failed to reveal good clinical outcomes due to the immunosuppressive microenvironment of the PDAC and the cancer-associated fibroblast (CAF). In this study, two strategies were proposed to enhance the immunosuppression effects: activating the CIK and regulating the immunosuppressive microenvironment of PDAC and CAF. We showed that a small molecule, n-butylidenephthalide (BP), with multi-targeting characteristics including inhibiting the immune checkpoint PD-1 on immune cells, suppressing CD47 on cancer cells, as well as down-regulating the immunosuppressive cytokines including IL-6 and IL-1α/β secreted by CAFs. To further explore the feasibility of BP in the treatment of immunosuppressive PDAC, a CAF-PDAC xenograft murine model was established. Besides, a biocompatible wafer containing BP with slow-releasing characteristics was designed for the in-situ regulation of the local environments. It was found that the combination of wafer implantation and CIK injection could significantly reduce the CAF-PDAC tumor growth when compared to the CIK-only or the wafer-only group. It was proven that local delivery of BP successfully regulated the CAF-PDAC microenvironment and enhanced the therapeutic effects of CIK. These findings demonstrated that BP might have the potential to break through the limitation of CIK therapy for PDAC patients.

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Keywords: Activation of cytokine induced killer cells, Treatment for pancreatic cancer, Regulation of cancer microenvironment

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LINEAGE TRACING OF 3D-CULTURED T CELL DEVELOPMENT REVEALS TRANSCRIPTIONAL SIMILARITY BETWEEN T-IPS-T CELLS AND HUMAN THYMOCYTES

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T cells induced from induced pluripotent stem cells (iPSCs) derived from antigen-specific T cells (iPS-T cells) are an attractive tool for T cell immunotherapy. In CAR-transduced T cells, primary CD8 + T cells in combination with CD4 + T cells showed superior antitumor reactivity in vivo. It indicates potential antitumor efficacy of CD8+ iPS-T therapy combined with CD4+ iPS-T cells. We previously reported the induction method of cytotoxic T cell derived iPS-T (T-iPS-T) cells from hematopoietic progenitor cells induced from iPSCs(iHPCs). After that, the cytotoxic T-iPS-T cell induction is well established, however, the induction of T-iPS-T cells which have helper potential is still challenging. Recently, 3 dimensional (3D) organoid culture made it possible to induce CD4+ iPS-T cells from iPSCs, but this method requires the use of a murine cell line, making it difficult to develop a large-scale production. We successfully induced mature CD4+ T-iPS-T cells and CD8+ T-iPS-T cells from iHPCs by 3D organoid culture. To find key genes for the induction of CD4+ T-iPS-T cells in feeder-free CD4+ differentiation culture, we performed lineage tracing analysis of 3D-cultured T-iPS-T cell maturing process by scRNA-seq and compared the result with a previously reported dataset of human thymocytes. Weekly UMAP plot of T-iPS-T cells showed gradual maturation confirmed by reported transcriptional markers. In merged plots of T-iPS-T cells and human thymocytes, T-iPS-T cells were plotted on the maturation process depicted by human thymocytes. It revealed transcriptional similarity in maturing process between T-iPS-T cells and human thymocytes and implied gene candidates for inducing CD4+ T-iPS-T cells. These results may promote the clinically applicable production of helper T-iPS-T cells.

Keywords: iPS-T, scRNA-seq, CD4

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REGENERATION OF INVARIANT NKT CELLS FROM IPS CELLS WITH 3D CULTURE SYSTEM

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Recently, cancer immunotherapies have received much attention, but their efficacy against solid tumors has been difficult to achieve. invariant NKT (iNKT) cells can be expected to have therapeutic effects on solid tumors due to their adjuvant effects and ability to improve the immunosuppressive microenvironment of tumor. However, acquiring sufficient numbers of iNKT cells from patients is an obstacle to iNKT cell-based immunotherapy because their frequency in the total lymphocyte population is only 0.01-0.1%. To solve this problem, we have reported the regeneration and expansion of human iNKT cells via induced pluripotent stem cells (iPSCs) with 2D culture system (2D-re-iNKT cells). 2D-re-iNKT cells can mature dendritic cells (DCs) and subsequently induce cytotoxic T cells. However, 2D-re-iNKT cells have some characteristic deviations from primary iNKT cells, such as the lower proliferative capability and the Th1-skewed profile. We hypothesized that these differences resulted from inefficiencies in the in vitro differentiation procedure. In this study, we differentiated human iPSCs into iNKT cells by 3D organoid culture system (3D-re-iNKT cell). 3D-re-iNKT cells expressed the specific TCR of iNKT cells and showed a higher proliferative capacity than 2D-re-iNKT cells when stimulated with αGalCer, an antigen widely used in iNKT cell research and clinical trials. Furthermore, when immature DCs and 3D-re-iNKT cells were co-cultured to investigate the adjuvant effect on 3D-re-iNKT cells, the expression of mature markers of DCs was up-regulated and the production of IL-12 was detected. These results together indicate regeneration of iNKT cells from iPSCs by 3D culture system gives rise to regenerated iNKTs with functions comparable to primary iNKT cells and provides a better cell source for iNKT cell-therapy.

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Keywords: T cell, iPS cell, cell therapy

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EZH2 INHIBITION IMPROVES DEFINITIVE HEMATOPOIETIC STEM CELL DIFFERENTIATION FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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De novo generation of human hematopoietic stem cells (HSCs) from induced pluripotent stem cells (iPSCs) holds immense potential for cellular therapy of blood disorders. However, current protocols induce iPSCs to produce immature progenitors with limited multilineage engraftment capacity in vivo. Emerging evidence indicates that acquisition of a definitive HSC phenotype during ontogeny is restricted by a distinct epigenetic barrier. In particular, the Polycomb Repressor Complex 2 (PRC2) participates in transcriptional repression during hematopoietic development via H3K27me3 methylation at target genes. PRC2 consists of multiple core subunits, including EZH2 and its homolog EZH1. Knockdown of EZH1 during iPSC differentiation was shown to promote the emergence of hematopoietic cells with multipotentiality but their functionality in vivo was not demonstrated. In another study, inhibition of EZH2 overcame the protracted cellular maturation of newly born neurons in iPSC cultures. Here, we hypothesized that repression of EZH2 by addition of a small molecule inhibitor (GSK126) at various stages of human iPSC differentiation might enable molecular maturation of primitive hematopoietic progenitors in culture and elicit a definitive HSC phenotype. To determine the impact of GSK126 on the extent of HSC maturation, we first tracked the CD45+CD34+CD45RA-CD90+ cell surface phenotype customarily accepted to define a population enriched in HSCs. Inhibition of EZH2 at the endothelial-to-hematopoietic transition stage of iPSC differentiation significantly increased percentages (12% to 24%) of phenotypically-defined HSCs at day 12 of differentiation. Next, we assessed the self-renewal and proliferative capacities of iPSC-derived cells in secondary clonogenic assays. Notably, we observed a significant (up to 30-fold) increase in replating efficiency from cells arising from GSK126-supplemented cultures compared to controls. Ongoing single-cell transcriptomics and transplantation analyses will further confirm HSC identity and function in vivo. Hence, inhibition of key epigenetic regulators may be required to promote the full functionality of HSCs engineered ex vivo.

Keywords: induced pluripotent stem cell (iPSC), hematopoietic stem cell (HSC), EZH2

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INVESTIGATING TRANSCRIPTIONAL NETWORKS INVOLVED IN BLOOD LINEAGE COMMITMENT

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Direct reprogramming of somatic cells could represent a safer therapeutic alternative than the differentiation of iPSCs, bypassing a pluripotent state to generate patient-specific blood cells for regenerative medicine. However, this potential is hindered by the stochastic nature and low efficiencies of reprogramming. To investigate dynamic cellular processes involved in reprogramming MEFs to HSPCs we developed a dox-inducible system to reprogram MEFs by overexpressing only two transcription factors, TAL1 and LMO2. Reprogrammed cells are positive for hematopoietic cell surface markers, have colony-forming potential and express many HSPC marker genes. iHSPCs generated can differentiate into cells of myeloid, erythroid and megakaryocyte lineages. Bulk RNA sequencings at regular intervals (Days 2, 4 and 14) during reprogramming reveal the upregulation of hematopoietic genes and the downregulation of fibroblast genes as early as day 2. When integrated with ATAC sequencing data, our results show that accessibility and expression of several TFs required for specifying haematopietic phenotype such as Gata1 and Fli1, are increased. Single-cell RNA sequencing analyses were performed to define the specific population of successfully reprogrammed cells in starting fibroblasts as wel as early stage reprogrammed cels to determine the factors driving their success. Trajectory analysis uncovered a small subpopulation of cells which transitioned from fibroblast towards a hematopoietic state and exhibited increased expression of endothelial to hematopoietic transition (EHT) marker genes such as Sox7, Gata2 and Procr. These cells are distinct from the remaining population due to their specific expression of hematopoietic factors and the cell cycle phase in which they reside. Preliminary data indicates expression of Heparin binding growth factor by fibroblasts is associated with a higher likelihood of successful reprogramming. Further validations are underway to determine whether the expression of these genes can reduce the stochasticity of reprogramming and increase its efficiency.

Keywords: Direct reprogramming, Induced HSPCs, scRNA Seq

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DEVELOPING IPSC-DERIVED MACROPHAGES (IMACS) FOR OFF-THE-SHELF CELL THERAPY - A VERSATILE NEW CELL TYPE IN THE EVOCELLS PLATFORM FOR IMMUNO-ONCOLOGY AND BEYOND

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Induced pluripotent stem cell (iPSC)-derived immune cells have opened a wide range of possibilities for cell therapy ranging from applications in the field of immuno-oncology to regenerative medicine. Off-the-shelf immune cells derived from iPSC offer the opportunity to overcome many of the challenges connected to autologous approaches including limitations in patient material, as well as manufacturing and logistic complexity. Recently, macrophages have gained increasing attention for clinical translation due to their unique plasticity and immunomodulatory characteristics. This is also reflected in the emergence of CAR-directed autologous macrophages as a new potential treatment for solid tumors, due to their characteristics beneficial for accessing and reprogramming the immunosuppressive tumor microenvironment. We aim to generate genetically modified iPSC-derived macrophages (iMACs) as an innovative, off-the-shelf source for cell therapies. Using a validated GMP iPSC line, we have established a proprietary feeder- and cell sorting-free 3D differentiation protocol that enables robust and scalable production of iMACs. To ensure reproducible high quality and safety of our cell product, we perform stringent monitoring of all process stages using flow cytometry, transcriptome analysis (scRNAseq), as well as functional assays comparing iMACs to blood-derived counterparts.

Keywords: Macrophages, iPSC, Cell Therapy

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HEMATOPOIETIC STEM CELL-DERIVED PLATELET PROGENITORS POSSESS STRIKING HETEROGENEITY THAT CHANGES WITH AGE

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Hematopoietic stem cells differentiate into all blood cells, including Megakaryocyte progenitors (MkPs) and subsequently, platelets. Recently, our lab uncovered molecular and functional differences between young and old MkPs. This finding has led us to define two phenotypic subpopulations of MkPs; canonical (cMkPs), and rare non-canonical (ncMkPs). We have also discovered that this initial MkP heterogeneity changes with age, as ncMkPs dramatically and specifically increase in number and frequency. Deepening our understanding of platelet generation has the potential to inform cardiovascular and thrombotic disease, a leading global health concern among the aged population. We sought to uncover underappreciated heterogeneity among young, steady-state MkPs, and if that changed with age. First, we asked what surface proteins define young, unperturbed MkPs. We performed targeted flow cytometry analysis informed by bulk RNAseq of MkPs to identify and test candidate markers. We found that specific cell surface proteins exhibited differential expression among steady-state MkPs, indicating greater heterogeneity than previously observed. To better visualize steady-state heterogeneity, we combined nine candidate markers that demonstrated bimodal or highly variable expression across MkPs and applied dimensionality reduction analysis (UMAP) to our standardized flow cytometry data. We observed striking heterogeneity among young MkPs and defined multiple clusters of phenotypically-unique subpopulations. Second, we assessed MkP heterogeneity of old mice in parallel to young. Interestingly, aged MkPs also demonstrated elevated heterogeneity at both individual marker and combinatorial (UMAP) levels. Further, specific subclusters of MkPs were expanded with advancing age. Finally, we assessed human MkPs for features analogous to the mouse. We found that, similar to mice, human MkPs possess phenotypic heterogeneity that changes with age. Collectively, this new data defines phenotypically distinct subpopulations of MkPs in both mice and humans that change with age. Excitingly, these findings may advance our understanding of platelet generation by specific MkP subpopulations, whose changes with age could contribute to uncovering basic biology underlying thrombotic and cardiovascular disease.

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Keywords: Stem and progenitor cell heterogeneity, Aging, Megakaryocyte progenitor and platelet

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TUNABLE OUTCOMES IN VASCULAR DIFFERENTIATION USING COMPUTATIONALLY DESIGNED PROTEIN AGONISTS

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Cells progress through a highly specific series of lineages during development that are spatially and temporally controlled. This process relies on the secretion and reception of extracellular cues through complex signaling networks that control vital biochemical, transcriptional and structural processes. Growth factors and cytokines achieve this by causing self-association of receptor pairs in the plasma membrane; however, the precise signaling requirements for differentiation can be studied in more detail using synthetic ligands that either 1) drive receptor association in novel geometries and valencies, or 2) exhibit specificities for receptors of signaling pathways not achievable by native growth factors. In this study we designed robust and hyperstable cyclic homo-oligomers with up to 8 subunits using repeat protein building blocks that can be modularly extended. Cryo-electron microscopy structures and solution x-ray scattering data closely match the design models. By incorporating a designed fibroblast growth factor receptor (FGFR) c-isoform specific binding module, we use these scaffolds to drive FGF receptor association and observe potent valency- and geometry-dependent activation of Ca2+ and MAPK pathways. We also showed that these synthetic agonists have a capacity to drive endothelial cell differentiation through an FGF-mimetic trajectory. Interestingly, we demonstrate that the c-isoform specificity of our designs allows us to control the outcomes of vascular differentiation into either endothelial or perivascular fates, and into highly specific arterial, venous or lymphatic endothelial fates. Our results highlight the potential of using highly specific designed agonists to precisely investigate the signaling requirements for cellular differentiation processes, towards the development of better regenerative strategies.

Keywords: Vascular differentiation, Computationally designed proteins, FGF signaling

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EXTRAMEDULLARY HEMATOPOIESIS AND IMMUNE TRAINING DRIVE INFLAMMATION AND ASSOCIATED ORGAN DAMAGE IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Extramedullary hematopoiesis (EMH) is an emerging player in autoimmune disorders to meet the increased demand for effector cells in the periphery. In Systemic Lupus Erythematosus (SLE), deregulation of hematopoiesis is characterized by myeloid skewing and priming of proinflammatory hematopoietic stem/progenitor cells (HSPCs). Here, we use the NZBW/F1 model to explore the contribution of EMH to SLE pathogenesis. EMH takes place in the F1-L (NZBW/F1-Lupus) spleen and correlates with lupus nephritis (LN) activity. Transcriptomic analysis of splenic HSPCs reveals a higher inflammatory potential than their bone marrow (BM) counterparts. β-glucan -an inducer of innate immunity- exacerbated splenic EMH, increased neutrophil production and worsened LN. Mechanistically, the methylome of BM-HSPCs supports epigenetic tinkering towards their myeloid skewing. Transcriptomic signatures of HSPCs in patients with high disease activity show similar changes to the ones observed in mice. EMH and trained immunity contribute to SLE pathogenesis by sustaining and amplifying the inflammatory response and increasing the risk for flare.

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Keywords: Hematopoietic Stem and Progenitor Cells, Autoimmunity, Extramedullary Hematopoiesis

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IDENTIFICATION OF GENE REGULATORY NETWORKS ESSENTIAL FOR HEMATOPOIETIC PROGENITOR CELL AND T CELL EMERGENCE FROM HUMAN PLURIPOTENT STEM CELL VIA CRISPR SCREENS

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Established cell-based therapies using haematopoietic stem and progenitor cells (HSPCs) and T cells have demonstrated a tremendous potential for treating blood disorders and cancer. However, access to the therapy is limited by the shortage of compatible donors and the complexity of patient-specific manufacturing processes. Human pluripotent stem cells (hPSCs) differentiation represents a renewable way to generate HSPCs and T cells in vitro. While there have been attempts to differentiate hPSCs into various haematopoietic cell types, the intrinsic and extrinsic regulatory mechanisms that control the generation of definitive HSPCs and T cells are not fully known. Our lab has recently developed a chemically defined and robust protocol to generate T cell competent HSPCs from hPSCs. Using single-cell RNA-sequencing, we have identified differentially expressed genes (DEGs) across hemogenic endothelial cell, HSPC, mast cell, myeloid cell, erythroid cell, and progenitor T cell populations. To improve T cell yield and to understand the impact of individual transcription factors (TFs) on HSPC emergence and the establishment of T cell competence, we performed a CRISPR-Cas9 knockout (KO) screen targeting 70 TFs. In our preliminary analysis, we have confirmed the importance of known T-cell specific TFs GATA3 and TCF7 during T cell emergence. Specifically, removal of these two genes reduced the output of CD5+, CD7+ T cell progenitors while not affecting the emergence and expansion of CD43+, CD45+ HSPCs. We have also identified additional top candidate genes including YBX1 to be essential for T cell development. Building on this foundation, we plan to perform Perturb-seq to further examine the regulatory mechanisms pioneering TF candidates on T cell specification. We have also utilized the computational methods such as NicheNet and IQCELL to infer ligand-receptor-target links and identify signalling pathways upstream of the effector TFs. Preliminary studies suggest that NOTCH and PPAR signalling pathways are strong enhancers for T-lineage specification. This study will contribute to our understanding of the gene interactions that orchestrate HSPC and T cell emergence, and underpin new strategies to scale up the production of therapeutically relevant cell types in future stem cell therapy.

Keywords: Hematopoietic Stem and Progenitor Cells and T cell emergence, CRISPR screens, Gene regulatory networks

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BOOSTING MITOCHONDRIAL RECYCLING REJUVENATES HEMATOPOIETIC AND IMMUNE SYSTEMS

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Hematopoietic stem cells (HSCs) generate all blood lineages and ensure the correct homeostasis between myeloid and lymphoid lineages during the entire life of an organism. However, HSCs decrease their regenerative potential and undergo toward a myeloid-biased fate skewing with age. This phenomenon leads to a reduction of B and T cell compartments and immune dysfunction. Consequently, elderly patients are more susceptible to severe viral and fungal infections. It was reported that HSC aging is associated with defective autophagy and accumulation of damaged mitochondria. And emerging studies showed that dysregulation of mitochondria metabolism correlates closely with hematopoietic aging process. However, the clear causal relationship between mitochondrial metabolism and HSC aging remains unknown. We were wondering if metabolic modulator can rejuvenate aged HSCs, rescue loss-of-function of aged HSCs and improve overall immune response. We used mito-QC reporter mice and mitochondrial stain to analyze mitochondrial network upon in vitro treatment of metabolic modulator. In vivo long-term blood reconstitution assays and LCMV infection model were used to estimate the effect of metabolic modulator on HSC functionality. Here we reported that interventions aimed to modulate mitochondria recycling and metabolism can rejuvenate aged HSCs and thus preserving the lymphoid compartments and immune function. In vitro administration of the mitophagy inducer Urolithin A on aged

HSCs restore their blood and immune reconstitution capability. Moreover, Urolithin A supplemented in the mouse diet, improves bone marrow functionality, expands the lymphoid compartment and boost immune response against viral infection in old mice. Finally, we discovered that HSCs rejuvenation is associated with mitophagy induction and re-establishment of mitochondria homeostasis and fitness in aged HSCs. In summary, we identified mitophagy as potent regulator of aging process and as valuable target for rejuvenating approaches in HSCs.

Keywords: hematopoietic stem cell (HSC), mitochondrial metabolism, HSC aging

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GENERATION OF LARGE NUMBERS OF MEGAKARYOCYTES FROM HUMAN PLURIPOTENT STEM CELLS

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Platelets (PLTs) are generated by the differentiation of hematopoietic stem and progenitor cells (HSPCs) into megakaryocytes (MKs), a rare subset of large polyploid bone marrow cells. Methods that promote the differentiation of human pluripotent stem cells (hPSCs) into MKs and PLTs could be used to alleviate PLT shortages and reduce transfusion-related complications. Here, we describe an efficient feeder- and serum-free culture system that promotes the differentiation of hPSCs into polyploid MKs with high yields and the ability to generate PLT-like particles (PLPs). The 17-day protocol consists of two stages. First, hPSC clumps were cultured in Matrigel®-coated plates for 12 days, during which they underwent mesoderm induction and then hematopoietic differentiation using a multi-stage protocol and respective media supplementation. Next, non-adherent cells were harvested, replated, and cultured for 5 additional days for megakaryocytic differentiation. Finally, cells were assessed for MK marker expression (CD41/CD42), DNA ploidy, and PLP production by flow cytometry and immunofluorescence (IF) microscopy. Two embryonic stem cell lines (H1 and H9) and two induced pluripotent stem cell lines (1C and R038) were used in this study. On average, 85% of the cells expressed CD41 (range: 80 - 90%), 69% co-expressed CD41 and CD42 (range: 56 - 77%), and 302 CD41+CD42+ cells were generated per seeded hPSC (range: 223 - 425; n = 12 - 29 for 4 hPSC lines). DNA ploidy profiles of the CD41+ cells showed on average 25% and 10% for 4N and 8N+ ploidy, respectively (n = 6 - 28 for 4 hPSC lines). The PSC-derived MKs produced on average 3.5 PLPs per MK (range: 3.2 - 5.1; n = 12 - 28 for 4 hPSC lines; gating based on blood PLTs). The culture system was then validated for scale-up in three dimensional (3D) rotary suspension cultures where hPSC aggregates were maintained and differentiated into MKs and PLPs in the absence of Matrigel®. This 3D hPSC culture generated MKs/PLPs with similar efficiency and yields as the original 2D protocol (average of 56% CD41+CD42+ cells, 48% 4N+ cells, 5.6 PLPs per MK, and a potential yield of ~150 million CD41+CD42+ cells in a 100 mL bioreactor; n = 2 - 3 for 1C and H9). In conclusion, we have developed a culture system capable of generating high numbers of polyploid, PLP-shedding MKs that is amenable to scale-up method development.

Keywords: hPSC, megakaryocyte/platelet, differentiation

TOPIC: KIDNEY

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NEPHRON PROGENITOR FATE SPECIFICATION IS CONTROLLED BY THE RENIN-ANGIOTENSIN SYSTEM IN HUMAN IPSC-DERIVED KIDNEY ORGANOIDS

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Nephron progenitor cells (NPCs) are multipotential cells responsible for generating podocytes, proximal tubules, loops of Henle, and distal tubules. Nephrogenesis is dependent upon NPCs maintaining a balance between self-renewal and differentiation controlled by SIX2 and WNT, respectively. The molecular mechanisms that underpin NPC sensitivity to WNT and differentiation are unclear. The renin-angiotensin system (RAS) is critical for normal kidney development as in-utero exposure to RAS inhibitors leads to kidney dysgenesis. Using induced pluripotent stem cell (iPSC) derived kidney organoids, we sought to understand the impact of RAS on NPC fate specification. We hypothesize that angiotensin (Ang II) and angiotensin receptor blockers (eg. Losartan) can regulate NPC fate specification in our organoid model. Human iPSC-derived kidney organoids were generated using the Takasato protocol. Organoids were treated between days 7 to 16 of the protocol (during nephrogenesis) with vehicle control, Ang II, losartan, or Ang II and losartan. Alternatively, organoids were treated for only 24hrs. To ascertain the effect on NPC differentiation, we analyzed the organoids using single-cell RNA sequencing and confocal imaging. Organoids expressed the major RAS genes within expected cell populations. By day 16, Ang II and losartan-treated organoids were smaller than control. However, Ang II increased the number of glomeruli, the size of each glomerulus, and the number of Ki67- NEPHRIN+ cells. Losartan treatment decreased the size of each glomeruli but maintained the number of proliferating NEPHRIN cells. NPCs and podocyte precursors were the most transcriptionally sensitive cells to Ang II and losartan, respectively. Ang II upregulated podocyte differentiation genes (WNT4, FOXC2, PAX8), depleted NPCs, and increased the number of differentiated podocytes. Losartan downregulated

podocyte differentiation genes, increased SIX2 expression, and increased the number of NPCs. Overall, our preliminary data suggest that NPC fate specification is controlled by RAS as Ang II accelerated podocyte differentiation and AGTR1 inhibition stalled podocyte differentiation. Our work provides new insights into the role of RAS in nephrogenesis and improved organoid protocols for the study of glomerular diseases in vitro.

Funding Source: KRESCENT and CIHR

Keywords: Organoid, Nephron progenitor, Podocytes

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UNDERSTANDING THE MECHANISM BEHIND WNT7B REGULATION OF MEDULLARY FATE AND FORMATION IN MAMMALIAN KIDNEYS

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The mammalian kidney is a complex organ in which the radial organization reflects different functional compartments. This organization underlies the corticomedullary axis which allows for the generation of an osmolarity gradient within the medulla. This gradient enables the concentration of the primitive urine, a key function of the kidney. Within the medulla, epithelial (nephron and collecting ducts), interstitial, and vascular cell populations work together to generate the osmolarity gradient. Previous work in the McMahon lab has shown that the Wnt ligand Wnt7b is required for the formation of the medullary compartment of the kidney, controlling oriented cell division and regulating apoptosis in the epithelium as well as controlling medullary capillary development. Strikingly, mice that lack Wnt7b have no medulla and die soon after birth, while collecting duct-specific knockouts of Wnt7b have an attenuated phenotype compared to the ubiquitous knockout and eventually die at P10 due to dehydration. While the data suggest that Wnt7b is critical for medullary formation, its mechanism of action, the specific populations responding to its signal, and the temporal requirement for Wnt7b signaling are not well understood. Since Wnt7b is expressed in the epithelial populations of the kidney, we characterized the transcriptional effect of ubiquitous, collecting duct-specific, and nephron-specific loss of Wnt7b using mouse models, finding that the phenotypic severity varies depending on the knockout population. To understand what non-epithelial populations may be responsive to Wnt7b, we utilized single cell transcriptomic data to identify putative target populations of interstitial and endothelial cells that may directly respond to Wnt7b signal through canonical Wnt signaling. To understand whether Wnt7b is required during the establishment of the medullary osmolarity gradient or during adulthood, we characterized inducible knockouts of Wnt7b from P7 and adult mice. Taken together, we aim to further our understanding of the mechanism behind Wnt7b regulation of medullary fate and function during development and adulthood in the mammalian kidney.

Keywords: Wnt signaling, kidney, development

TOPIC: LIVER

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KUPFFER CELL-DERIVED IL-6 IS REPURPOSED FOR HEPATOCYTE DEDIFFERENTIATION VIA ACTIVATING PROGENITOR GENES FROM INJURY-SPECIFIC ENHANCERS

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Stem cell-independent reprogramming of differentiated cells, also known as in vivo dedifferentiation and transdifferentiation, has been lately uncovered as an important paradigm to repair injured tissues. Following injury, mature hepatocytes re-activate reprogramming/progenitor-related genes (RRG) and dedifferentiate to liver progenitor-like cells (LPLC) in both mice and humans, which contribute remarkably to liver regeneration. However, it remains largely unknown which and how external factors trigger hepatocyte reprogramming. Here, by employing single-cell transcriptional profiling and lineage-specific depletion tools, we uncovered that periportal-specific formation of LPLC in biliary injuries was initiated by regionally activated Kupffer cells, but not peripheral blood monocytes-derived macrophages. Unexpectedly, using in vivo screening, the proinflammatory factor IL-6 was identified as the key niche signal repurposed for RRG induction via activation of STAT3, which drove RRG expression through binding to their pre-accessible enhancers in mature hepatocytes. Notably, RRG were activated from injury-specific rather than liver embryogenesis-related enhancers. Collectively, these findings depict an injury-specific niche signal and the inflammation-mediated transcription in driving the conversion of hepatocytes into progenitor phenotype.

Keywords: Kupffer cell-derived IL-6, in vivo hepatocyte reprogramming, transcriptional regulation of genes

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HEPATOCYTE PLASTICITY AND METABOLIC REPROGRAMING UPON DEREGULATION OF WNT BETA CATENIN ACTIVITY

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The WNT/β-catenin pathway regulates multiple cellular processes upon injury and during nonalcoholic steatohepatitis–hepatocellular carcinoma (NASH-HCC) development and serves as one of the most frequently activated pathways (up to 50% HCCs).

The underlying molecular mechanisms of how the WNT/β-catenin pathway initiates and drives NASH-HCC progression are still largely unknown. The ZRNF3/RNF43-LGR4/5 axis can fine-tune WNT/β-Catenin activity and plays a key role during liver regeneration and liver metabolic zonation. We have recently shown that the deletion of ZNRF3/RNF43 leads to the activation of the WNT/β-catenin pathway and results in increased hepatocyte proliferation, the conversion of all hepatocytes to a pericentral phenotype, and, eventually, to massive HCC formation (Sun et al., 2021, Cell Stem Cell). The molecular determinants during this pathogenesis process have remained elusive. We hypothesize that the deregulation of WNT/β-catenin activity levels shapes cell fate by boosting cell fitness adaption and enabling the pathological outcomes. Using a combined approach of gene expression profiling, spatial transcriptomics profiling, in vivo clonal tracing, characterization of injury models, and WNT/β-catenin pathway modulation in mice, we now show that there is a zonal selection of cell stress and proliferation upon deregulation of WNT/β-catenin activity. RNF43 /ZNRF3 in mutant mice results in metabolic reprogramming of periportal hepatocytes and induces clonal expansion in a subset of hepatocytes, ultimately promoting liver tumors, while the same deletion in pericentral hepatocytes leads to the elimination of these clones in the liver. The cross- talk between nutrient sensing and WNT/β-catenin pathway may drive this zonal selection of cell growth. This highlights the importance of safeguarding mechanisms that constrict uncontrolled WNT/β-catenin signaling and proper liver metabolic zonation, which is necessary to ensure liver homeostasis. Together, ZNRF3 and RNF43 cooperate to safeguard liver homeostasis by spatially and temporally restricting WNT/β-catenin activity, balancing metabolic function and hepatocyte proliferation. Zonal-based deregulation of WNT/β-catenin activity drives the initiation and progression of NASH-HCC.

Keywords: metabolic reprogramming and tumor initiation, liver metabolic zonation, cell identity and plasticity

TOPIC: MESENCHYMAL STROMAL CELLS, ADIPOSE, AND CONNECTIVE TISSUE

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ACTIVATING TRANSCRIPTION FACTOR-2 SUPPORTS THE ANTIOXIDANT CAPACITY AND ABILITY OF HUMAN MESENCHYMAL STEM CELLS TO PREVENT ASTHMATIC AIRWAY INFLAMMATION

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Glutathione (GSH), an abundant nonprotein thiol antioxidant, participates in several biological processes and determines the functionality of stem cells. A detailed understanding of the molecular network mediating GSH dynamics is still lacking. Here, we show that activating transcription factor-2 (ATF2), a cAMP-response element binding protein (CREB), plays a crucial role in maintaining the level and activity of GSH in human mesenchymal stem cells (MSCs) by crosstalking with nuclear factor erythroid-2 like-2 (NRF2), a well-known master regulator of cellular redox homeostasis. Priming with ascorbic acid 2-glucoside (AA2G), a stable vitamin C derivative, increased the expression and activity of ATF2 in MSCs derived from human embryonic stem cells and umbilical cord. Subsequently, activated ATF2 crosstalked with the CREB1-NRF2 pathway to preserve the GSH dynamics of MSCs through the induction of genes involved in GSH synthesis (GCLC and GCLM) and redox cycling (GSR and PRDX1). Accordingly, shRNA-mediated silencing of ATF2 significantly impaired the self-renewal, migratory, proangiogenic, and anti-inflammatory capacities of MSCs, and these defects were rescued by supplementation of the cells with GSH. In addition, silencing ATF2 attenuated the ability of MSCs to alleviate airway inflammatory responses in an ovalbumin-induced mouse model of allergic asthma. Consistently, activation of ATF2 by overexpression or the AA2G-based priming procedure enhanced the core functions of MSCs, improving the in vivo therapeutic efficacy of MSCs for treating asthma. Collectively, our findings suggest that ATF2 is a novel modulator of GSH dynamics that determines the core functionality and therapeutic potency of MSCs used to treat allergic asthma.

Keywords: Mesenchymal stem cell, activating transcription factor-2(ATF2), Glutathione

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FINDING A NOVEL TGF-BETA1 SIGNALING CAPABLE OF MANIPULATING THE LINEAGE SPECIFICATION OF BONE-MARROW MESENCHYMAL STEM CELLS

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SMAD signalings play crucial roles in regulating cell fate decision of mesenchymal stem cells (MSCs) in the bone marrow. TGF-β1, the main factor for activating SMAD signalings, is the most abundant cytokine in bones. It not only promotes the expansion of MSCs but also hinders their differentiation into osteoblasts or adipocytes. Surprisingly, in addition to inhibition of adipogenesis via its canonical SMAD2/3 signaling, we here found that TGF-β1 also activates SMAD1/5/8 signaling, which is typically induced by BMPs to promote MSC differentiation toward osteoblasts or adipocytes. As TGF-β1 coexists with BMPs in the bone marrow but counteracts BMP-driven differentiation, we thus hypothesize that the cross of TGF-β1 into SMAD1/5/8 would manipulate the fate decision of MSCs that differs to BMPs in the bone niche. Indeed, in diverse MSCs, blockage of SMAD1/5/8 signaling, but not SMAD2/3 signaling, activated by TGF-β1 merely relieved its effect on the inhibition of BMP-promoted osteogenesis without impacting its effect on the suppression of adipogenesis. Such a signal manipulation also increased the calcification capacity in both in vitro MSC culture and ex vivo bone culture models. Mechanism dissection revealed that TGF-β1 primarily activates SMAD1 phosphorylation via receptor complexes formed by TGFBR2 and ALK5. Interestingly, reporter assay and quantification of osteogenesis-related genes indicated that this signaling initially activates BMP-responsive genes but turns to suppression in a time-dependent manner. ELISA, co-immunoprecipitation, and ChIP results further profiled that such a transition is due to formation of mixed SMAD1-SMAD2/3 complexes, which would further allow recruitment of specific repressors. In conclusion, our find-

ings highlight a novel TGF-β1-mediated signaling pathway that specifically hinders osteogenesis through mixed SMAD complexes. These findings also provide a way to fine-tune the balance between osteogenesis and adipogenesis in the aging bone or adipocyte-enriched bone microenvironment.

Keywords: Mesenchymal stem cell, SMAD, TGF-β

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INTERNAL FORCE POLARIZATION ALIGNED BY TISSUE GEOMETRY DICTATES STEM CELL FATE

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The presence of unbalanced mesenchymal stem cell (MSC) differentiation is a recognized cause of decreased bone formation and also a hallmark of osteoporosis. Hence, developing approaches to control MSC osteogenesis in the bone microstructures, such as bone geometric features, is of great interest. The focal adhesions (FAs) sense mechanical cues and transduce these into biological signals, but how FA-mediated signals control MSC lineage differentiation remains largely unknown. Here, we have determined the biochemical role of FAs in geometric cue-controlled MSC lineage commitment. By imposing geometric constraints via micropatterning, we were able to prove that FA organization can control the distribution of actomyosin-mediated intracellular tension, the nuclear-actomyosin force balance, and changes in nuclear deformability. This mechanism controls nucleus translocation of the transcription co-activator YAP, and in turn modulates the switch in MSC commitment between adipogenic and osteogenic fates. These discoveries led us to suggest that tuning FA organization by using geometric variations could contribute to the fundamental mechanisms of osteoporosis treatment.

Keywords: mesenchymal stem cells, focal adhesions, nuclear deformation

TOPIC: MUSCULOSKELETAL

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RECAPITULATING BOVINE MUSCLE TISSUE DEVELOPMENT FOR ENHANCEMENT OF CULTIVATED MEAT PRODUCTION

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The overall footprint of meat and its associated impacts on the environment and public health has increased the need for new and complimentary production systems that are in balance with their use in natural resources. In the last decade the new field of cellular agriculture has emerged. Meat produced from animal cells in cell culture permits the optimized production of only the edible parts of the animal. This allows consumers to maintain the culinary and sensory qualities of the meat, while substantially lowering greenhouse gas emissions by 92%, reducing land use by 95% and water use by 78%. To meet consumer expectations for higher quality products, production methodology better recapitulate the natural developmental processes in cow. These processes consist of formation of tissue consisting of extra cellular matrix (ECM), adipocytes and skeletal muscle cells. Various differentiation protocols have been established in cow cells originating from mesenchymal stem cells and satellite cells. However, bovine embryonic stem cells (bESCs) exhibit the most reliable and eternal source for production of cultivated meat, allowing an efficient and reproducible differentiation process, suitable for mass production. Aleph Farms has developed in-house, short and efficient, animal component-free bESCs differentiation protocol towards muscle cells in 2D and 3D cultures. Our short, and scalable protocol mimics natural developmental pathways. Activation of TGF-beta pathway alongside inhibition of GSK3 pathway yield a mesodermal progenitor cell population that upon further induction can differentiate to muscle cells, adipocyte and ECM producing cells. Commitment to mesodermal lineage occurs in 3D culture in organoid like structures, thus bioreactor scalable. We show that our mesoderm progenitor cells highly express TBXT, TBX6, MSGN1 and PAX3 while losing OCT4 and NANOG expression. We show that further differentiation of this progenitor population results in cells exhibiting muscle fiber morphology expressing Six1, MyoD1, MyoG, MYH3, MYH7, MYH8, Myoglobin (MB) and Myomaker (MYMK). Our work demonstrates an innovative, scalable method to reach cultivated meat production from bESC through activation/inhibition of classical developmental pathways.

Keywords: Cultivated meat, Cellular agriculture, bovine embryonic stem cells (bESC)

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THE ROLE OF EPIGENETICS AND CHROMATIN DYNAMICS IN REGULATION OF SKELETAL MUSCLE MYOFIBERS AND THEIR ASSOCIATED MUSCLE STEM CELLS

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Epigenetic mechanisms have an integral role in gene expression and proper tissue and cell functioning including in skeletal muscle. While skeletal muscle has a great regenerative capacity, this ability is highly compromised in muscle wasting diseases. Muscle integrity and regeneration is dependent on the proper functioning of muscle stem cells (MuSCs) and their associated niche components including the myofibers. Therefore, studying the chromatin state and epigenetic mechanisms regulating myofibers and MuSCs will provide insights into proper skeletal muscle regeneration as well as identifying novel therapeutic avenues for muscle wasting diseases. We have developed a novel method that can identify the accessible chromatin at a single myofiber resolution. This method can be used to identify the differences in the epigenome of myofibers under various physiological or disease stimuli. Application of this technique revealed significant chromatin differences in myofibers under regeneration as well as myofibers in a dystrophic niche. The complete mechanistic insights of muscle function and muscle regeneration also requires understanding of the epigenetic regulation of MuSCs. We have discovered that the master transcriptional repressor REST/NRSF is required for the maintenance of skeletal MuSC pool as the genetic deletion of REST in the mouse MuSCs leads to loss of lineage identity and induction of apoptosis and subsequent depletion of the MuSC pool. We have determined that in MuSCs and their progenitors, REST regulates heterochromatin at non-muscle and developmental genes. Overall, our work provides novel findings into the epigenetic mechanisms mediated by REST that regulate MuSC survival and muscle regeneration.

Funding Source: Canadian Institute of Health Research (CIHR) Natural Sciences and Engineering Research Council of Canada (NSERC) Richard and Edith Strauss Foundation

Keywords: Epigenetics, Muscle Stem Cells, Muscle Regeneration

TOPIC: PANCREAS

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METHIONINE METABOLISM REGULATES PLURIPOTENT STEM CELL THROUGH ZINC MOBILIZATION

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Pluripotent stem cells (PSCs) exhibit a unique feature that requires S-adenosylmethionine (SAM) for the maintenance of their pluripotency. Methionine deficiency in the medium reduced SAM in undifferentiated PSCs, altered their epigenetic state and thus rendering PSCs in a state potentiated for differentiation (Shiraki et al., Cell Metab 2014)(Ozawa et al., Stem Cells 2022). In this study, we find that methionine deprivation triggers a reduction in intracellular protein-bound Zn content and upregulation of Zn exporter SLC30A1 in PSCs. Culturing PSCs in Zn-deprived medium results in decreased intracellular protein-bound Zn content, reduced cell growth, and potentiated differentiation, which partially mimics methionine deprivation. PSCs cultured under Zn deprivation exhibit an altered methionine metabolism-related metabolite profile (Sim et al., Cell Reports 2022). We conclude that methionine deprivation potentiates differentiation partly by lowering cellular Zn content. We establish a protocol to generate functional pancreatic β cells by applying methionine and Zn deprivation. Our results reveal a link between Zn signaling and methionine metabolism in the regulation of cell fate in PSCs.

Keywords: methionine, zinc, metabolism

TOPIC: NEURAL

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SPATIOTEMPORAL ORCHESTRATION OF MULTICELLULAR TRANSCRIPTIONAL PROGRAMS AND IN SITU CELL-CELL COMMUNICATION IN ACUTE SPINAL CORD INJURY

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Spinal cord injury (SCI) involves a complex cascade of cellular and pathological changes that last for months to years. However, the most dramatic molecular responses and overwhelming reorganization occur in the first few days, which determine the overall progression and prognosis of SCI, yet remain poorly understood. Here, we resolve the comprehensive spatiotemporal architecture of multicellular gene expression in a mouse model of acute SCI. The spatial transcriptomic analyses unveil dynamic and coordinated transcriptional programs and regulatory networks, identify and quantify in situ cell-cell interactions from gene expression, and highlight the "early-reacting" spatial clusters, gene modules and cell types. For example, we reveal a distinct population of white matter-originated, Igfbp2-expressing reactive astrocytes, which migrate to the grey matter and express multiple axon/synapse supporting molecules that might foster spinal cord recovery. Together, our dataset and analyses not only showcase the spatially-defined molecular features endowing the cell (sub) types with new biological significance, but also provide a molecular atlas for disentangling the spatiotemporal organization of the spinal cord and the dynamic landscape of gene expression and cell communication in SCI.

Keywords: spatial transcriptomics, spinal cord injury, astrocyte

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IDENTIFYING KEY REGULATORS IN THE DIRECT CONVERSION OF HUMAN SKIN FIBROBLASTS TO NEURONS BY GENE REGULATORY NETWORK ANALYSIS

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Transdifferentiation between cell types has relied on knowledge-based search for optimal reprogramming factors. Our recent study found that the overexpression of ASCL1, miR9/9*-124, nPTB shRNA, and p53 shRNA efficiently converted human skin fibroblasts to neurons. By analyzing longitudinal RNA-seq data of human skin fibroblasts being converted with various combinations of these reprogramming factors, we constructed gene regulatory network (GRN) models capturing the high order information important for neuronal conversion. Examination of gene communities and transcription factors (TFs) in the GRNs identified OTX2 and LMX1A as the key regulators of conversion to neurons, as they had strongest connections to genes functionally associated with neuronal development and differentiation. We confirmed the critical roles of OTX2 and LMX1A experimentally as their knockdown markedly impaired the conversion. The study shows that GRN models are effective in augmenting empirical discovery of optimal reprogramming factors in the transdifferentiation of human skin fibroblasts to neurons. Further improvements in this approach may identify a generally applicable principle for direct cell-fate conversion.

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Keywords: neuronal transdifferentiation, gene regulatory network, transcription factors

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THE TRANSCRIPTIONAL AND REGULATORY LANDSCAPE OF CELLS IN THE DEVELOPING HUMAN CEREBELLUM

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Human cerebellar development is precisely orchestrated by molecular regulatory networks to finally achieve cytoarchitecture and coordinate motor and cognitive functions. However, the understanding of cell type- and time-specific gene expression patterns and the regulatory landscape of human cerebellar development is limited. Here, we combined single-cell transcriptomics and chromatin accessibility states to systematically depict an integrative landscape of the molecular features and cellular composition of the developing human cerebellum covering GW 12-27. We identified 80 cell subtypes and described their developmental trajectories. The multiomic data reveal molecular networks governing progenitor differentiation and cell fate determination along the trajectories. Our data indicate that cell lineage regulation occurs in a hierarchical manner and that a combination of several transcription factors is required for fate divergence, offering an informative regulatory map to show how and when cell fates are determined. We found that the progenitors of these cells highly expressed primate-specific genes such as TMEM14B and ARHGAP11B, which are key regulators of human neocortex expansion and folding. In addition, we analyzed cell-cell interactions and communication to reveal how different types of neurons and glial cells differentiate and function in a synchronized and coordinated manner. In particular, our data revealed that Purkinje cells and Bergmann glia may regulate granule cell migration via different ligand-receptor pairs. We finally illustrated that genes and SNPs of cerebellar dysfunction-related diseases could be mapped onto cell types, indicative of the cellular basis and possible pathogenesis mechanisms of neuropsychiatric disorders.

Keywords: Cerebellum development, Single cell sequencing, Purkinje Cell

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LUNG PERIBRONCHIAL LGR5+ FIBROBLASTS COLLOCATE WITH R-SPONDIN SECRETING NEURONAL CELLS IN HUMANS AND PIGS

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LGR5, a well known marker for stem cells including follicular and intestinal stem cells, is expressed in two distinct lung populations in humans but not mice. Using a transgenic pig model that allows identification of LGR5 expressing cells (LGR5-H2BGFP), we previously demonstrated that one of these populations, peribronchial LGR5-expressing fibroblasts (LGR5-PeriBF), located just under the airway basement membrane, is a prominent cell type in pig lungs. These LGR5-expressing peribronchial fibroblasts are similarly located along human lung airways and are associated with lung diseases such as chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF). Following our IHC evidence that LGR5-PeriBF are located in close proximity to S100B+ and PGP9.5+ neuronal cells, we decided to test the hypothesis that neuronal cells play a role in potentiating the LGR5-PeriBF canonical WNT pathway. Exploration of human single-cell RNA-seq data revealed that LGR5-PeriBF are the only cell type that expresses LGR5 in mature adult lungs. Moreover, R-spondin (RSPO) ligands for LGR5 originate almost entirely from nearby neuronal cells including perineurial (PeriNAF), endoneurial (EndoNAF) and epineurial (EpiNAF) fibroblasts as well as non-myelinating Schwann cells (nmSC). To further understand this LGR5-PeriBF to neuronal cell association, cell-cell junctions in the peribronchial region were examined for commonality. It was found that, in humans, cadherin-11 (CDH11) uniquely associates LGR5-PeriBF and PeriNAF cell types. Additionally, in our transgenic LGR5-H2BGFP pigs, CDH11 and cadherin-13 (CDH13) were differentially over-expressed in bulk RNAseq data of LGR5-PeriBF sorted cells. This adherens junction colocalization of LGR5-PeriBF with PeriNAF lung airway cells may constitute an RSPO signaling niche for an LGR5 potentiated WNT/β-catenin pathway in lung diseases and injury repair.

Funding Source: NIH R21OD019738 (JAP)

Keywords: Lung, peribronchial, perineurial, endoneurial, Schwann, LGR5, RSPO, WNT, CDH11, CDH13, S100B, PGP9.5

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DEFINING THE MECHANISMS OF DYSFUNCTIONAL FERRITINOPHAGY IN BETA PROPELLER PROTEIN-ASSOCIATED NEURODEGENERATION

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Beta-propeller protein-associated neurodegeneration (BPAN) is a congenital neurodegenerative disorder and subtype of neurodegeneration with brain iron accumulation (NBIA), manifesting with childhood intellectual disability followed by progressive parkinsonism, dystonia, and dementia in adolescence or early adulthood. BPAN is X-linked and caused by mutations in WDR45/WIPI4, a critical effector of vesicle maturation in the highly conserved degradation pathway of autophagy. Although prior studies have found that mutations in WDR45/WIPI4 lead to impairments in autophagic flux and abnormal accumulation of degradative cargo, the molecular mechanisms underlying brain iron accumulation and neuron-selective degeneration in BPAN remain undefined. In the present study, we evaluate cell type-specific derangements in ferritinophagy, a selective form of autophagy that regulates intracellular iron content and turnover. To do this, we use a novel cell model of BPAN comprised of human induced pluripotent stem cells edited by CRISPR/Cas9 to knock-in a BPAN-related mutation (c.C52>T, p.Gln18X), from which we generate isogenic neurons, astrocytes, and skeletal muscle cells. In comparing mutant against wild-type cells and neurons against non-neurons, we are assessing immunocytochemical, Western, and qPCR analyses of ferritinophagy machinery: ferritin, an essential intracellular carrier of iron, and NCOA4, a selective autophagy receptor for degrading ferritin. We expect that ferritinophagy proteins fail to undergo normal autophagic degradation and are mislocalized in BPAN cells, and that these derangements are most severe in neurons. By clarifying how and to what extent ferritinophagy is dysfunctional in BPAN and on a cell type-specific basis, we will further our understanding of BPAN pathogenesis and the neurotoxic effects of dysfunctional autophagy. In so doing, our studies will provide the basis for investigating novel and neuron-specific mechanisms of rescuing dysferritinophagy and inform treatment strategies for BPAN and other neurodegenerative autophagiopathies.

Keywords: BPAN, autophagy, ferritin

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NEURONAL ACTIVITY-DEPENDENT TRANSCRIPTOME AND CHROMATIN PROFILE OF HUMAN FETAL NEURONS

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Primate and human pluripotent stem cells (hPSCs) allow for the investigation of unique and clinically relevant aspects of human brain development and neurobiology that are not represented in traditional rodent model systems. To employ human pluripotent stem cell-based models effectively, we must compare differentiated cells to the relevant primary human tissues to accurately assess cellular identity and maturity level. However, there are limited resources available for such comparisons. The human developing brain contains dozens of different cell types that undergo multiple protracted maturation stages, and neuronal activity is required for the proper development and differentiation of certain neuronal subtypes within the neocortex. Most recent single-cell transcriptional data sets of human brain are from adult tissue. However, hPSC-derived neurons are immature and most closely resemble early-mid embryonic stages of brain development. Furthermore, neuronal activity-dependent gene expression may reveal additional cellular complexity that is not apparent at a basal state. In this study, we aim to provide a deeper understanding of the cellular identities within the developing human neocortex and the multitude of neuronal activity-dependent gene expression profiles present at this stage of development. To do this, we are generating a multiomic, single-cell dataset of primary human fetal cortical cultures and their activity-dependent transcriptional profiles. This data set will provide the transcriptional identity and chromosome accessibility profiles of neuronal and non-neuronal cell types of the mid-gestational developing neocortex alongside their neuronal depolarization-dependent gene expression patterns.

Keywords: Neuronal Development, Activity-dependent gene expression, Single-cell profiling

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AGING MICROGLIA AND THEIR IMPACT ON NEURAL STEM CELL FUNCTION IN THE SVZ

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Neurogenesis, the production of new neurons, occurs throughout life in brain regions known as neural stem cell (NSC) niches. There are two niches in the brain, the hippocampus and subventricular zone (SVZ). The niches are specialized to provide support for NSC survival and proliferation. The SVZ is the largest NSC niche in the rodent brain and is responsible for producing neurons that integrate into the olfactory bulbs and producing progenitors that migrate to areas of ischemic or traumatic brain injury. Neurogenesis drastically declines with age which contributes to the decrease in cognitive capacity and reduced injury repair seen in aging. The causes of age-related NSC dysfunction are not fully understood but reduced number of NSCs and progenitors in the niche is associated with age. However, apoptosis does not increase significantly in the SVZ during aging. We show that microglia, the immune cells of the brain, are morphologically distinct from neighboring striatum in young and aged mice. During aging, microglia in the SVZ become activated before reduced NSC numbers are observed. This pro-inflammatory activation occurs much earlier in the niche and to a greater degree than microglia in other brain regions. We show that young SVZ microglia have reduced phagocytic capacity compared to whole brain microglia in vitro suggesting that these niche microglia are specialized not to phagocytize stem cells and progenitors. However during aging SVZ microglia exhibit increased phagocytosis becoming more similar to microglia from other brain regions. Furthermore, in young microglia we observe very little evidence of phagocytosis in the niche in vivo. During aging we show that there is a significant increase in the number of microglia that have NSC and progenitor markers within the lysosomes, strongly suggesting that during aging microglia begin to engulf NSCs and progenitors. Using anti-inflammatory and C3 complement inhibitory interventions we see an increase in SVZ proliferation in aged mice. Interestingly while we see decreased amounts of SVZ microglial activation with these interventions, we see an increase in microglia phagocytosis of progenitor cells. This indicates that SVZ microglia could be a key regulator of neurogenesis in the aged SVZ.

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Keywords: neurogenesis, microglia, neural stem cell

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INVESTIGATING THE DRIVING GENETIC FACTORS OF BRAIN OVERGROWTH IN AUTISM SPECTRUM DISORDER

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Autism spectrum disorder (ASD) is a common and complex neurodevelopmental disorder characterized by impairments in social interaction and communication, as well as repetitive behaviors. Approximately 15-20% of individuals with ASD have disproportionate megalencephaly (ASD-DM), with disproportionate enlargement in both gray and white matter volume. Individuals with ASD-DM have more severe behavioral and cognitive impairments and are less responsive to standard therapeutic interventions, leading to very poor prognoses relative to individuals with ASD and normal head circumferences. Increases in brain size often precede clinical symptoms, suggesting that understanding the underlying mechanisms regulating brain overgrowth could provide a window of opportunity for intervention or mitigation of symptoms. Here, we use reprogrammed peripheral blood mononuclear cells (PBMCs) to generate induced pluripotent stem cells (iPSCs) and derive neural progenitor cells (NPCs) from autistic individuals with early brain enlargement (ASD-DM), autistic individuals with normal brain size (ASD-N), and typically developing control individuals with normal brain size (TD-N). We demonstrate substantial changes in cell proliferation, cell cycle, and DNA damage repair pathways following RNA-sequencing and gene network analyses of the NPCs in ASD. P53 signaling and programmed cell death are particularly down-regulated in ASD-N vs ASD-DM. Furthermore, by integrating this cellular and gene expression data with structural magnetic resonance imaging (MRI) and behavioral and clinical data on the same individuals, we identify candidate driver genes associated with brain overgrowth and specific clinical features. This work begins to provide a direct link from the cellular basis to the neural and behavioral manifestations of the ASD-DM phenotype and highlight potential therapeutic targets in ASD.

Keywords: Autism spectrum disorder, Brain overgrowth, iPSCs

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TRANSCRIPTOMICS OF ENTERIC NEURAL CREST IN THE SOX10DOM MOUSE MODEL OF HIRSCHSPRUNG DISEASE IDENTIFY DEFECTS IN ENTERIC PROGENITORS PROCEEDING ALTERED NEURONAL ALLOCATION

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The Enteric Nervous System (ENS) derives from the neural crest and is essential for normal gastrointestinal motility. Sox10 is an essential transcription factor for normal formation of the ENS and mutant alleles of this gene lead to notable defects in ENS development including absence of enteric ganglia in the distal bowel, a hallmark of Hirschsprung disease. Our prior studies of Dominant megacolon (Sox10Dom) mutants found that these mice also exhibit altered transit of luminal gcontents in the small intestine due to abnormal neuron subtype ratios. This was unexpected since SOX10 is expressed in the neural crest progenitors that colonize the fetal intestine and is downregulated as neuronal lineages mature. We hypothesized that SOX10-regulated gene networks in ENS progenitors influence enteric neuron subtype allocation in early ENS development. We conducted single-cell RNA-Sequencing (scRNA-Seq) on ENS progenitors during fetal mouse development. Sox10 (Tg(Sox10-HIST2H2BE/Venus)ASout) and Phox2b (Tg(Phox2b-HIST2H2BE/Cerulean)1Sout) transgenic lines that label enteric progenitors and developing neurons respectively were incorporated into crosses with the Sox10Dom allele to comprehensively label developing ENS populations. Fluorescent activated cell sorting was used to isolate developing progenitors and forming enteric neurons at 15.5 days post-coitus (dpc) that were then encapsulated using 10XGenomics methods for subsequent paired-end next-generation sequencing. Analysis of wild-type populations revealed three neuronal trajectories, suggesting a previously unappreciated level of complexity during early ENS lineage divergence. RNA velocity analysis identified transit-amplifying and intermediate progenitor-to-neuronal transition zones. Cross-comparisons of wild-type and Sox10Dom scRNA-Seq data sets revealed a shift in cell distribution and the presence of a novel progenitor cluster exclusively within the Sox10Dom populations. Decreased cell distributions were also observed along the transit-amplifying transition zone of Sox10Dom lineages. These results indicate that three neuronal lineages are initially present at 15.5 dpc and that SOX10 influences neuronal subtype allocation between two of these major lineages through effects in a transient amplifying population.

Keywords: Enteric Development, Neural Crest Lineage, Sox10

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INDIVIDUAL VARIATION IN THE EMERGENCE OF ANTERIOR-POSTERIOR NEURAL IDENTITIES FROM HUMAN PLURIPOTENT STEM CELLS

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Variability between human pluripotent stem cell (hPSC) lines remains a challenge and opportunity in biomedicine. We identified differences in early lineage emergence across hPSC lines that can be mapped to the anterior-posterior axis of embryonic development. Dynamic lineage-related and stable cell line-specific transcriptional signatures in pluripotency interacted to regulate early fates. Stable line-specific signatures predicted retinoic acid (RA) response and regional bias in forebrain vs. hindbrain development. Replicate hPSC lines and paired adult donor tissue demonstrated that cells from individual humans expressed unique transcriptional signatures throughout life, which are associated with evolutionarily recent genes. In contrast, replicate lines from one donor showed divergent gene expression and differentiation phenotypes linked to distinct chromatin states, showing that epigenetic mechanisms also contribute to stable transcriptional and neural fate differences. This variation in lineage bias and its correlation with RA signaling was also observed in a large collection of hPSC lines. These results indicate that stable transcriptional differences between hPSC lines initiate human neuronal diversity.

Keywords: Pluripotent stem cells, Human variation, Retinoic acid signaling

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EPIGENETIC REGULATION OF DEVELOPMENTAL TIMING IN HUMAN EMBRYONIC STEM CELLS

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Embryonic development follows a series of developmental events at a particular sequence and tempo. While the order is evolutionarily conserved across species, the timescale of many developmental processes is considerably longer in humans compared to rodents. This difference in tempo is recapitulated in human and mouse embryonic stem cells (ESCs) in vitro, suggesting cell-autonomous mechanisms that act as intrinsic developmental timers. Although protein turnover rates and metabolic rates have been shown to scale with developmental tempo, we do not know the genetic basis of these parameters and how they differ between species. By performing a whole-genome CRISPR screen during neural conversion of human ESCs, we uncovered epigenetic regulators that act as speed limits for this process, including a co-factor for H3K4 methyltransferase, and core subunits of Polycomb Repressive Complex 2 (PRC2). Loss of these epigenetic regulators leads to premature expression of a subset of bHLH transcriptional factors specific for neural lineage and accelerates neural conversion. Specifically, loss of core PRC2 subunits erases H3K27me3, resulting in a broader, smoothening effect on the epigenetic landscape that allows for a more rapid response of developmental genes. Additionally, we found PRC2 inhibition appears to accelerate later neuronal differentiation and other germ layer differentiation as well. Our results highlight a deterministic role of the epigenetic landscape in setting the human developmental tempo. Future works will focus on understanding differential landscapes between species and potential crosstalk between metabolism and epigenome. Collectively, this work will shed light on the general principles of timing mechanisms and set the stage for more efficient differentiation protocols for disease modeling and stem cell-based therapies.

Keywords: developmental timing, human embryonic stem cells, epigenetics

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INVESTIGATING HOW BMP SIGNALING ALTERS CHROMATIN ACCESSIBILITY AND GENE EXPRESSION DURING DIFFERENTATION OF MOUSE EMBRYONIC STEM CELLS INTO SPINAL DORSAL INTERNEURONS

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Spinal dorsal interneurons (dIs) mediate somatosensation: our ability to sense the environment, permitting us to react to pain or touch, and move in a coordinated manner. When dIs are damaged, after injury or disease, individuals are no longer able to interpret sensory information from the periphery. Spinal dIs are generated when discrete domains of neural progenitor cells arise along the dorsal-ventral axis of the neural tube. Growth

factors, including the bone morphogenic protein (BMPs) family, drive the transcriptional changes needed to specify at least six populations of dIs (dI1-dI6). BMP signaling is transduced by two R-Smads, Smad1 and Smad5, during spinal cord development. Previous studies have demonstrated that activated R-Smads bind to both pre-acetylated nucleosome depleted sites, as well as closed chromatin. However, it remains unknown how Smad1/5 binding modulates the overall architecture and accessibility of chromatin during dI specification. To assess the consequences of BMP signaling on global chromatin composition, we are using previously defined directed differentiation protocols that derive bona fide dIs from mouse embryonic stem cells (mESCs). Using RNA-Seq and ATAC-Seq, we have profiled gene expression and open chromatin regions across the timeline of our protocols, at the key developmental decision points. These studies will uncover the regulatory genomic regions responsible for patterning and dI specification during spinal cord development. Currently, our pluripotent stem cell dI differentiation protocols do not direct the formation of homogeneous populations of dI subtypes. We thus anticipate that information from this study will be used to generate pure populations of dIs for drug screen platforms and/or cellular replacement therapies seeking to restore somatosensation

Keywords: spinal dorsal interneurons, BMP signaling, in vitro differentiation

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SINGLE NUCLEUS MULTI-OMICS REVEALS EPIGENETIC AND 3D GENOME STRUCTURE MEMORY OF ALZHEIMER DISEASE IN DIRECTLY REPROGRAMMED HUMAN NEURONS AND PRIMARY ENTORHINAL CORTEX

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Since partial maintenance of age-dependent molecular signatures, induced neurons directly converted from patients' fibroblast provide an unique opportunity to study the biological events occurring along with aging in many neurodegenerative disorders. Like late-onset Alzheimer's disease is typically diagnosed as the sporadic form and age-dependent way. Its molecular biomarker and pathogenesis mechanism are still challenging to explore due to the limited brain samples from live patients which are molecularly diagnosed. Here, by using single-cell multiomics technologies, we identified the conserved AD specific DNA methylation signatures in human fibroblast and fibroblast-induced neurons from sporadic AD patients. The comparison between these in vitro cell models with the corresponding primary entorhinal cortex brain tissues from the same individuals is showing that they shared the epigenetic biomarkers and molecular processes. Further analysis on the multimodal measurements of transcriptional and 3D genome structure configuration, our single nucleus multi-omics data reveal the linkage of expression regulations and chromosome interactions at the AD specific hotspots, which collectively demonstrate the recapitulation of age-related epigenetic features in induced neurons and its modeling application for biomarker detection and neuronal diseases.

Keywords: SINGLE NUCLEUS MULTI-OMICS, INDUCED NEURONS,, EPIGENETIC BIOMARKER

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NOVEL FUNCTION OF PRO-APOPTOTIC BH3-ONLY PROTEINS BIM AND BIK IN NEURONAL DIFFERENTIATION

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Human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) are of significant importance for their widespread application in disease modelling and clinical therapeutics. Considerable research has focused on developing efficient models of neuronal differentiation but the dynamic changes in fundamental cell biological pathways that occur during neuronal differentiation remain less explored. The apoptotic pathway undergoes striking changes during neuronal differentiation. While the undifferentiated hESCs are highly sensitive to apoptotic stimuli such as DNA damage, these cells become markedly resistant with neuronal differentiation. However, less is known about the mechanisms by which the apoptotic pathway becomes restricted during neuronal differentiation. Here, we examined the expression of the various apoptotic genes during hESC differentiation into neurons (e.g. cortical, nociceptive or dopaminergic neurons). As expected with neurons becoming more resistant to apoptosis, we found expression of the pro-apoptotic proteins BAX and BAK to decrease and the anti-apoptotic proteins BCL-2 and BCL-XL to increase during neuronal differentiation. In contrast, the pro-apoptotic BH3-only gene family exhibited unexpected patterns of expression. The BH3-only gene family are the first components of the core apoptotic pathway that are activated in response to apoptotic stimuli. We were surprised to find that the pro-apoptotic BH3-only proteins BIM and BIK increased their expression both at RNA and protein level even when the cells were becoming more resistant to apoptosis. To examine if these proteins might have functions unrelated to apoptosis, we generated CRISPR knockout hESCs for BIM and BIK as well as BIM/BIK double knockouts. All three knockout hESCs appeared indistinguishable from wild-type cells at the undifferentiated stage. Strikingly however, upon cortical neuronal differentiation, all three knockout cells exhibited thicker axons in comparison to the wild type neurons. In ongoing studies, we are examining this phenotype in detail. Together, our

results point to a previously unknown function of the pro-apoptotic proteins BIM and BIK in regulating axonal architecture during neuronal differentiation.

Keywords: BH3-only proteins, non-apoptotic, Axonal architecture

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DISSECTION OF SAFETY AND IDENTITY PROFILE OF HIPSC-DERIVED NSCS BY TRANSCRIPTIONAL AND REGULATORY ANALYSES: IMPLICATIONS FOR CELL THERAPY APPROACHES TO NEURODEGENERATIVE DISORDERS

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Human iPSC-derived neural stem/progenitor cells (hiPSC-NSCs) are a promising source for cell/gene therapy approaches for neurodegenerative disorders with an unmet clinical need. A comprehensive evaluation of hiPSC-NSCs identity is key to establishing efficacy and safety criteria for prospective clinical application. To this end, we combined the global evaluation of the transcriptional (by RNA-seq and scRNA-seq) and epigenetic (by H3K27Ac ChIP-seq) signatures in hiPSCs, hiPSC-NSCs, and human fetal-derived NSCs (hfNSCs; a clinically-relevant, somatic NSC source) with functional in vivo studies aimed to assess the behavior of hiPSC-NSCs upon transplantation in the murine brain. The results of RNA-seq and ChIP-seq analysis revealed a complete switch-off of pathways involved in pluripotency, cell cycle, and Myc-signalling and concomitant acquisition of a distinct "neural signature" in hiPSC-NSCs. The hiPSC to hiPSC-NSC transition is characterized by a dramatic change in the usage of cell-specific enhancers, which contact genes involved in NSC commitment and maintenance. In addition, hiPSC-NSC enhancers are decorated with Transcription Factor Binding Sites (TFBSs) of NSC-associated Transcription Factors. Transcriptional and epigenetic differences between hiPSC-NSCs and hfNSC were not associated with aberrant differentiation or reactivation of pluripotent "memory" and could be mostly ascribed to different culture conditions and maturation stages. Single-cell RNA-seq analyses confirmed the heterogeneity of hiPSC-NSC and hfNSC lines, which are mainly composed of radial glia-like cells at different stages of maturation and glial-committed progenitors, with only a small percentage of neuronal progenitors, preferentially in hiPSC-NSCs. Upon intraventricular transplantation in immunodeficient neonatal mice, hiPSC-NSCs showed robust engraftment, widespread migration and integration in the brain parenchyma, and predominant differentiation into oligodendrocytes and astrocytes, with no evidence of abnormal proliferation or re-expression of pluripotency markers up to 10 months after the transplant. The results of this study support the use of fully-characterized hiPSC-NSC populations for cell-based approaches to treat neurodegenerative and demyelinating disorders.

Keywords: Neural Stem Cell, hiPSC, Identity

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SCALABLE DIFFERENTIATION OF HYPOTHALAMIC ARCUATE NUCLEUS NEURONS FROM HUMAN PLURIPOTENT STEM CELLS

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The hypothalamus is the main integration site of endocrine and neuronal systems regulating autonomous and behavioral responses related to sleep, circadian rhythm, thermoregulation, hormone release, arousal, reproductive cycling, thirst, hunger, and satiety. Neuroendocrine dysfunction of hypothalamic neurons in the arcuate nucleus is associated with obesity, type 2 diabetes, hyperlipidemia, high blood pressure, and cardiovascular disease. Proopiomelanocortin (POMC) neurons in the arcuate nucleus of the hypothalamus respond to postprandially secreted hormones (e.g., insulin) to inhibit appetite and increase energy expenditure. However, access to human hypothalamus for translational research related to identification of new drugs for weight loss remains very limited. Here we report controlled, efficient, and scalable differentiation of human pluripotent stem cells (hPSCs) into pure populations of POMC neurons with arcuate nucleus identity. Combined use of several small molecules that modulate different developmental pathways specifies hPSCs into ventral diencephalon followed by a hypothalamic identity. Time-course gene expression analysis (RNA-seq) of independent hPSC lines revealed strong upregulation of typical hypothalamus cell-type specific transcription factors over the course of 21 days (e.g., NKX 2.1, RAX, OTP, ARX, ISL1). Importantly, no upregulation of dorsal forebrain markers (e.g., PAX6 and FOXG1) was observed. These findings were further validated by protein expression analysis (immunocytochemistry, Western blot). Comparison of the top differentially expressed genes of stem cell-derived neurons to human brain tissue confirmed a distinct hypothalamic molecular signature. Additional phenotypic characterization revealed inhibitory properties (GABA, GAD65/67) and subpopulation of tyrosine hydroxylase positive (TH+) dopamine neurons. Administration of human recombinant insulin to cultures of electrically active neurons increased firing rate in dose dependent manner. Lastly, this differentiation approach was automated using a robotic cell cul-

ture system enabling the production of large numbers of hypothalamic neurons for translational applications.

Keywords: Hypothalamus, Proopiomelanocortin, Obesity

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A MIR-124-MEDIATED TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL MECHANISM CONTROLLING THE CELL FATE SWITCH OF ASTROCYTES TO INDUCED-NEURONS

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Direct astrocytic reprogramming holds therapeutic promise for the amelioration of neuronal loss during neurodegeneration or brain trauma. For this, many neurogenic factors, mainly transcription factors (TFs) and to some extend miRNAs or chemical compounds have been employed for the direct conversion of astrocytes to induced neurons (iNs) in a variety of in vitro and in vivo approaches. The miRNA miR-124 has been employed supplementary to neurogenic TFs and other miRNAs to enhance direct neurogenic conversion, however its reprogramming capacity and independent mechanism of action haven't been studied in depth. Here we show that miR-124 is a potent driver of the reprogramming switch of primary mouse cortical astrocytes towards an immature neuronal fate by repressing many astrocytic regulatory genes and upregulating gene signatures characteristic of intermediate progenitor cells (IPCs) and cortical layer neurons. Identification of miR-124 direct targets in our analysis using publicly available Ago HITS-CLIP data from mouse cortex, revealed the RNA-binding protein Zfp36l1 – implicated in ARE-mediated mRNA decay – as a prominent target. Further experiments highlighted the importance of miR-124:Zfp36l1 interaction during the neurogenic conversion as inferred by the de-repression of the neurogenic interactome of Zfp36l1 at the post-transcriptional level. To this end miR-124 contribution in iNs' production largely recapitulates endogenous neurogenesis pathways, being further enhanced upon addition of the neurogenic compound ISX9, which greatly improves iNs' functional maturation. Additionally, gene regulatory network analysis revealed the major TFs that dictate the reprogramming process at the transcriptional level. Importantly, AAV-mediated overexpression of miR-124 in a mouse model of cortical trauma combined with BrdU treatment pointed towards a capacity of miR-124 in directly converting reactive astrocytes to immature iNs of cortical identity, while ISX9 supplementation conferred a survival advantage to newly produced iNs, still without reinforcing their maturation state. However, certain downstream effectors of miR-124 and ISX9 identified here bear the potential to amplify their combined in vivo reprogramming action and enhance iNs' maturation.

Keywords: Direct reprogramming of astrocytes to induced neurons using neurogenic miRNAs, In vitro and in vivo reprogramming capacity of miR-124, RNA binding protein Zfp36l1

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SINGLE-CELL RNA-SEQUENCING IDENTIFIES DISEASE-ASSOCIATED OLIGODENDROCYTES IN MALE APP NL-G-F-A AND 5XFAD MICE

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Alzheimer's disease (AD) is associated with progressive neuronal degeneration as amyloid-beta (Aβ) and tau proteins accumulate in the brain. Glial cells were recently reported to play an important role in the development of AD. However, little is known about the role of oligodendrocytes in AD pathogenesis. Here, we describe a disease-associated subpopulation of oligodendrocytes that is present during progression of AD-like pathology in the male AppNL-G-F and male 5xFAD AD mouse brains and in postmortem AD human brains using single-cell RNA sequencing analysis. Aberrant Erk1/2 signaling was found to be associated with the activation of disease-associated oligodendrocytes (DAOs) in male AppNL-G-F mouse brains. Notably, inhibition of Erk1/2 signaling in DAOs rescued impaired axonal myelination and ameliorated Aβ-associated pathologies and cognitive decline in the male AppNL-G-F AD mouse model.

Keywords: Alzheimer's Disease, Single-cell anaylsis, Oligodendrocyte

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UNDERSTANDING AND EXPLORING NEW STRAGIES TO INDUCE HUMAN PLURIPOTENT STEM CELLS DIFFERENTIATION INTO INTERNEURON SUBTYPES

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Deficiencies in interneurons have been linked to the development of various neurodevelopmental and neurodegenerative disorders, such as autism spectrum disorder, epilepsy, schizophrenia, and Alzheimer's disease. The derivation of mature subtypes of human interneurons from patients can serve as a foundation for exploring the causes and potential treatment targets for diseases related to interneuron dysfunction. Transcription factor (TF) - mediated cell fate programming represents a promising avenue for improving disease modeling in vitro and generating clinically relevant cell types for potential use in cell replacement therapies. Direct programming involves utilizing one or more TFs to force the establishment of a distinct transcriptional network, leading to a new cell fate. We have previously reported a significant transcriptional shift in human pluripotent stem cells (PSCs) resulting from the overexpression of Ascl1 and Dlx2. In the current study, we evaluated two critical components of this transformation: the trajectory of the programming process, the uniformity of the terminal cell population, and their similarity to their corresponding cell types in vivo. Using single-cell RNA-seq, we profiled the differentiation over time. When organizing the cells according to differentiation pseudo-time, a unidirectional trajectory was observed with no apparent branching points or roadblocks from pluripotency (e.g., POU5F1) to GABAergic neuron states (e.g., MAP2, DCX, DLX5, GAD1/2). Next, we analyzed the neurons at their mature stage and characterized their regional and subtype identities. Such insights allowed us to improve our method further to rapidly generate cortical GABAergic neurons, which serves as a basis for developing more specific and relevant models in the future.

Keywords: human Pluripotent stem cell, GABAergic neuron, reprogramming

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ENHANCED MATURATION OF HUMAN STEM CELL DERIVED INTERNEURONS BY MTOR ACTIVATION

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The use of stem cell derived neurons for cell-based therapies is limited by a protracted maturation. We present a novel approach for accelerating the post-mitotic maturation of human stem cell derived interneurons via the constitutive or transient activation of mTOR signaling. For constitutive activation, Lox sites were placed within PTEN, a key mTOR inhibitor, in a cortical interneuron (CIn) reporter line in which GFP is expressed by the Lhx6 locus. Following directed differentiation and purification by FACS, the CIns were exposed to Cre-expressing lentivirus, then transplanted into mouse neocortex or plated onto cultured rat neocortex. Input synaptogenesis and dendritogenesis was greatly enhanced in the PTEN-deleted CIns. Whole-cell recording of the PTEN-deleted CIns in slices of transplanted neocortex revealed multiple indices of enhanced maturation. Finally, we observed similar effects using transient, doxycycline-inducible activation of AKT. Enhanced maturation of neurons was further characterized by single nuclei RNA-seq (sNuc-Seq) and pseudotime analysis of neurons dissociated from medial ganglionic eminence like IPSC-derived spheroids with doxycycline-inducible activation of AKT. We thus present an inducible, reversible approach for accelerating the maturation of human stem cell derived CIns, and to study the influences of this disease-related signaling system in human neurons.

Keywords: maturation, AKT, PTEN

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P53 SUPPORTS HIGH EFFICIENCY CELLULAR REPROGRAMMMING

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The tumor-suppressor protein p53 regulates proliferation and transcription, binds nucleosome-dense regions, and maintains genomic integrity. In reprogramming, interference with p53 increases reprogramming rates. Putatively, p53 inhibits cell-fate transitions. However, we find that p53 plays a central role in supporting high efficiency reprogramming. We recently developed a chemogenetic cocktail that increases reprogramming rates 100-fold via a p53 mutant. Curiously, mutant p53 induces accumulation of native p53 in rapidly proliferating cells. To track this population through the reprograming process, we developed a live-cell sensor of p53 accumulation by which we could identify and isolate cells. Even with high levels of p53, rapidly proliferating cells reprogram at high efficiency, suggesting p53 does not limit reprogramming. Knockdown of native p53 reduces accumulation of p53 and eliminates the mutant-induced boost in reprogramming efficiency. We hypothesize that accumulation of p53 protects reprogramming cells from genotoxic-levels of replication stress,

enabling cells to persist and reprogram despite transcription-associated genomic stress. To test our hypothesis, we developed synthetic constructs that tune the level of native p53 accumulation. We find that reprogramming rates correlate with the levels of p53 accumulation. We hypothesize that p53 mitigates DNA damage induced by transcription-associated genomic stress. Using mathematical modeling, we examined the p53 response to transcription-associated genomic stress. By limiting the transactivation activity of native p53, mutant p53 induces accumulation of p53 to support high rates of reprogramming. To test this prediction, we inhibited the ATR-Chk1 replication stress-response that triggers p53 activation. Inhibition of Chk1 reduced p53 accumulation and reprogramming yield. Together, our data suggest a dual role for p53 in cell-fate transitions, serving to gate proliferation and limit genomic stress. By biasing the p53 response towards repair during high rates of transcription and proliferation, mutant p53 co-opts native p53 to promote reprogramming.

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Keywords: reprogramming & direct conversion, molecular mechanisms of plasticity, p53

TOPIC: PANCREAS

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INVESTIGATING THE ROLES OF TEASHIRT GENES IN THE PANCREATIC ENDOCRINE SPECIFICATION

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The generation of insulin-producing β cells from the in vitro differentiation of human pluripotent stem cells (hPSC) both for disease modelling and the treatment of diabetes has been pursued aggressively in recent years. Current protocols heavily rely on our understanding of pancreatic development in the mouse and other vertebrates. Fundamental differences in the gene regulatory networks governing mammalian pancreatic development and the specification of endocrine cell types do however exist amongst different species. Pancreatic and duodenal homeobox 1 (PDX1) encodes a well-characterized transcription factor crucial for human pancreatic organogenesis: homozygous loss of PDX1 results in pancreatic agenesis, while heterozygous mutations are associated with β cell dysfunction. Despite its importance in pancreatic specification and glucose homeostasis, surprisingly little is known about the identity of the transcriptional targets downstream of PDX1. To address this knowledge gap, we developed a comparative bioinformatics pipeline incorporating human in vitro and in vivo gene expression data sets as well as PDX1 ChIP-seq and epigenome data (H3K27ac at active enhancers) to identify candidate effectors of PDX1. From this pipeline, we identified teashirt zinc finger homeobox 2 and -3 (TSHZ2/3) as potential downstream targets of PDX1 transcriptional regulation. During the directed differentiation of hPSC towards the pancreatic progenitor fate and consistent with the predictions of our pipeline, TSHZ2/3 expression is observed soon after the activation of PDX1. Significantly, TSHZ2/3 expression is decreased in hPSC lacking PDX1 and PDX1 ChIP-seq confirms that PDX1 binds to regions in both genes whose putative enhancers are marked by H3K27ac. Lastly, TSHZ2/3 expression has been documented in the human pancreatic primordium. Here, we outline experimental strategies and preliminary data, including the inactivation of TSHZ2/3 by CRISPR-Cas9, that we have undertaken to elucidate the roles of TSHZ2/3 in the development of the human pancreas.

Keywords: teashirt, pancreas, differentiation

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USP7: A CRITICAL REGULATOR OF NGN3 STABILITY AND PANCREATIC ENDOCRINE LINEAGE DEVELOPMENT IN MOUSE AND HUMAN INDUCED PLURIPOTENT STEM CELLS (IPSC) ORGANOID MODELS

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Understanding the key elements of beta-cell development is crucial for devising treatments to generate functional beta cells for diabetes patients. The transcription factor NGN3 plays a crucial role in endocrine progenitors' commitment and beta-cell differentiation. In this study, we search for new NGN3 binding partners and identified the deubiquitinating enzyme USP7 as a crucial regulator of NGN3 stability. Our findings indicate that USP7 stabilizes NGN3 through deubiquitination, ensuring proper endocrine development and beta-cell differentiation. In mice, deletion of Usp7 in the embryonic pancreas resulted in reduced islet formation and hyperglycemia in adulthood due to hindered NGN3-mediated endocrine specification. scRNA-Seq analysis of the human embryonic pancreas at different gestational weeks further suggested

that this mechanism operates in human pancreas development. Furthermore, inhibition of USP7 during beta-cell differentiation in human iPSCs-derived pancreas progenitor organoids led to a decline in NGN3-expressing progenitor cells and impaired beta-cell differentiation. These results highlight the central importance of the USP7-NGN3 axis in beta-cell development, opening avenues for novel therapeutic approaches for diabetes

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Keywords: Pancreas development, iPSCs organoids beta cell differentiation, Neurogenin3 stability

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CELL REPLACEMENT THERAPY FOR TYPE 1 DIABETES

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Type 1 diabetes can be treated with transplantation of cadaveric pancreatic islets, but this approach is limited by shortage of donor islets and low viability of islets after transplantation. Transplantation of pluripotent stem cell derived pancreatic islets (SC-islets) into the anterior chamber of eye (ACE) can potentially solve these problems by utilizing a renewable cell source and the possibility of non-invasive longitudinal evaluation post transplantation. Because successful stem cell therapy for type 1 diabetes relies on large-scale production of high-quality functional islets, we in this study developed a highly efficient protocol for differentiation of multiple hPSC lines into functional pancreatic islets. As compared to SC-islets generated using other protocols, our SC-islets showed strong glucose responsiveness and good proportions of mono-hormonal a and b cells, but very low proportions of poly-hormonal cells and proliferating cells. To understand if ACE is suitable for clinical transplantation, we transplanted SC-islets into ACE of a large-eyed pre-clinical animal model. Our results show that the injected SC-islets were integrated onto the iris tissue and became vascularized. The transplants closely resemble human islets in both size and morphology, and contain mainly mono-hormonal endocrine cells expressing either insulin, glucagon or somatostatin. Human insulin c-peptide could be detected in both serum and anterior chamber fluid collected after transplantation. We also transplanted SC-islets into both ACE and kidney capsule of a diabetic mouse model. Two months after transplantation, SC-islets reversed diabetic symptoms in mice. Taken together these results show that ACE transplantation of SC-islets generated using our new protocol could be an ideal solution for treatment of type 1 diabetes.

Keywords: Stem cell therapy for type 1 diabetes, in vitro pancreatic islet differentiation, human pluripotent stem cells

TOPIC: NO TISSUE SPECIFICITY

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HOMOGENEITY OF XEN CELLS IS CRITICAL FOR GENERATION OF CHEMICALLY INDUCED PLURIPOTENT STEM CELLS

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In induced pluripotent stem cells (iPSCs), pluripotency is induced artificially by introducing the transcription factors Oct4, Sox2, Klf4, and c-Myc. When a transgene is introduced using a viral vector, the transgene may be integrated into the host genome and cause a mutation and cancer. No integration occurs when an episomal vector is used, but this method has a limitation in that remnants of the virus or vector remain in the cell, which limits the use of such iPSCs in therapeutic applications. Chemical reprogramming, which relies on treatment with small molecule compounds to induce pluripotency, can overcome this problem. In this method, reprogramming is induced according to the gene expression pattern of extra-embryonic endoderm (XEN) cells, which are used as an intermediate stage in pluripotency induction. Therefore, iPSCs can be induced only from established XEN cells. We induced XEN cells using small molecules that modulate a signaling pathway and affect epigenetic modifications, and devised a culture method in which can be produced homogeneous XEN cells. At least 4 passages were required to establish morphologically homogeneous chemically induced XEN (CiXEN) cells, whose properties were similar to those of XEN cells, as revealed through cellular and molecular characterization. Chemically induced pluripotent stem cells (CiPSCs) derived from CiXEN cells showed characteristics similar to those of mouse embryonic stem cells (mESCs). Our

results show that the homogeneity of CiXEN cells is critical for the efficient induction of pluripotency by chemicals.

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Keywords: Chemical reprogramming, Reprogramming, Extra-embryonic endoderm cells, XEN

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NOVEL PLURIPOTENT CELL IN THE OSKM-MEDIATED DIRECT REPROGRAMMING

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Direct reprogramming refers to the conversion of somatic cells to target cells without passing through induced pluripotent stem cells (iPSCs). This process is faster than iPSC reprogramming and avoids the risk of tumorigenesis associated with generating pluripotent stem cells, making it well-suited for regenerative medicine. Thus, we investigated the pluripotent factor-mediated direct reprogramming (PDR) process, with a focus on the intermediate stage. Our previous results showed that the cytoskeletal linker Desmoplakin (Dsp) has stage-specific expression during PDR and that knockdown of Dsp impedes direct reprogramming and fin regeneration in zebrafish. Notably, reduced expression of Dsp impaired the formation of granular-shaped intermediate cells (gICs) during reprogramming, as well as a blastema, which are multipotent cells that form during regeneration (Ha et al., Science Advances, 2022). In addition, we confirmed that the gICs identified in our study differ from iPSCs due to their low expression of pluripotent marker genes. We aimed to verify whether gICs have a high differentiation potential similar to blastema. We isolated gICs and induced spontaneous differentiation, which resulted in the differentiation of gICs into cells representing all three germ layers. Furthermore, when we injected gICs and iPSCs into an NSG mouse, iPSCs showed 100% tumor incidence, while gICs showed less than 20%. These results suggest that we may have isolated blastema-like cells in mammals and that it is possible to produce pluripotent cells with characteristics distinct from conventional iPSCs for regenerative medicine.

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Keywords: Direct reprogramming, Regeneration, Pluritpotent stem cells

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TRANSIENT METHIONINE DEPRIVATION TRIGGERS HISTONE MODIFICATION AND POTENTIATES DIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) possess the capacity for unlimited self-renewal and the potential to generate many functionally differentiated cell types, which are expected to be useful as alternative cell source for cell replacement therapy, disease modeling, and drug discovery. As cells exhibit lineage-specific nutrient requirements that are dependent on endogenous metabolic network, elucidating the metabolic mechanisms underlying cellular physiology will provide novel possibilities for manipulating cell fate by developing optimized media formulations. hPSCs exhibit a unique metabolic state in which they exhibit a high requirement for S-adenosyl methionine (SAM), which is generate through methionine (Met). Met restriction triggers rapid metabolic changes followed by a significant transcriptional response with epigenetic changes, rendering hPSCs in a biased state for differentiation. In this research, we addressed the unknown mechanism underlying the potentiation of differentiation. The rapid decrease in intracellular SAM results in a significant reduction in H3K4me3 marks, particularly in specific genes encoding pluripotency core transcription factors and components of the cholesterol metabolic pathway. During differentiation, loss of H3K27me3 occurs in definitive endodermal signature genes, including GATA6, CER1, and EOMES, switching from a bivalent to monovalent state. The results suggest that these genes are key regulators of the transi-

tion from a pluripotent state to definitive endoderm lineage commitment. Our results show the molecular mechanism of the potentiation of differentiation by Met deprivation. Taking advantage of the differences in metabolic features between hPSCs and their derivatives, the metabolic selection is a simple but effective solution for cost-effective manufacturing highly purified hPSCs-derived differentiated cells.

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Keywords: Methionine, Histone modification, Induced Pluripotent Stem Cells

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ADRENALINE STIMULATES BODY-WIDE STEM CELL ACTIVATION FOR LIMB REGENERATION

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Many species throughout the animal kingdom naturally regenerate complex body parts following amputation. Most research has focused on identifying mechanisms that influence cell behaviors in the remaining stump tissue, adjacent to the injury site. Roles for activation steps that occur outside of the injury site remain largely unexplored, yet they may be critical for regeneration. Here, we discovered a novel role for peripheral nervous system in stimulating a body-wide activation response to amputation that readies progenitor cells for regeneration in salamanders. Notably, this systemic response is mediated by adrenaline and mTOR signaling. This work challenges the predominant conceptual framework centering the injury site alone in the regenerative response, arguing instead for body-wide stem cell activation as a primary step upon which molecular cues at the injury site build tissue. Our results provide a foundation for future studies of body-wide regenerative responses in species with limited regenerative prowess, which may fuel the ultimate development of therapeutic approaches for stimulating human limb regeneration.

Keywords: systemic responses, adrenaline, limb

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CELLULAR POPULATION DYNAMICS SHAPE THE ROUTE TO HUMAN PLURIPOTENCY PROMOTED BY A PARACRINE HGF-MET-STAT3 AXIS

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The route to induced pluripotency via human cellular reprogramming is characterized by plastic intermediate stages whose features are still largely unknown. This has long hindered the efficiency of human reprogramming. By taking advantage of a microfluidic approach, we perform reprogramming with high efficiency, thus highlighting an active and pivotal role of a confined microenvironment. To profile the dynamic reciprocity between reprogramming cells and their microenvironment, we profile the time-course of secretome and single-cell transcriptome. This reveals the functional role of extrinsic pathways across subpopulations emerging along reprogramming trajectories. This protein communication dynamically shapes a permissive extracellular environment resembling primitive node formation during embryo development. Among other factors, we pinpoint the HGF/MET/STAT3 axis as a potent enhancer of reprogramming, which acts via HGF accumulation within the confined system of microfluidics, and in conventional dishes needs to be supplied exogenously to enhance efficiency. Our data integrate the notion of human cellular reprogramming as a transcription factor-driven process with the concept that it is deeply dependent on extracellular context and cell population determinants.

Keywords: hiPSCs, Microfluidic reprogramming, multi-comics

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PROSPECTIVE ISOLATION OF TOTIPOTENT MURINE EMBRYONIC STEM CELLS

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Embryonic stem cells (ESCs) isolated from the blastocyst inner cell mass (ICM) possess in their majority developmental pluripotency. In contrast, a recently identified subpopulation of 2C-like ESCs possesses totipotency, with a unique capacity to give rise not only to all embryonic lineages, but also to extra-embryonic placental tissue. A scarcity of cell surface markers suitable for isolation has limited the further study of 2C-like ESCs to date. Here, we show that 2C-like ESCs can be prospectively isolated from murine ESC cultures using monoclonal antibody-mediated receptor engagement and cell sorting based on high expression of the cell surface stem cell determinant, ATP-binding cassette member B5 (Abcb5). Purified Abcb5-positive ESCs not only expressed higher levels of the pluripotency markers Oct4, Klf4 and Nanog compared to Abcb5-negative ESC bulk populations, but also expressed higher levels of the 2C-like subpopulation-defining totipotency inducer Klf5 and its transcriptional regulators Tbx3 and Dppa2. Additionally, Abcb5-positive ESCs displayed developmental totipotency upon isolation, as shown by a capacity for trophoblast induction and, following in vivo transplantation, for teratoma formation with additional expression of the placental markers Pl1 and Mash2. A newly created transgenic Abcb5 lineage tracing mouse model confirmed that developmental tissues of all three germ layers and the placenta are derived from 2C-like Abcb5-positive ESCs. Successful prospective isolation of totipotent Abcb5-positive 2C-like ESCs paves the way towards further investigation of mechanisms of totipotency and advances utilization of mouse ESCs towards novel placental applications.

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Keywords: Totipotency, Embryonic stem cells, ABCB5

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BENCHMARKING AND IMPROVING ALGORITHMS THAT AID CELL FATE ENGINEERING WITH COMPFORCE AND IMCELL

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One reason that designing cell fate engineering (CFE) protocols is difficult is because the search space of entities to perturb (e.g. cytokines, growth factors, small molecules, transcription factors (TFs)) and when to perturb them is vast. Ad hoc strategies to reduce the search space typically leverage knowledge from development and differential expression analysis. Recently, several computational methods have been devised that formally integrate gene regulatory networks, for example by adjusting TF scores based on network properties. However, the relative performance of these methods and the extent to which they offer gains over differential expression analysis alone is an open question. To answer this question, we developed a computational platform, CompForce, which consists of (1) a standardized re-implementation of the most prominent computational CFE prediction tools, and (2) literature-based and synthetic gold standards. In our comprehensive benchmarking, we found that existing network-based tools do not offer clear improvements over differential expression. This is due to their reliance on local network centrality metrics. These results inspired us to consider other strategies to improve CFE prediction tools. The first is to avoid individual metrics of network centrality. The second is to return optimized TF sets rather than ranked lists, which cannot adequately account for redundancy or synergism among TFs. Our new method, IMCell, returns optimized TF sets that maximize the activation or repression of specified target programs by solving a modified version of the Influence Maximization problem. Using CompForce, we show that IMCell vastly out-performs differential expression and existing network-based tools. We also extend IMCell to dynamic contexts to predict multi-step differentiation protocols that transition cells through specified intermediate states. We are now prospectively testing IMCell predictions to engineer synovial joint progenitor populations.

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Keywords: cell fate engineering, gene regulatory networks, reprogramming

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A FAST AND ROBUST PROTOCOL FOR NON-INTEGRATIVE GENERATION OF MONOCLONAL INDUCED PLURIPOTENT STEM CELLS FROM HUMAN SKIN BIOPSIES WITHIN FOUR WEEKS

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Human induced pluripotent stem cells (iPSCs) with their potential to differentiate into countless different cell types in vitro and in vivo hold great potential for allogenic as well as autologous cellular therapy. However, many traditional techniques used for generation of iPSCs from somatic cells entail the risk for genomic integration and therefore limit their use in a clinical setting. In addition, the established protocols are often lengthy and require the subsequent screening for unwanted vector integration. Transient mRNA-based reprogramming eliminates this risk and is one of the fastest and most efficient reprogramming technologies available today. We hereby present a fast and robust protocol to generate monoclonal iPSCs of high quality from human skin punch biopsies within four weeks. Fibroblasts from healthy donors were isolated from abdominal skin via combined mechanical and enzymatic dissociation. The cells were expanded for only one passage before starting the reprogramming routine, eliminating the need for extensive upstream expansion of fibroblasts and thus minimizing the risk for cultivation induced aberrations. Cells were then transfected for five consecutive days with an mRNA cocktail containing the transcription factors POU5F1 (OCT3/4), SOX2, KLF4, MYC, NANOG and LIN28A. During a short growth phase iPSC colonies formed with high efficiency and could be further expanded into stable monoclonal cell lines after an optional isolation step. Due to the optimized reprogramming protocol and mRNA mixture, no feeder cells, conditioned medium or B18R protein were necessary. Generated iPSCs showed typical stem cell morphology and marker expression. Classic pluripotency markers like Oct3/4, Sox2, Tra-1-60, SSEA-5 and SSEA-4 were expressed at high levels, while cells showed minor expression of differentiation marker SSEA-1. Moreover, the generated iPSCs exhibited a normal karyotype in passage four after reprogramming and successfully differentiated into the three germ layers endoderm, ectoderm and mesoderm. In summary, the described protocol allows for efficient and reliable integration-free generation of high quality monoclonal iPSCs from human skin punch biopsies within only four weeks and will therefore facilitate especially the generation of iPSCs from individual donors.

Keywords: Reprogramming, mRNA, Transfection

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THE EFFECTS OF DOPAMINE ON BREAST CANCER STEM CELL BEHAVIOUR

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Tumour-host interactions are essential for cancer progression. In particular, primary tumour sympathetic innervation has been reported to promote breast cancer cell growth. However, the effects of specific neurotransmitters on cancer cell plasticity are not fully elucidated. Here we investigated the role of a series of neurotransmitters in mammosphere formation and propagation to unravel potential impact on cancer stem cell like behaviour. Surprisingly, exposure of cancer cells to dopamine and not other transmitters resulted in increased mammosphere propagation. While the role of dopamine in the brain has been extensively characterized, its peripheral impact on cancer behaviour is less understood. We confirmed the presence of dopamine within hyperplasias and fully formed tumours in genetic spontaneous models of breast cancer progression. Exposure of cancer cells to dopamine increased stem cell associated markers SOX9, SOX2 and KLF4 via the D1-like dopamine receptor family. Pharmacological inhibition of D1 receptors led to loss of stem markers, while a D1 receptor agonist increased their expression. In order to investigate the long-term effects of dopamine on tumour growth, we treated cancer cells with dopamine in vitro and examined their in vivo behaviour. Orthotopical injections of cancer cells pre-exposed to dopamine on syngeneic mice grew larger tumours compared to controls. Accordingly with an increase in aggressive tumour growth, we detected an increased resistance to chemotherapy. Surprisingly, when pre-treated cells were injected intravenously to form experimental metastasis, we detected reduced metastatic growth initiation. After isolation of cancer cells from the lung, cells pre-exposed to dopamine displayed increased colony formation in vitro, indicative of increased intrinsic stem cell potential. How pre-exposure to dopamine impacts cancer cell behaviour on these different contexts is being currently investigated. Collectively, our results indicate an unexpected role of peripheral dopamine on breast cancer progression by imposing an altered cellular behaviour with increased stem cell like characteristics.

Keywords: cancer stem cells, breast cancer, epigenetics

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TRANSCRIPTIONAL PROGRAMMING FOR THE DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS INTO FOREBRAIN NEURONS

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Embryonic stem cells can differentiate into neural stem cells. The differentiation potential of neural stem cells is spatiotemporally restricted to neuronal and glial lineages. This complex process is tightly controlled by many factors including transcription factors. The Sine Oculis Homeobox 3 (SIX3) is a transcription factor that controls gene expression in a spatiotemporal specific manner.

SIX3 functions in Shh signaling, Wnt signaling, postnatal ependymal cell maturation, and in post-proliferative neurons of the hypothalamus and pituitary. However, we still do not know what regulates SIX3 expression during development in human brain. Also, it is unclear how SIX3 controls its targets in different developmental stages. More than 60 mutations in SIX3 have been causally associated with Holoprosencephaly (HPE), which occurs during the first few weeks of pregnancy in one in 5,000 live births. HPE is characterized by many signs including brain malformation, seizures, and developmental delay. Mutations in SIX3 in HPE are of unknown penetrance. SIX3[G34R] mutation was carried by the healthy mother to cause different phenotypes in the progeny: HPE in the male offspring but not pathogenic in his half-sister. Our lab obtained experimental findings suggesting that SIX3 gene is epigenetically regulated by the UTX-53BP1 axis during human neural differentiation. UTX is a lysine-27-specific demethylase of histone 3, and 53BP1 is a factor that binds to specific histones at double-strand break sites and contributes to the maintenance of heterochromatin and genome stability. Therefore, our hypothesis is that SIX3 alterations differentially affect the expression of its target genes and epigenetic mechanisms during different stages of forebrain development. To address these questions, we are investigating SIX3 functions in UTX-KO human neural stem cells cultured in forebrain differentiation media, the effects of SIX3 suppression by CRISPRi, and the effects of SIX3[G34R] mutagenesis at 11, 17, and 24 days of forebrain neural differentiation. The successful completion of these experiments will illuminate the molecular and developmental targets of SIX3 during neurogenesis and forebrain development and contribute with more insights about the relationship between the UTX-53BP1 axis and SIX3.

Funding Source: 3R01GM134358-03S1

Keywords: forebrain neuronal differentiation, Sine Oculis Homeobox 3, mutagenesis

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TRANSCRIPTIONAL COUPLING WITH RNA PROCESSING CONTROLS ZYGOTIC GENOME ACTIVATION AND TOTIPOTENT REPROGRAMMING

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After fertilization, the zygote activates its genome, which is initially transcriptionally quiescent, to enable gene expression, a process known as zygotic genome activation (ZGA), which subsequently initiates development. Transcription promiscuously occurs across the genome at the basal level in the zygote and is shifted to zygotic genes during ZGA at the late two-cell stage of mouse embryos. Despite the importance of this critical transition, the mechanisms governing the remodeling of RNA polymerase (Pol) II binding from intergenic regions to genes are largely unknown. Regulated phosphorylation of repeated heptads in the carboxyl terminal domain (CTD) of the largest subunit of Pol II controls its binding and elongation activity on chromatin. Serendipitously, we found that truncation of the Pol II CTD reprograms pluripotent mouse embryonic stem cells (ESCs) into a totipotent-like state. In particular, gene-centric transcription in ESCs shifted to noncoding intergenic regions, resembling promiscuous transcription prior to ZGA. In addition, these reprogrammed cells exhibit characteristics of two-cell embryos in terms of gene signature, epigenetic state, nuclear architecture, and cell cycle, and also possess the ability to give rise to embryonic and extraembryonic lineages when transplanted into early embryos. Mechanically, the CTD promotes gene transcription but inhibits random transcription of intergenic regions. Deletion of the CTD decoupled genic transcription and RNA processing, leading to precocious release of Pol II from the promoter into the gene body, thereby reducing elongation. In contrast, in intergenic regions, deletion of the CTD abolished the ability of Pol II to sense nascent RNA, thereby enhancing its binding and translocation activity to upregulate intergenic RNA production. Taken together, these results demonstrate the critical role for the Pol II CTD in coupling transcription and RNA processing to activate gene expression during ZGA, providing unprecedented insights linking fundamental nuclear events to embryonic development. This work also provides a new means for generating totipotent cells.

Keywords: ZGA, totipotent reprogramming, RNA Pol II CTD

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REGULATION OF KEY TRANSCRIPTS BY RNA EXOSOME IMPACTS PROLIFERATION AND DIFFERENTIATION IN STEM CELLS AND CANCER

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Self-renewal and tissue differentiation programs involve networks that balance proto-oncogenes (promoting self-renew-

al), gate-keeping tumor suppressors (limiting self-renewal), and care-taking tumor suppressors (maintaining genomic integrity). Therefore, it is vital to understand how these mechanisms are preserved to ensure faithful cell proliferation and tissue differentiation. The eukaryotic RNA exosome complex is an essential and conserved protein complex that can degrade or process RNA substrates. While the importance of the housekeeping function of the core exosome subunits in RNA processing is known, the role of the different exosome components in cell fate transitions is not clear. Our previous work suggested that lower expression of one of the exosome subunits due to aging strongly correlated with poor DNA damage response and genomic instability, known features of RNA exosome-deficient disease phenotypes. In this study we find and describe the dominant downstream mRNA targets of the RNA exosome and elucidate how they balance proliferation and differentiation in stem and progenitor cells. We show that the regulation of certain transcripts is highly conserved, and deregulation of RNA exosome is a selection mechanism in colorectal cancer.

Keywords: RNA exosome, stem cells, differentiation

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A ZINC-FINGER C2H2 TYPE PROTEIN ZNF REGULATES PLURIPOTENCY OF HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) and human induced pluripotent stem cells (hiPSCs) are characterized by its abilities to proliferate infinitely (self-renewal) and differentiate into almost all cell types in our body. However, the ultimate functions of controlling pluripotency by zinc-finger protein family remain unknown. Here, an uncharacterized protein ZNF, a zinc-finger C2H2 type family member protein, was found enriched in hPSCs. ZNF knockdown impaired the pluripotency of hPSCs which were shown by down-regulated key pluripotent markers such as OCT4, SOX2, NANOG, KLF4, PODXL. Meanwhile, ZNF overexpression can partially delay cell differentiation. ZNF knockdown also significantly inhibited iPSC reprogramming. Inducible ZNF knockout approach also blocked the pluripotency of hPSCs and the teratoma formation in the mice model. Using protein sequence alignment, the ZNF protein shared 92% similarity with CTCF. Moreover, ZNF possessed DNA-binding ability through ChIP-seq analysis. Combined with transcriptomic target gene analysis after loss of ZNF, ZNF ChIP-seq indicated that ZNF was potentially involved in chromatin remodeling in hPSCs.

Keywords: Embryonic stem cell, DNA binding protein, chromatin

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DYNAMIC RNA REGULATION ORCHESTRATES WNT/BETA-CATENIN-DRIVEN MESODERMAL CELL FATE SPECIFICATION

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Post-transcriptional regulation orchestrates diverse cellular mechanisms that regulate pluripotent stem cell biology and pattern early mammalian development. We have recently shown that post-transcriptional regulation of Profilin-2, an actin / dynamin-binding protein, enables FGF signaling, ERK activation, and early stem cell differentiation by controlling endocytosis. In the present study, we use three-dimensional embryoid cell models and chimeras to illuminate coordinate control of the Profilin-2 transcript by RNA-binding proteins Ago2 and IRP1/2 and reveal a critical point at the axis of cell signaling, cell adhesion (structure) and cell fate. We dissect the role for post-transcriptional regulation of Profilin-2 and subsequently reveal an essential function in the initial specification of mesodermal lineages, due to effects on the localization of beta-catenin during critical pluripotent cell state transitions.

Funding Source: This work was supported by NIH/Eunice Kennedy Shriver National Institute of Child Health and Human Development Grant K08HD105017.

Keywords: Wnt, mesoderm, post-transcriptional regulation

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DDX18 COORDINATES NUCLEOLUS PHASE SEPARATION AND CHROMATIN ORGANIZATION TO CONTROL THE PLURIPOTENCY OF HUMAN EMBRYONIC STEM CELLS

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Pluripotent stem cells are unique in their large nucleus and manifestly open chromatin structure with hypertranscriptional activities. How the nucleolus, the largest membraneless and phase-separated subnuclear organelle, maintains its structural integrity in preserving pluripotent chromatin remains incompletely understood. By studying nucleolus-specific DEAD-box RNA helicase-18 (DDX18) in human embryonic stem cells, we discover mechanisms controlling nucleolus phase separation and chromatin organization. Specifically, the interaction and droplet miscibility between DDX18 and NPM1 in granular component (GC) of the nucleolus, facilitated by RNA species, maintain nucleolar structural integrity in restricting centromere clustering and perinucleolar heterochromatin (PNH) formation. DDX18 depletion disrupts the coexistence of immiscible dense fibrillar component (DFC) and GC. Finally, we develop the NoCasDrop technique to achieve the tethering of centromere clustering around nucleolus for enhanced PNH formation with the derepression of development-related genes. Together, we establish DDX18 as a critical factor coordinating nucleolus phase separation and chromatin organization to maintain pluripotency.

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Keywords: DDX18; pluripotency; nucleolus; phase separation; multiphase immiscibility, ribosome biogenesis; centromere; perinucleolar heterochromatin;, chromatin interaction; NoCasDrop

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HISTONE LYSINE METHYLTRANSFERASE NSD2: AN ESSENTIAL REGULATOR OF HUMAN ERYTHROPOIESIS

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Erythropoiesis is the process of hematopoietic stem cell proliferation and differentiation to form mature erythrocytes. Steady-state erythropoiesis is coordinated by various transcription factors and epigenetic regulators. Histone methyltransferases (HMTs) are critical epigenetic regulators that drive activation and repression during lineage progression by modifying histone proteins. Nuclear receptor-binding SET domain protein 2 (NSD2) is H3K36 methyltransferase (HKMT) that regulates B cell differentiation and mature B cell function during normal hematopoiesis. Mutation in NSD2 induces Wolf-Hirschhorn syndrome, while knockdown (KD) of NSD2 leads to abnormal lymphocytes and deficiency in antibody production. However, the biological function of NSD2 in human erythropoiesis is yet to be explored. Thus the aim of present study was to understand the role of NSD2 during erythroid differentiation. Lentiviral mediated NSD2 knockdown was done during ex-vivo erythroid differentiation in CD34+ HSPCs (hematopoietic stem and progenitors). 70-80% NSD2 KD was obtained at mRNA level post 72 hrs of induction. It was observed that NSD2 KD lead to significant decrease in β-globin gene expression suggesting that NSD2 regulates the expression of β-globin gene during erythroid differentiation. Furthermore, NSD2 KD lead to reduced formation of Megakaryocyte Erythroid progenitors (MEP) populations at day 4 of erythroid differentiation. NSD2 KD lead to decrease in 75-80% BFU-E and significant reduction in CFU-E cells as observed in Methocult assay and by FACS. NSD2 KD at later stages lead to block in terminally differentiating (Polychromatic and orthochromatic) erythroblasts with increase in CD71 population along with proerythroblasts and basophilic erythroblasts. NSD2 KD decreases cell proliferation while inducing apoptosis on early and maturing erythroblasts. NSD2 KD alters erythroid specific (GATA1, FOG1, KLF1) and heme biosynthesis genes (PBGD, FECH) as revealed by gene expression analysis. Our find-

ings suggest that NSD2 regulates the process of erythropoiesis and is crucial for normal erythroid differentiation.

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Keywords: Nuclear receptor-binding SET domain protein 2 (NSD2), Erythroid differentiation, Flowcytometry

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SINCMAT : A SINGLE-CELL BASED METHOD FOR PREDICTING MATURATION TRANSCRIPTION FACTORS

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One of the major goals of regenerative medicine is to generate tissue-specific mature and functional cell types for clinical applications. However, current direct cell differentiation/reprogramming strategies are still unable to systematically produce fully mature and functional cells, especially in in vitro systems. To address this important challenge in the field, we have developed SinCMat, a single-cell based computational method for predicting cell maturation transcription factors (TFs) for in vitro use. A large body of evidence indicates that the acquisition of cell identity is necessary, but not sufficient, for the generation of functionally mature cells. Emerging evidence has demonstrated that environment signals play a significant role in the cell maturation process by modifying the gene expression through Signal-dependent Transcriptions Factors (STFs), a class of TFs expressed in a broadly manner across cell types and activated by external stimuli. SinCMat is based on a recently proposed concept where Identity Transcription Factors (ITFs), along with epigenetic modifiers, set a cell-type specific epigenetic landscape during cell differentiation, allowing a further transcriptional regulation. Once differentiated, STFs can be activated in response to environment signals during cell maturation, therefore honing the expression of cell-restricted genes. In particular, with the help of prior TF-target information, SinCMat leverages the co-targeting property of ITFs and STFs on functional genes. We applied SinCMat to a wide range of cell types using the publicly available single-cell RNA sequencing data from the Mouse Cell Atlas and the Human Cell Landscape. To assess the performance of SinCMat, we created SinCMatDB, a manually curated database providing a total of 1209 experimentally validated cell maturation cues, including TFs as well as exogenous treatments. SinCMat was able to identify known maturation TFs and predicted novel candidates for further experimental validations. In conclusion, we have developed the first single-cell RNA-seq based computational tool for predicting cell maturation TFs that considers both identity and environment components. We expect SinCMat to be an important resource for studies aiming at producing functionally mature cells for clinical applications.

Funding Source: Supported by the Luxembourg National Research Fund (C19/BM/13624979)

Keywords: Functional cell maturation, Reprogramming, Cellular therapy

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A NON-SYNDROMIC OROFACIAL CLEFT RISK LOCUS TETHERS TO A TRANSCRIPTIONAL AND A TRANSLATIONAL REGULATOR IN NEURAL CREST CELLS

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Non-syndromic orofacial clefts (nsOFC) affecting the lip and/or palate are one of the most common craniofacial defects in newborns. Such face-specific dysmorphisms arise when the development of cells responsible for the morphogenesis of the human face is perturbed. The multipotent neural crest cells (NCCs) are the cell type that contributes to most of the tissues that comprise the vertebrate face. Defects in NCCs are thought to underlie nsOFC, but in most cases, the etiological mechanism that causes nsOFC is still elusive. We use nsOFC-associated risk loci reported in GWAS to identify new genes linked to nsOFC. We find a topologically associated domain shared by the protein-coding genes DDX1 and MYCN. We show that NCC-specific knockouts of DDX1 or MYCN in chicken embryos result in craniofacial malformations that resemble nsOFC. Since the MYCN transcription factor has previously been reported to be associated with facial dysmorphisms including nsOFC, we focus on DDX1. DDX1 belongs to the DEAD-box family of proteins that control multiple steps of RNA metabolism in cells. We define and characterize DDX1 as a new regulator of protein synthesis in NCCs. Molecularly, DDX1 cooperates with RTCB to control tRNA metabolism in NCCs. Our findings identify a mechanism by which defects in tRNA metabolism can affect NCC maintenance, which positions the misregulation of translation as a contributor to the complex nature of nsOFC.

Keywords: Transcription, Translation, Neural Crest

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THE EPITHELIAL TO MESENCHYMAL TRANSITION (EMT) IN EARLY DIFFERENTIATION: INTEGRATING AND INTERROGATING CELL ORGANIZATION, CELL BEHAVIOR, AND CELL IDENTITY

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The Allen Institute for Cell Science aims to understand the principles by which human induced pluripotent stem cells (hiPSC) establish and maintain robust dynamic localization of cellular structures, and how cells transition between states during differentiation and disease. The epithelial to mesenchymal transition (EMT) is a state change that occurs in both normal and pathological contexts such as development and cancer metastasis, respectively. EMT has been described as a change in behavior from largely non-motile to migratory, a change in organization from apical-basal to front-back polarity, and a change in identity by protein/transcript expression profile. However, to understand the dependencies and relationships between these three aspects of EMT that are currently unknown, we are employing a large-scale, multi-modal data integration approach using hiPSC from the Allen Cell Collection (allencell.org). We are developing a standardizable framework to combine live imaging of dynamic cell behavior with cell identity characterization via multiplexed immunolabeling or RNA-FISH. In parallel, we have performed a single cell RNA-seq (scRNA-seq) experiment with high temporal resolution over the duration of EMT. Psuedotime trajectory analysis on this data shows more variation in the pseudotime assignment at timepoints associated with dramatic behavioral changes. We are now using this data to identify novel probes to perform labeling of fixed cells after live-cell imaging as described above. This integrative approach allows us to formally compare EMT in different contexts. For example, we have compared EMT induced in hiPSC by two methods- upregulating the WNT signaling pathway with a small molecule inhibitor of GSK3 beta vs. treatment with growth factors BMP-4 and Activin-A. We have also tested how different extracellular matrix proteins within the substrate and/or solution surrounding hiPSC, including those leading to the formation of lumenoids, affect EMT. We believe that this multi-modal, multi-scale approach might serve as a template for studying EMT in various contexts and aid in understanding cell states and transitions more generally.

Keywords: EMT, State, Differentiation

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NUTRITIONAL REQUIREMENTS OF HUMAN INDUCED PLURIPOTENT STEM CELLS

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Since 2004, the use of DMEM/F12 as basal media for human pluripotent stem cell culture has remained largely consistent, being the backbone of commercial media and chemically defined alternatives such as our own B8. Even though DMEM/F12 is a complex formulation, few experiments have been published to compare suitable alternatives for hiPSC culture. One of those, indicated that a relatively simpler medium, MEMa, could sustain similar levels of cell growth, indicating that a complex basal medium might not be essential. Hence, over the past three years, we performed extensive analysis to understand which of the 52 components of DMEM/F12 are needed for hiPSC growth and maintenance, and at which conditions and concentrations they are required. We then developed a novel formulation called BMEM, comprised of only 39 components, all demonstrated to be essential for hiPSC culture. BMEM-cultured cells present robust cell proliferation, expression of pluripotency markers, and differentiation potential into multiple lineages as compared to those cultured in B8-supplemented DMEM/F12. Additionally, hiPSC cultured in BMEM consistently have enhanced expression of pan-pluripotency markers such as POU5F1 and NANOG over those cultured in DMEM/F12, along with increased expression of markers of the primed state and reduced expression of markers of the naïve state. Combined, this suggests BMEM maintains hiPSC in a higher primed pluripotent state. We have cultured hiPSC in BMEM for >80 passages, including the generation of >20 lines in BMEM, and successfully transitioned >40 lines from DMEM/F12-based media to BMEM. Lastly, BMEM can be generated in-house at large scale with basic equipment for 1/250th of the cost of commercial media.

Funding Source: American Heart Association Postdoctoral Fellowship (874276)

Keywords: Human Induced Pluripotent Stem Cells, Cell Culture Media, Nutritional Requirements

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TELOMERE LENGTH IN HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSC), albeit human induced pluripotent stem cells (hiPSC) or human embryonic stem cells (hESC), are a powerful tool in research and also hold great potential for clinical applications. One of their basic properties is active telomerase, which leads to telomere length maintenance or even lengthening in low passages (Zeng Sicong et al., 2014). Short telomeres can lead to early senescence in cells differentiated from hPSC. Therefore we used Southern blot analysis of terminal restriction fragments (TRFs) to test if the telomere length changes during reprogramming and culture of hiPSC in low passages. Contrary to previously published work (Yehezkel Shiran et al., 2011), we did not find any trend within the tested hiPSC lines. We next tested the possible impact of culture conditions on telomere length. We compared the telomere length of hPSC lines cultured for 15 passages in feeder-dependent and feeder-free systems. We also tested the effect of hypoxic/normoxic culture conditions. We did not prove any influence of culture conditions on the telomere length.

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Keywords: telomeres, hPSC, hiPSC

TRACK: MODELING DEVELOPMENT AND DISEASE (MDD)

Session 1: Even

6:45 PM - 7:30 PM

TOPIC: CARDIAC

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ALTERNATIVE SPLICING MEDIATED BY RNA-BINDING PROTEIN RBM24 FACILITATES CARDIAC MYOFIBRILLOGENESIS IN A DIFFERENTIATION STAGE-SPECIFIC MANNER

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Mutations in genes encoding sarcomeric proteins lead to failures in sarcomere assembly, resulting in cardiomyopathies that are a leading cause of morbidity and mortality worldwide. Splicing variants of sarcomeric proteins are crucial at different stages of myofibrillogenesis, accounting for sarcomeric structural integrity. RBM24 (RNA-binding motif protein 24) is known as a tissue-specific splicing regulator that plays an essential role in cardiogenesis. However, it had been unclear if the developmental stage-specific alternative splicing facilitated by RBM24 contributes to sarcomere assembly and cardiogenesis. Our aim is to study the molecular mechanism by which RBM24 regulates cardiogenesis and sarcomere assembly in a temporal-dependent manner. By ablating RBM24 from human embryonic stem cells (hESCs) using CRISPR/Cas9 techniques, the derived RBM24−/− hESCs still differentiated into sarcomere-hosting cardiomyocytes. Nevertheless, the knockout cell lines exhibited disrupted sarcomeric structures with punctate Z-lines due to impaired myosin replacement during early myofibrillogenesis. Transcriptomics revealed >4000 genes regulated by RBM24. Among them, core myofibrillogenesis proteins (eg, ACTN2 [α-actinin 2], TTN [titin], and MYH10 [non-muscle myosin IIB]) were misspliced. Consequently, MYH6 (muscle myosin II) cannot replace nonmuscle myosin MYH10, leading to myofibrillogenesis arrest at the early premyofibril stage and causing disrupted sarcomeres. Intriguingly, we found that the ABD (actin-binding domain; encoded by exon 6) of the Z-line anchor protein ACTN2 is predominantly excluded from early cardiac differentiation, whereas it is consistently included in human adult heart. CRISPR/Cas9-mediated deletion of exon 6 from ACTN2 in hESCs, as well as forced expression of full-length ACTN2 in RBM24−/− hESCs, further corroborated that inclusion of exon 6 is critical for sarcomere assembly. Overall, we have demonstrated that RBM24-facilitated inclusion of exon 6 in ACTN2 at distinct stages of cardiac differentiation is evolutionarily conserved and crucial to sarcomere assembly and integrity. To conclude, RBM24

acts as a master regulator to modulate the temporal dynamics of core myofibrillogenesis genes and thereby orchestrates sarcomere organization.

Keywords: Actins, Alternative Splicing, Sarcomeres

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NEW HIGH THROUGHPUT PHENOTYPIC SCREENING PLATFORM FOR HYPERTROPHIC CARDIOMYOPATHY DRUG DISCOVERY USING HUMAN IPS CELL DERIVED CARDIOMYOCYTES

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Hypertrophic Cardiomyopathy (HCM) is mainly associated with mutations in sarcomere protein genes, such as MYH7 and MYBPC3. Although new small molecules such as sarcomere modulators are being extensively developed in clinical trials, novel drug discovery research is still warranted due to still-unsatisfied unmet needs, e.g., direct anti-hypertrophic effect. To identify novel candidate therapeutic targets, we have developed 384 well-based high-throughput phenotypic screening platforms using iPS cell-derived cardiomyocytes (iPSC-CMs). Human iPSCs harboring HCM-causing mutations in MYH7 (MYH7-R719Q) or MYBPC3 created by genome editing technology and patient-derived iPSCs, were differentiated to cardiomyocytes then disease-characteristic morphological phenotype (hypertrophy by cell area, cell number, sarcomere orientation) was measured using a high content imaging system combined with computational analysis method. We also established “regression assay” to evaluate the reverse effect of identified compounds against hypertrophied cell size based on the fluorescence immunostaining signal patterns of cardiac troponin. Our highly diverse compound library including drug repositioning and annotated compounds provided new active compounds and novel candidate therapeutic targets, and some targets were validated using HCM patient-derived cells. Whereas sarcomere modulators did not show any anti-hypertrophic effect in our assay system, clinically approved statin’s anti-hypertrophic effect has been extensively compared, indicating our platform’s rigid data outcome which is important for selecting candidate therapeutic targets as novel therapy against HCM. Using this platform, we identified several targets which should be promising toward further compound optimization and clinical application.

Keywords: Hypertrophic Cardiomyopathy (HCM), High-throughput Phenotypic Screening, iPS cell-derived cardiomyocytes

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CRISPR-CAS9 KNOCKOUT OF PITX2C IN HPSC DERIVED ATRIAL CARDIOMYOCYTES AS AN IN VITRO TARGET DISCOVERY ASSAY FOR ATRIAL FIBRILLATION

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Genome-wide association studies have identified Pitx2 locus at 4q25 as the strongest risk locus associated with atrial fibrillation (AF) in human patients. Pitx2 is a paired like homeodomain 2 transcription factor, and Pitx2c is the cardiac specific isoform of Pitx2 that plays important roles in cardiac asymmetric development. Pitx2c is expressed in the adult left atria, and downregulation of Pitx2 in transgenic mouse models increases the susceptibility to AF. However, the lack of a comprehensive understanding of Pitx2c downstream regulated target genes and atrial selective Pitx2c interactome hinders the development of a cellular assay for the identification of druggable targets for AF. To investigate the function of Pitx2c in atrial cardiomyocytes, we first performed a CRISPR/Cas9 knockout (KO) assay of Pitx2 in human pluripotent stem cell (hPSC) derived atrial cardiomyocytes (aCMs) using multiple guide RNAs targeting different exons of the Pitx2 gene and analyzed the resulting cellular structures by cell painting. We found that a CRISPR guide RNA specifically targeting Pitx2c resulted in dramatic changes of cell structure and morphology. Functional validation of Pitx2c KO in aCMs by optical cell voltage mapping showed a shortened atrial action potential duration that provides a vulnerable substrate promoting AF. These findings offer us a valuable in vitro cellular assay for AF target discovery. To further develop an in vitro AF cellular assay, we also successfully generated a Pitx2c heterozygous (Het) KO hPSC line and will perform cell painting and functional studies in Pitx2c Het hPSC derived aCMs.

Keywords: Atrial Fibrillation, CRISPR/Cas9 KO, In vitro model

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DNA-PKCS INHIBITOR EXACERBATES DOXORUBICIN-INDUCED CARDIOTOXICITY

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The DNA protein kinase catalytic subunit (DNA-PKcs) is critically important for DNA repair. DNA-PKcs inhibitors, which sensitize tumour cells to DNA damaging agents, are currently in clinical trials with doxorubicin (DOX) to treat advanced solid tumours and leukaemia. Although effective against cancer, DOX can cause severe cardiotoxicity through DNA damage and mitochondrial dysfunction. The combined effects of DOX+DNA-PKcs inhibitor treatments on cardiotoxicity are unknown. We used a clinically relevant, DOX-sensitive human pluripotent stem cell (hPSC)-cardiomyocyte (CM) model, as well as an in vivo mouse model to evaluate the cardiotoxicity of AZD7648, a specific DNA-PKcs inhibitor currently in clinical trials.AZD7648 was found to exacerbate cardiotoxicity caused by DOX in vitro and in vivo. In hPSC-CMs, AZD7648 suppressed DNA-PKcs phosphorylation, blocked DNA repair and caused DNA damage to persist. Consequently, AZD7648 worsened DOX-induced mitochondrial function, and compromised viability. To confirm the role of DNA-PKcs in AZD7648 induced cardiotoxicity, we used siRNA to knockdown DNA-PKcs. Compared to random siRNA control, siRNA against DNA-PKcs reduced mitochondrial function of hPSC-CMs at different doses of DOX and at different time points. This suggests that cardiotoxicity may be a common feature of DNA-PKcs inhibitors when used with DOX. Lastly, we examined the cardiotoxicity of DOX and DOX+AZD7648 in mice. Body weight was lower in the DOX+AZD7648 than DOX only cohort. A decrease in heart weight was also observed in DOX+AZD7648 group relative to DOX only, showing that DOX+AZD7648 can damage the heart in vivo. Together, our results reveal cardiac safety concerns associated with the use of DNA-PKcs inhibitors in combination therapy and highlight the importance of further investigations with in vivo animal models and active clinical monitoring to ensure safe treatment for cancer patients.

Funding Source: Hong Kong Children's Cancer Foundation
Hong Kong Research Grants Council Early Career Scheme

Keywords: Human pluripotent stem cell-cardiomyocyte, DNA Damage, Cardiotoxicity

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DECODING EXTRACELLULAR VESICLE SECRETION DURING MYOCARDIAL INFARCTION USING HUMAN IPSC-DERIVED CARDIOMYOCYTES

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Extracellular vesicles (EVs) are phospholipid-bilayer enclosed particles secreted by all cells and mediate cellular crosstalk by intercellular transfer of cargo molecules, such as nucleic acids and proteins. EVs have a fundamental role in regulating physiological and pathological processes and hold great promise as early biomarkers, therapeutic agents, and drug delivery vehicles. EVs released by cardiac cells after myocardial infarction (MI) have been described to shape inflammation in a mouse model. Furthermore, injection of mitochondria-containing cardiomyocyte EVs into the mouse heart improved cardiac function after MI. Despite the clear relevance of cardiomyocyte EVs to cardiac repair following MI, the molecular mechanisms governing cardiomyocyte EV formation and release are unknown, mainly due to the absence of translational in vitro models and challenges in robust EV isolation from small cell numbers. Here, we developed a human iPSC-derived cardiomyocyte model expressing tetraspanin-NanoLuc reporters that robustly quantify EV release without the need of EV isolation. This allowed us to screen for drugs that modulated EV secretion and revealed multiple cardiomyocyte EV biogenesis mechanisms. We found that the kinase ROCK and the enzyme nSmase2 are involved in EV production by human cardiomyocytes. Moreover, nutrient starvation reduced EV release, whereas hypoxia and stimulation with TNF-alpha increased EV secretion. Further characterization revealed that human iPSC-derived cardiomyocytes secrete distinct EV subpopulations under normoxic and hypoxic conditions. Finally, proteomic analysis showed that hypoxia changes the cargo of cardiomyocyte EVs. To conclude, human iPSC-derived cardiomyocytes expressing tetraspanin-NanoLuc reporters offer a robust method to study cardiomyocyte EV biogenesis under physiological and pathological conditions, which may ultimately guide new therapeutic strategies to promote cardiac repair after MI.

Funding Source: This work was funded by the European Research Council (ERC) consolidator grant EVICARE (#725229).

Keywords: iPSC-derived cardiomyocytes, Extracellular vesicles, Myocardial infarction

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MOLECULAR AND METABOLOMIC CHARACTERIZATION OF HIPSC-DERIVED CARDIAC FIBROBLASTS TRANSITIONING TO MYOFIBROBLASTS

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Cardiac fibrosis is a central risk factor for arrhythmias and heart failure. Fibrosis disrupts matrix protein turnover resulting in increased matrix deposition, which affects cell-to-cell communication, elevates myocardial stiffness, and serves as a substrate for promoting arrhythmogenesis. The underlying cause of fibrosis is the phenotype conversion of fibroblasts to myofibroblasts, which is triggered by mechanical stress and/or pathological signaling. Cardiac fibroblasts derived from hiPSC (hiPSC-CFbs) have the potential to hold greater clinical relevance and in precision disease modeling than murine fibroblasts. However, it is unclear how passaging promotes the phenotype transition of hiPSC-Fbs to myofibroblasts and the signaling mechanisms involved. Thus, we hypothesize that passaging of hiPSC-CFbs promotes genes responsible for the myofibroblasts phenotype and increased mitochondrial metabolism. We passaged and collected hiPSC-CFbs from passage 0 to 3 (P0 to P3) and treated P0 with TGFβ1 to promote a myofibroblast phenotype. For transcriptomic profiling, qPCR analysis and NanoString analysis with a fibrosis codeset were used. For proteomic profiling, we used mass spectrometry. A Seahorse assay was used for metabolomic profiling across the different passages of cells. Immunofluorescent imaging was performed to see the phenotypic changes and mitochondrial content intracellularly after passaging. We observed that the passaging and TGFβ1 treatment of hiPSC-CFbs promoted a gradual increase in the genes responsible for myofibroblast phenotype, including collagen, periostin, fibronectin, and collagen fiber processing enzymes while downregulating the proliferation markers. Most importantly, TGFβ1 canonical and hippo signaling pathways were influenced by passaging. The Seahorse assay revealed that passaging and TGFβ1 treatment increased mitochondrial respiration consistent with the notion that the phenoconversion process requires high energy demand. Based on these data, hiPSC-CFbs passaging enhanced the fibroblast phenoconversion to myofibroblasts, influenced fibrotic signaling pathways, and enhanced mitochondrial metabolism. Thus, hiPSC-CFbs can mimic the in vitro cardiac fibrotic condition, which may help test and identify potential anti-fibrotic therapies.

Keywords: Cardiac Fibrosis, Cardiac Fibroblast, Myofibroblast

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ALPHA KINASE 3 SIGNALLING AT THE SARCOMERIC M-BAND IS REQUIRED FOR CARDIAC FUNCTION

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Pathogenic variants in alpha kinase 3 (ALPK3) cause cardiomyopathy and musculoskeletal disease. How ALPK3 mutations result in disease remains unclear because little is known about this atypical kinase. Using a suite of engineered human pluripotent

stem cells (hPSCs) we show that ALPK3 localizes to the M-Band of the sarcomere. ALPK3 deficiency disrupted sarcomeric organization and calcium kinetics in hPSC-derived cardiomyocytes and reduced force generation in cardiac organoids. Phosphoproteomic profiling identified ALPK3-dependant phospho-peptides that were enriched for sarcomeric components of the M-band and the ubiquitin-binding protein SQSTM1. Analysis of the ALPK3 interactome confirmed binding to M-band proteins including SQSTM1. Importantly, in hPSC-derived cardiomyocytes modeling ALPK3 deficiency and cardiomyopathic ALPK3 mutations, sarcomeric organization and M-band localization ofSQSTM1 were abnormal. These data suggest ALPK3 has an integral role in maintaining sarcomere integrity and proteostasis in striated muscle. We propose this mechanism may underly disease pathogenesis in patients with ALPK3 variants.

Funding Source: The Novo Nordisk Foundation Center for Stem Cell Medicine, reNEW.

Keywords: cardiomyopathy, sarcomere, cell signalling

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PROTEIN-ENCAPSULATED DOXORUBICIN REDUCES CARDIOTOXICITY IN HIPSC-CARDIOMYOCYTES AND CARDIAC SPHEROIDS WHILE MAINTAINING ANTICANCER EFFICACY

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The chemotherapeutic doxorubicin (DOX) detrimentally impacts the heart during cancer treatment. This necessitates development of non-cardiotoxic delivery systems that retain DOX anticancer efficacy. We utilized human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), endothelial cells (hiPSC-ECs), cardiac fibroblasts (hiPSC-CFs), multi-lineage cardiac spheroids (hiPSC-CSs), patient-specific hiPSCs, and multiple human cancer cell lines to compare the anticancer efficacy and reduced cardiotoxicity of single protein encapsulated doxorubicin (SPEDOX-6) to standard unformulated (UF) DOX. Cell viability assays and immunostaining in human cancer cells, hiPSC-ECs, and hiPSC-CFs revealed successful uptake of SPEDOX-6 and efficacy in killing these proliferative cell types. In contrast, hiPSC-CMs and hiPSC-CSs exhibited substantially lower cytotoxicity during SPEDOX-6 treatment compared to UF DOX. SPEDOX-6-treated hiPSC-CMs and hiPSC-CSs maintained their functionality, shown via sarcomere contractility assessment, calcium imaging, and multielectrode arrays. This study demonstrates the potential of SPEDOX-6 to alleviate cardiotoxic side effects associated with UF DOX, while maintaining its anticancer potency.

Funding Source: American Heart Association, National Institute of Health

Keywords: Cancer, Cardiotoxicity, iPSC

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EARLY ONSET OF CARDIAC PROGENITOR MATURATION LEADS TO THEIR DEPLETION AND CARDIAC PATHOLOGY DEVELOPMENT IN DUCHENNE MUSCULAR DYSTROPHY

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Duchenne muscular dystrophy (DMD) is a rare, X- linked neuromuscular disorder caused by mutations of the dystrophin gene resulting in progressive skeletal muscle loss followed by cardiomyopathy. Current medical care for DMD is palliative, and a better understanding of DMD pathologies is needed for targeted interventions and improved therapies. In addition to the widely accepted role of dystrophin in myocytes, we have previously shown that DMD derived pluripotent stem cells present impaired self- renewal as well as elevated DNA damage. The damage was at least partially caused by deregulation of nitric oxide synthase (NOS) and subsequent production of reactive oxygen/nitrogen species. Here we present that dystrophin deficient cells show impaired cardiac differentiation efficacy as illustrated by forming fewer spontaneously contracting organoids with higher rate of cardiomyocyte (CM) death, increased content of collagen and altered transcriptional program during the differentiation process. Characterization of the developing cardiovascular progenitor (CP) population shows both higher and earlier activation of DMD CP markers with subsequent attenuation of their transcription. Furthermore, earlier onset of transcription of genes associated with maturation compared to wild type (WT) coincides with a decrease in proliferation in the organoid. DMD CP population also presents higher levels of inflammation and DNA damage, thus recapitulating phenotype of mdx mouse and human DMD hearts. NOS inhibition attenuates DNA damage and improves beating organoid formation; however, it does not prevent CM death or significantly affect transcription of cardiac development related genes. Therefore, NOS inhibition may be tested as a complementary treatment

to current medical care and as a target for further molecular-level investigation and modulation.

Funding Source: Funded by National Institute for Research of Metabolic and Cardiovascular Diseases (Programme EXCELES, ID LX22NPO5104), by the European Union – Next Generation EU and by Ministry of Health of the Czech Republic, grant NU20-06-00156.

Keywords: Cardiac progenitor, Duchenne muscular dystrophy, Cardiac pathology

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STUDYING AUTOIMMUNE MEDIATED MYOCARDITIS USING AN HIPSC-MODEL OF THE HUMAN HEART

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Myocardial injury develops in approximately 25-50% of systemic lupus erythematosus (SLE) patients and presentation ranges from asymptomatic to heart failure. While the diverse landscape of patient autoantibodies may explain a significant fraction of clinical heterogeneity, their direct contribution to myocardial injury remains unknown. Here, we used a human iPSC-derived model of the human heart to study the effects of autoantibodies isolated from SLE patients with and without myocardial inflammation. For a subset of patients with myocardial inflammation and systolic dysfunction, the autoantibodies exhibited increased reactivity with cardiac tissues, resulting in decreased viability, altered calcium handling, tissue remodeling, and differential gene expression. We further demonstrated that autoantibodies isolated from these patients led to increased oxidative stress and impaired respiration. The model further allowed us to demonstrate that autoantibodies which target the cell surface delineate these patients from the other patient groups, and to identify potential disease-related autoantibodies. We therefore propose that the human tissue model of SLE-induced myocardial inflammation described here can provide a framework to characterize the molecular mechanisms of autoantibody-mediated injury and explain clinical heterogeneity.

Keywords: Cardiac tissue engineering, Disease modeling, Autoimmune

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MULTIPLEXED GENERATION AND FUNCTIONAL ANNOTATION OF HYPERTROPHIC CARDIOMYOPATHY-ASSOCIATED MYH7 MISSENSE VARIANTS IN ISOGENIC GENE-EDITED HIPSC-DERIVED CARDIOMYOCYTES

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Hypertrophic cardiomyopathy (HCM) is an inheritable cardiac condition affecting 1:200-500 people and is characterized by idiopathic left ventricular hypertrophy that can cause sudden cardiac death and/or heart failure. Autosomal-dominant, missense mutations in MYH7 (encoding the sarcomeric thick filament MHC-β) account for 33% of genotype+ HCM cases. Despite this, 75% of clinically reported MYH7 missense variants are variants of unknown significance (VUS). Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are a powerful tool to study the effect of MYH7 VUS, however, the identification of new variants by genetic testing outpaces methods to gene edit hiPSCs. Thus, we developed a novel method called CRISPRa On-Target Editing Retrieval (CRaTER) which increased successful gene-editing by 25-fold compared to current methods. We leveraged CRaTER to enrich for hiPSCs edited with a library of MYH7 single nucleotide variants (SNVs) and recovered 113 different SNVs. As deleterious mutations can reduce protein stability, we flow sorted MYH7 variant hiPSC-CMs into bins based on differing MHC-β abundance followed by next generation sequencing of each bin to calculate variant abundance scores. This multiplexed assay identified 31 MYH7 missense variants as functionally abnormal (lower abundance scores than synonymous controls), including all tested pathogenic variants and VUS. This MHC-β depletion phenotype was validated in clonal hiPSC-CMs and is supported by western blotting of myocardium from a patient with a pathogenic MYH7

missense mutation. Additionally, single-cell contractility analyses of hiPSC-CMs with a known pathogenic MYH7 variant and reduced MHC-β abundance revealed hyperdynamic physiology relative to isogenic controls, suggesting MHC-β loss could be involved with systolic dysfunction. Together, CRaTER enables the generation of isogenic variant hiPSC lines at unprecedented scale for applications in disease modeling. Functional analysis of MYH7 variant hiPSC-CMs has revealed a novel correlation between loss of MHC-β protein and clinical pathogenicity that is useful for stratifying previously untested MYH7 variants in hiPSC-CMs at scale. Further investigation into the consequences of MHC-β loss will be key to elucidating MYH7-based HCM pathogenesis.

Keywords: MYH7, hypertrophic cardiomyopathy, disease modelling

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SINGLE-CELL RNA SEQUENCING REVEALS MATURATION TRAJECTORY IN HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES IN 2D AND 3D ENGINEERED CARDIAC TISSUES AND CHAMBERS

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Human engineered cardiac tissue models are increasingly used for in vitro studies as stem cell-derived cardiomyocytes (CMs) have become more accessible with optimized differentiation. However, the resulting derived CMs often have inconsistent and varying levels of post-differentiation maturity, and are typically still much less mature than their in vivo counterparts. It is, therefore, important to understand what additional factors, such as in vitro environmental complexity, can affect the maturity of these cells. In this study, the transcriptomes of human pluripotent stem cell-derived CMs (healthy hESC and hiPSC) were compared by performing single-cell RNA sequencing across CMs that had been cultured in different engineered cardiac tissue configurations: 2D human ventricular cardiac anisotropic sheet, 3D human ventricular cardiac tissue strip, and 3D human ventricular cardiac organoid chamber (hvCOC). Spontaneously formed 3D cardiac spheroid aggregates (CS) were used as a control. Post differentiation, CMs in fabricated tissues were cultured in their respective constructs for ~15 days prior to dissociation of the tissues into single cells for RNA isolation. Libraries were constructed using the 10X Chromium system and sequenced using the Illumina NovaSeq 6000 and Illumina NextSeq500 Systems. To identify cell phenotypes in the stem cell-derived tissue constructs, the 2,000 most variable genes were selected using the Seurat R package, and the cells were projected onto a two-dimensional space. Clustering of CMs and fibroblasts was performed separately for both cell types. Our results suggest that maturity increases with the complexity level of the engineered tissues, with CS exhibiting the fewest markers of maturity while hvCOC exhibited the most. It is also interesting to note the order in which hallmarks of maturity develop: the contractile components developed first, followed by electrophysiology and oxidative metabolism. Outcomes of this study demonstrated the application of single-cell RNA-sequencing for evaluating maturation in engineered cardiac tissues, and the finding that more complex engineered tissue models provide a more physiologically mature in vitro cardiac tissue model for drug screening and disease modeling.

Keywords: Development Modeling, Human Engineered Cardiac Tissues, Single-Cell RNA Sequencing

TOPIC: EPITHELIAL INCLUDES SKIN GUT LUNG

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AUTOPHAGY-RELATED COMPOUND SCREENING FOR COVID-19 DRUG DEVELOPMENT

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It is essential to develop not only drugs that target viral proteins but also drugs that target host proteins to deal with broad spectrum coronavirus including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It has been reported that autophagy is deeply involved in coronavirus infection. However, to our knowledge, a comprehensive anti-SARS-CoV-2 drug evaluation using an autophagy compound library has not been performed. In this study, we performed drug screening using airway organoids and an autophagy compound library, aiming to identify compounds that could treat broad spectrum coronavirus infection. Among 80 autophagy compounds, cycloheximide and thapsigargin reduced SARS-CoV-2 infection efficiency in a dose-dependent manner. Cycloheximide treatment reduced the infection efficiency of not only 6 SARS-CoV-2 variants (B.1.1.214, B.1.617.2, BA.1, BA.2, BA.2.3, and BA.5), but also human coronavirus (HCoV)-229E and HCoV-OC43. Cycloheximide treatment could cancel the viral infection-mediated induction of innate immune response. However, even low-dose (1 μM) cycloheximide treatment altered the

expression profile of ribosomal RNAs, thus side effects, such as inhibition of protein synthesis in host cells should be noted. These results suggest that cycloheximide showed broad spectrum anti-coronavirus activity in vitro.

Funding Source: This research was supported by the iPS Cell Research Fund, the JSPS KAKENHI (Grant Number: 21H03795), and the AMED (Grant Number: JP20fk0108533, JP21fk0108492, and JP21gm1610005).

Keywords: drug screening, airway organoids, SARS-CoV-2

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IN VITRO THYMIC EPITHELIAL PROGENITOR DEVELOPMENT FROM IPSCS AS A MODEL FOR DISSECTING GENETIC THYMIC DISORDERS

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Thymic epithelial cells (TECs) play a critical role in the development of T cells as they provide essential cues to hematopoietic progenitors for proliferation and differentiation in the thymus. For this reason, genetic mutations that affect TECs prevent development of T cells. We have previously reported that T cell development is blocked in patients with hematopoietic-intrinsic genetic defects using an artificial thymic organoid system, but this model does not allow to test for thymic defects. To address this, we have generated multiple induced pluripotent stem cell (iPSC) lines from patients carrying mutations in genes involved in thymic stromal development and/or function, including TBX1, HOXA3, FOXI3, PAX1, AIRE, EXTL3, and TP63. Previously published differentiation protocols mostly started from embryonic stem cells and we have optimized them for iPSCs, with the ultimate goal of differentiating these patient-derived lines into TECs and assess whether these mutations alter TEC generation. Analysis of the developmental progression of these lines from definitive endoderm to thymic epithelial progenitors using RNA-seq and ATAC-seq will provide insights into the transcriptional network governing this process in homeostasis and disease. Moreover, once we successfully generate TECs, we plan to incorporate them into our artificial thymic organoid system to create a model that is based solely on the patients' cells to provide an accurate reproduction of T cell development in the thymus of patients with T cell immunodeficiencies.

Funding Source: This work was supported by the Division of Intramural Research, NIAID, NIH.

Keywords: Thymic epithelial cells, Thymic development, T- cell Immunodeficiency model

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VALUABLE MODEL: SKIN-DERIVED PRECURSOR CELLS FROM HUMAN AUTOPSY-DERIVED SCALP FIBROBLASTS FOR NEURODEGENERATIVE DISEASE RESEARCH

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The Human Cells Core for Translational Research (HCCTR), established in 2018, takes advantage of the Brain and Body Donation Program (BBDP) tissue resource to build a human fibroblast banking program using postmortem scalp tissues. Tissues are used to obtain fibroblasts by directly culturing scalp explants, and 85 cases of various ages, APOE genotypes, and disease diagnoses have been banked. The banked fibroblasts were confirmed as fibroblast-specific using both immunofluorescence and RT-qPCR assays, as they tested positive for markers and genes associated with fibroblasts and negative for those associated with keratinocytes and epithelial cells. Here we report that human skin-derived precursors (hSKPs) were successfully isolated from banked fibroblasts and presented properties of self-renew and differentiation potentials. Stem cell characterization of mature hSKPs was confirmed through immunofluorescence and RT-qPCR. In vitro differentiation potential of mature hSKPs was demonstrated to have multilineage potential, resulting in the formation of fat-, cartilage- and bone-like cells. Furthermore, we demonstrated that the banked cells were successfully reprogrammed to human inducible pluripotent stem cells (iPSCs) and directly reprogrammed into induced neuronal cells (iN cells). The hiPSCs exhibited typical hiPSC morphology, a normal euploid karyotype, a high expression of crucial pluripotency markers, and can differentiate into three lineages. Additionally, these induced iN cells express a variety of neuron-specific proteins and have the ability to form functional synapses. Our study showed that routinely collect and bank fibroblasts from scalps of neuropathologically characterized donors, which can then be used to generate hSKPs, reprogram them into hiPSCs, and directly convert them into iN cells. Through this cell banking program, a valuable human cell resource is being created to improve our understanding of normal aging and age-related neurodegenerative diseases. It also provides an opportunity to investigate potential underlying mechanisms and establish effective disease modeling. These cryogenically preserved cells are accessible to researchers through a request on the program website of the Banner Sun Health Research Institute (BSHRI).

Funding Source: National Institute on Aging; Arizona Biomedical Research Commission; Arizona Department of Health Services; Sun Health Foundation.

Keywords: Skin-derived precursors (SKPs), Somatic stem cell, Induced pluripotent stem cells

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HUMAN AIRWAY EX VIVO MODELS: NEW TOOLS TO STUDY THE AIRWAY EPITHELIAL CELL RESPONSE TO SARS-COV-2 INFECTION

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Airway-liquid interface cultures of primary epithelial cells and of induced pluripotent stem cell-derived airway epithelial cells (ALI and iALI, respectively) are physiologically relevant models for respiratory virus infection studies because they can mimic the in vivo human bronchial epithelium. Here, we investigated gene expression profiles in human airway cultures (ALI and iALI models) infected or not with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) using bulk and single-cell transcriptome datasets. SARS-CoV-2 2 infection significantly increased the expression of interferon-stimulated genes and inflammatory genes at day 4 post-infection, indicating activation of the interferon and immune responses to the virus. Extracellular matrix genes also were altered in infected cells. 3D Confocal microscopy, flow cytometry and RT-qPCR analysis revealed a plasticity of the tissue to fight infection. Single-cell RNA sequencing data shows that SARS-CoV-2 infection damaged the respiratory epithelium, particularly mature ciliated cells. The expression of genes encoding intercellular communication and adhesion proteins also was deregulated, suggesting a mechanism to promote shedding of infected epithelial cells. These data demonstrate that ALI/iALI models help to understand the airway epithelium response to SARS-CoV-2 infection and are a key tool for developing COVID-19 treatments.

Keywords: iPS cells, Human airway models, SARS-CoV-2 infection

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INTESTINAL SUB-EPITHELIAL MYOFIBROBLASTS AUGMENT BMP SIGNALING TO PROMOTE STEM CELL REGENERATION

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Intestinal stem cells (ISCs) sustain and replenish a continually shedding epithelium and are housed in crypt invaginations of the mucosa. Here, ISCs self-replicate and produce daughter transit-amplifying cells, or progenitors, that are pushed towards the tops of crypts into finger-like protrusions called villi where they terminally differentiate. During intestinal damage, including inflammatory bowel disease, the epithelium regenerates ISCs by de-differentiation of crypt progenitors. A crucial question in stem cell biology is how regeneration is achieved. At homeostasis, ISCs depend on a surrounding mesenchymal environment termed "the niche". The niche sustains the proper balance between ISC self-replication and differentiation via signaling gradients, including Bone Morphogenetic Proteins (BMPs), which promote epithelial differentiation. The niche is composed of a heterogenous mixture of cells that are anatomically, molecularly, and functionally distinct, including PDGFRA+ fibroblasts and smooth muscle. PDGFRA+ sub-epithelial myofibroblasts (SEMFs) occur closest to the epithelial-mesenchymal barrier, concentrate at tops of crypts, produce abundant BMPs, and promote in vitro epithelial differentiation. Sub-cryptal localized trophocytes and smooth muscle provide ISC sustaining factors, including BMP inhibitors, and are required to maintain ISCs in vivo and in vitro. While the niche sustains epithelial homeostasis, it is unclear what role it plays during ISC regeneration. We find that the transcriptomes of sub-cryptal supporting cells are largely unchanged following epithelial damage and ISC loss. In contrast, SEMFs reduce expression of BMP ligands and increase expression of BMP antagonists. Epithelial BMP activity, reflected in nuclear pSMAD expression, becomes attenuated as a result. Using in vitro and in vivo perturbations, we detail a previously unappreciated role for SEMFs in ISC regeneration via modulation of peri-cryptal BMP signaling.

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Keywords: intestinal stem cell regeneration, stem cell-niche interactions, intestinal sub-epithelial myofibroblasts

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RAPID ASSESSMENT OF VIABILITY IN PATIENT-DERIVED CANCER ORGANOIDS

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Cancer cell lines grown in 2D have long served as experimental surrogates for cancers. In recent years, the 3D culture of cancer cells, often alongside other cell types in formats where they can form multi-layered structures, is enabling new models for cancer research that are considered more biologically relevant. Cancer organoids derived from patient tissue offer researchers a highly relevant disease model system, as these organoids and the patients from which they were derived have been shown to respond similarly to drugs. Recently, these models have become more widely utilized, thanks to standardization and scalability that have made available large numbers of uniformly sized and highly viable organoids. Characterization of organoid response to candidate drug treatment is a powerful research tool that provides a wealth of detailed information. While automation can help to scale up the process, screening a large number of compounds requires significant effort and hands-on time. Streamlining the process is important for rapid identification of compounds that can be followed up with more time-consuming studies. We demonstrate here the methods for analyses of key parameters such as cell viability or metabolic markers that allow rapid identification of effective drug candidates, and can be combined with more complex image analysis. We have worked with several colorectal cancer organoid lines derived from patient tissues. Organoids

were placed in 384-well microplates either manually or using an automated liquid handling system and treated with a set of twelve selected anti-cancer compounds, including romidepsin, cisplatin, doxorubicin, and trametinib, for 3 or 5 days. Organoid number and overall morphology were assessed by label-free transmitted light imaging, then organoids were lysed and assayed for viability using a luminescent ATP assay. The cell viability assay was used to quantitatively analyze drug responses, which were combined with automated image analyses for characterization of compound effects on organoid size and morphology. We demonstrated differential effects of various compounds on cell viability and structure. We demonstrated the usefulness of the microplate reader-based ATP assay and label-free imaging methods for rapidly gauging drug response in patient-derived organoids.

Keywords: patient-derived organoids, cancer, three-dimensional cell culture

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AUTOPHAGIC FLUX IS UNAFFECTED IN OCA1A PATIENT DERIVED RETINAL PIGMENT EPITHELIUM

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Oculocutaneous albinism type 1A (OCA1A) is caused due to mutations in the TYROSINASE gene and results in pigmentation defects of the skin, hair, and eyes. Retinal pigment epithelium (iRPE) derived from OCA1A patients via induced pluripotent stem cell (iPSC) technology exhibits pigmentation defects such as the accumulation of pre-melanosomes, absence of mature melanosomes, and increased presence of abnormal and degenerating melanosomes. Here, we investigated the levels of intracellular debris in OCA1A- iRPE due to defective melanosome accumulation and the subsequent impact on the autophagy pathway. All human iPSC work was approved by the NIH Institutional Review Board, protocol #11-E1-0245 (NCT01432847). Three control and two OCA1A iPSC lines used in the study have been reported earlier (George et al., 2022). An isogenic pair of iPSC composed of normal TYR gene and CRISPR/Cas9 mediated TYR knockout were also generated. Directed differentiation of iPSCs towards RPE was performed as described earlier (Sharma et al., 2021). Autophagosomes were studied using transmission electron microscopy (TEM). Protein expression of autophagy-related genes was studied using Western blotting. OCA1A patient-derived iRPE exhibited a lack of pigmentation and mature melanosomes. The number of autophagosomes in OCA1A-iRPE was increased, but no significant change was observed in protein levels of ATG5 and ATG7 between OCA1A and CTRL-iRPE. No changes were observed in the protein levels of LAMP2, a marker of lysosomes, whereas LAMP1 which localizes to lysosomes and melanosomes was significantly downregulated in OCA1A-iRPE. The autophagy marker protein LC3b remained unchanged, but p62 levels were significantly upregulated in OCA1A-iRPE, as studied by Western blotting post Bafilomycin A treatment. mTOR which plays a vital role in regulating autophagy and its phosphorylated form p-mTOR (Ser2448) were both down-regulated in OCA1A-iRPE, but the p-mTOR/mTOR ratio remained unchanged. OCA1A iRPE exhibits increased build-up of cellular debris in the form of enlarged autophagosomes and increased protein levels of p62, which could be due to abnormal melanosome accumulation. No changes in autophagic flux and p-mTOR/mTOR levels suggest that OCA1A-iRPE might be adapted to efficient processing of increased build-up of cellular debris.

Keywords: albinism, retinal pigment epithlium, autophagy

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EFFECTS OF LUNG MESENCHYME FROM BRONCHOPULMONARY DYSPLASIA PATIENTS ON LUNG EPITHELIAL ORGANOIDS

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Bronchopulmonary dysplasia (BPD) is a chronic lung disease which develops in infancy and can persist throughout life. BPD commonly occurs in premature newborns as the lungs continue to develop and mature up to, and after, a normal term birth. Thus, premature newborns have underdeveloped lungs and often require lifesaving oxygen supplementation and mechanical ventilation which can cause damage resulting in BPD. BPD patient lungs have larger and more simplified alveoli than healthy counterparts; however, the mechanisms leading to BPD are not fully understood. Alveolar development requires coordinated mesenchyme-epithelial interactions. Recent work has shown that hyperoxia can affect mesenchymal cell transcription and protein secretion, suggesting that perturbations to the mesenchyme may contribute to BPD. Despite this, there is still much unknown regarding how mesenchymal cells, through physical interactions or secreted factors, influence epithelial cell function during normal human development and in the context of BPD. To address this gap, our lab has developed several primary human tissue organoid and explants, and iPSC-derived lung organoids that allow us to interrogate undifferentiated epithelial progenitor cells, airway, and alveolar cells. Here, we use well-characterized lung mesenchyme from healthy and age-matched BPD patients. We functionally interrogate healthy and BPD patient mesenchymal cells to determine how they influence epithelial cell behavior, differentiation, and function using co-cultures with human tissue models. This work will aid in our understanding of the progression of BPD, specifically how the lung mesenchyme influences epithelial development.

Keywords: lung disease, mesenchyme, bronchopulmonary dysplasia

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RECAPITULATION OF THE PATHOPHYSIOLOGY OF INFLAMMATORY BOWEL DISEASE USING HUMAN IPS CELL-DERIVED COLONIC EPITHELIAL CELLS

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Inflammatory bowel disease (IBD) is a chronic inflammation of the gastrointestinal tract. To our knowledge, the pathogenesis of IBD remains unknown and there is no treatment to achieve complete recovery. There are only a few IBD models that allow us to analyze how colonic epithelial cells are damaged in IBD. Therefore, we tried to establish an IBD model using human induced pluripotent stem cells-derived colonic epithelial cells (iPS-CECs). We confirmed that the expression levels of colonic markers were expressed in the iPS-CECs at high levels suggesting that iPS-CECs have colonic characteristics. To recapitulate IBD, iPS-CECs were treated with pro-inflammatory cytokines, tumor necrosis factor-α (TNF-α), interferon-gamma (IFN-γ), and interleukin 1 beta (IL-1β), produced by immune cells in the intestine of IBD. The gene expression levels of inflammatory markers (e.g., IL-8 or IL-6) and human beta-defensin-2 (hBD-2) in iPS-CECs were increased by cytokines treatment. Consistently, ELISA analysis showed that the IL-8 production capacity of cytokines-treated iPS-CECs was 25.7 times higher than that of vehicle-treated iPS-CECs. These results suggest that IBD-related inflammatory responses could be reproduced by cytokines treatment. In addition, we found that lactate dehydrogenase (LDH) release in cytokines-treated iPS-CECs was 2.6 times higher than that in vehicle-treated iPS-CECs. We also evaluated the gene expression levels of markers of colonic epithelial cells (colonocytes, goblet cells, enteroendocrine cells, and intestinal stem cells). The cytokines treatment decreased the gene expression levels of colonocyte and goblet cell markers, while it increased the gene expression levels of intestinal stem cell marker. Taken together, these results suggest that cytokines treatment causes cytotoxicity and alters the cellular composition of iPS-CECs. In conclusion, we succeeded in recapitulating IBD pathophysiology using iPS-CECs.

Funding Source: This research was supported by the iPS Cell Research Fund, the Japan Agency for Medical Research and Development (AMED) (JP21gm1610005), and Japan Science and Technology Agency (JST), ACT-X (JPMJAX222A).

Keywords: inflammatory bowel disease, human iPS cells, colonic epithelial cells

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PERTURBATION OF EPITHELIAL CELL STEMNESS LEADS TO COLITIS IN CBP-AA-KNOCKIN MICE THROUGH ENHANCED BINDING OF CBP TO P53

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Inflammatory bowel diseases (IBDs) including Crohn's disease (CD) and ulcerative colitis (UC) are chronic inflammation of intestine to result in bloody diarrhea and severe abdominal pain. Its etiology involves dynamic alterations in multiple cell types including epithelial, stem, mesenchymal and immune cells. The single layer epithelium is composed of different specialized intestinal epithelial cells (IECs) characterized by high turnover rate to maintain barrier integrity. The IECs are tightly orchestrated by a rapid self-renew of intestinal stem cells (ISCs). Abnormalities in intestinal barrier integrity are associated with elevation of intestinal permeability and lead to intestinal inflammation. CBP is a transcriptional coactivator modulating DNA accessibility for transcription factors. We previously demonstrated that IKKa phosphorylating CBP at Ser1382/1386 (human) plays a critical role in regulating cell fate by enhancing CBP binding to NF-kB but suppressing its binding to p53. These suggested that post-translational modification (PTM) of CBP plays an important role in regulating cell fates. CBP-AA-knockin mice were therefore generated by replacing CBP Ser1383/1387 with alanines (AA) and exhibit a spontaneous colitis phenotype. Proximity ligation assay showed an abundant binding of CBP to p53 in colonic IEC of CBP-AA mice instead of binding to NF-kB (p65) in CBP-WT mice. The role of impaired CBP phosphorylation in regulating colonic stem cells was unveiled by reduced number of Lgr5-eGFP+ stem cells in CBP-AA/Lgr5-eGFP mice demonstrated by immunofluorescence (IF) staining and flow cytometry analysis. A near-physiological primary epithelial 3D organoids grown from a single Lgr5+ stem cell in colonic crypt and consisting of various differentiated epithelial lineages are established. CBP-AA organoids show reduced organoid-forming efficiency and organoid size, while those from CBP-AA/Vil-p53KO mice are not seen and colitis phenotype is diminished in these mice as well. These suggest that a defect in PTM of CBP phosphorylation might dampen IEC stemness through a CBP/p53-dependent pathway, contributing to spontaneous colitis in CBP-AA mice. A new insight that PTM of CBP phosphorylation modulates IECs stemness per se and its defect translated into a clinically important human IBD is explored.

Keywords: IBDs, Lgr5, CBP, P53, organoid, Intestinal epithelial cells (IECs), Intestinal stem cells (ISCs), Post-translational modification (PTM)

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DISSECTION OF PARTICULATE MATTER 10 EXPOSURE BASED ON 2D AND 3D INTESTINAL EPITHELIUM MODEL SYSTEM

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Particulate matter (PM) is classified as one of air pollution and cause of various diseases. PM through direct ingestion or inhalation has been a known cause of inflammatory disease and an imbalance of the gut microbiome in the GI tract. However, the role of PM10 as a risk factor for the exacerbation of inflamed intestinal epithelium has still unknown. Here, we established the chronic inflamed intestinal epithelium models using human pluripotent stem cells (hPSCs)-derived 2D and 3D intestinal epithelial models for the study of the deleterious effect of PM10. The inflamed 2D human intestinal epithelial cells (hIECs) and 3D human intestinal organoids (hIOs) exhibited pathology such as inflammatory response, decreased intestinal markers, and disturbed epithelial barrier function. The PM10 exposure affects additive disease severity and lead to altered gene expression in inflamed 2D hIECs and 3D hIOs. Importantly, we identified that the PM10 exposure impaired the calcium signaling pathway and protein digestion and absorption pathway in inflamed 2D IECs and 3D hIOs. These results demonstrated that the calcium signaling-mediated deleterious effect of PM10 such as intestinal dysfunctions in inflamed 2D and 3D intestinal epithelium models and may be used as promising therapeutic candidates.

Keywords: human intestinal epithelium, human intestinal organoid, Particulate matter 10

TOPIC: GERMLINE AND EARLY EMBRYO

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GENOME-WIDE ANALYSIS OF HAPLOINSUFFICIENCY DISORDERS UTILIZING HUMAN EMBRYONIC STEM CELLS AND NEURAL PROGENITOR CELLS

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Haploinsufficiency describes a phenomenon where one functioning allele of a gene in a diploid cell or organism is insufficient for a normal phenotype. Although haploinsufficiency underlies several human diseases, the effect of haploinsufficiency on human embryonic stem cell (hESC) growth and proliferation has not been thoroughly studied. Here, we aimed to identify genes affecting the normal growth of hESCs when one of their two alleles is lost. To establish a genome-wide loss-of-function screening for heterozygous mutations, we fused normal haploid cells with a library of mutant haploid hESCs. We have identified over 600 genes with a negative effect on hESC growth in a haploinsufficient manner and characterized them as genes that show less tolerance to mutations, more conservation during evolution and depletion from telomeres and X chromosome. Interestingly, a large fraction of these haploinsufficiency genes is associated with the extra-cellular matrix and the plasma membrane. We have revealed an enrichment of genes causing haploinsufficiency disorders within WNT and TGF-b signal transduction pathways. We could thus identify haploinsufficiency-related genes and pathways that show growth retardation in early embryonic cells, suggesting dosage-dependent phenotypes in hESCs. There are several neurodegenerative disorders affected by haploinsufficiency phenomenon, and many of them are related to autism. We thus differentiated the library of heterozygote mutations into neural progenitor cells (NPCs) and identified about the genes essential for their normal differentiation in a haploinsufficient manner. We were able to identify neuro-related dosage-sensitive pathways and detected several autism-related genes with a haploinsufficiency effect, suggesting that they can be modelled at early stages of differentiation. Overall, we have constructed a novel model system for studying haploinsufficiency in both hESCs and NPCs, and thus were able to characterize important dosage-dependent genes and pathways involved in their normal growth and pathology. The initial part of the research was published in Cell Reports (2022).

Keywords: Haploinsufficiency Disorders, Human Pluripotent Stem Cells, Neural Progenitor Cells

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INTER-GASTRULOID HETEROGENEITY REVEALED BY SINGLE CELL TRANSCRIPTOMICS TIME COURSE: IMPLICATIONS FOR ORGANOID BASED PERTURBATION STUDIES

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Recent advances in organoid and genome editing technologies are allowing for perturbation experiments at an unprecedented scale. However, before doing such experiments it is important to understand the gene expression profile in each of the organoid's cells, as well as how much heterogeneity there is between individual organoids. Here we characterise an organoid model of mouse gastrulation called gastruloids using single cell RNA-sequencing of individual organoids at half-day intervals between day 3 and day 5 of differentiation (roughly corresponding to E6.5-E8.75 in vivo). Our study reveals multiple differentiation trajectories that have hitherto not been characterised in gastruloids. Intriguingly, we observe that individual gastruloids displayed a strong bias towards producing either mesodermal (largely somitic) or ectodermal (specifically neural) cell types. This bifurcation is already seen at the earliest sampled time point, and is characterised by increased activity of WNT-associated pathways in mesodermally-biased gastruloids as compared to neurally-biased gastruloids.

Notably, at day 5, mesodermal gastruloids show an increase in the proportion of neural cells, while neural gastruloids do not produce notably more mesodermal cells. This is in line with previous studies on how the balance between these cell types is regulated. We demonstrate using in silico simulations that without proper understanding of the inter-organoid heterogeneity, perturbation experiments have either very high false positive or negative rates, depending on the statistical model used. Thus in future studies, modelling of inter-organoid heterogeneity will be crucial when designing organoid-based perturbation studies.

Funding Source: European Research Council (ERC)

Keywords: Gastruloid, Lineage Commitment, Multi-seq

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HUMAN PLURIPOTENT STEM CELL AS A PLATFORM FOR IN VITRO MODELING OF INTRACRANIAL GERM CELL TUMORS

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Intracranial germ cell tumors (iGCTs) are a rare pediatric and adolescent/young adult (AYA) tumor arising from primordial germ cells (PGCs). The paucity of in-vitro models for studying iGCTs has stifled research; however, the recent development of human PGC-like cells (hPGCLCs) generated from human pluripotent stem cells (hPSCs) opened novel avenues to explore the transformation of PGCs into iGCTs. iGCTs harbor few somatic mutations, but alterations in the KIT/RAS pathways are most common and play a key role in tumorigenesis. There is likely genetic predisposition for developing iGCT because individuals from East Asian nations are disproportionally diagnosed with iGCTs. Interestingly, a germline variant of the histone lysine demethylase JMJD1C has been identified in Japanese patients with iGCTs. The mechanism by which these mutations alter PGC development and lead to tumorigenic transformation is not fully understood, but the hPSC-to-hPGCLC method provides for the investigation of these mechanisms. We sought to develop an in-vitro model of PGC transformation into iGCTs using the hPSC-to-hPGCLC method. We hypothesize that hPSCs, engineered to harbor somatic and/or germline mutations found in iGCTs, can be differentiated into hPGCLCs and then meaningfully evaluated for tumorigenic potential. Two parental hPSC cell lines, one from a European Caucasian male (KOLF2.1J) and one a Japanese male (WTC11.1J), were genetically targeted, cultured, and differentiated. hPSCs harboring the JMJD1C mutation S880P and/or the KIT mutation D816V were genetically engineered using CRISPR/Cas9. Single mutants were generated as homozygous and heterozygous, while the double mutant lines have heterozygous mutations at both loci. For each engineered hPSC line, hPGCLCs were generated using the two-step differentiation method, in which hPSCs were first differentiated into incipient mesoderm-like cells (iMeLCs) before being formed into aggregates that contain hPGCLCs. Each engineered hPSC line was evaluated for their ability to maintain pluripotency and to differentiate into iMeLCs and PGCLCs. PGCLCs generated from hPSCs with iGCT-relevant mutations are an important tool for investigating the effect of these genes on hPGCLC transformation and developing targeted therapeutics for iGCTs.

Keywords: human pluripotent stem cells, human primordial germ cells, intracranial germ cell tumor

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GENERATION AND CHARACTERIZATION OF TRIPLOID HUMAN EMBRYONIC STEM CELLS AND THEIR DIFFERENTIATED DERIVATIVES

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Humans are diploid organisms, and only a few tissues in our body naturally harbor cells with different number of complete genomic copies. However, triploidy in human embryos is not very rare, as it is responsible for ~10% of spontaneous miscarriages, and surprisingly, some of these pregnancies proceed to term. These triploid infants suffer from many physical and mental deficiencies, usually leading to their early death. To investigate the impact of triploidy on human development, we generated triploid human embryonic stem cells (hESCs) by fusing haploid and diploid hESCs. This allowed us to study the behavior of triploid compared to diploid hESCs carrying the same genetic background. Triploid hESCs showed a typical morphology of hESCs, with a larger volume than the diploid cells (leading to decreased surface area to volume ratio). We analyzed the genome-wide transcription, methylation, and replication timing profiles, as well as the pluripotency of these cells. Both diploid and triploid hESCs express all pluripotent stem cell markers, although we identified differential expression of genes linked to several transport and metabolic processes. To explore the cell size's effect on triploid hESCs' transcriptome, we analyzed the expression of membrane-associated proteins in triploid cells, identifying a significant decreased expression of ion channels. Interestingly, we observed a very significant decrease in the ability of triploid hESCs to differentiate to the three germ layers and especially into the neural lineage, both in vivo and in vitro, compared to their diploid counterparts. This inhibition in differentiation may correspond to the downregulation of ion channels, as we show that many of these genes are essential in neural progenitor cells but not in hESCs. Notably, our research raises the possibility that triploidy-related pathologies may emerge due to the physical dimensions of the cells affecting their initial stages of development, and emphasizes our unique platform in studying the effects of ploidy on early human development.

Keywords: Human pluripotent stem cells, Polyploidy, Human development

TOPIC: HEMATOPOIETIC, IMMUNE, AND ENDOTHELIAL

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ENGINEERED THREE-DIMENSIONAL VESSELS-ON-A-CHIP TO MODEL INFLAMMATORY RESPONSES USING HIPSC-DERIVED VASCULAR CELLS AND MONOCYTES

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Inflammation is a major contributor to many vascular diseases leading to EC dysfunction and tissue damage. The hallmark of inflammatory responses is the infiltration of immune cells to the site of inflammation. Endothelial cells (ECs) that form the inner lining of all blood vessels facilitate the capture of circulating immune cells upon activation by local pro-inflammatory mediators. Leukocyte recruitment cascade has been extensively studied using EC monolayers, however these models fail to mimic the complex and multicellular composition of human vasculature. To this end, we recently developed a physiologically relevant in vitro human vascular model to study inflammatory responses. 3D Vessels-on-a-Chip (VoC) platform integrates hiPSC-derived ECs and hiPSC-derived mural cells into a lumen patterned in collagen I matrix using viscous finger patterning technique. The endothelial barrier is formed on the following day which is directly associated with the surrounding hiPSC-mural cells, recapitulating physiological cell-cell interaction. Deposition of the basement membrane components, such as laminin and collagen IV, by vascular cells was confirmed by the immunofluorescent staining. Upon treatment with pro-inflammatory mediators (TNFα, IL1β, IFNγ), vascular cells upregulated the surface expression of adhesive receptors (ICAM-1), and released potent proinflammatory mediators (IL-6, IL-8, MCP-1), that are essential for the leukocyte recruitment cascade. Pre-treated vessels were next perfused with hiPSC-derived monocytes under controlled flow at physiological shear stress using a microfluidic pump. Adhesion of hiPSC-monocytes was significantly higher to the inflamed endothelial barrier under flow. Furthermore, preliminary results suggest that continuous exposure of physiological shear stress to the captured hiPSC-monocytes promotes their breaching and extravasation across the endothelial barrier. In conclusion, we developed an entirely isogenic hiPSC-derived 3D VoC model recapitulating key physiological aspects of vascular biology, which is valuable to study inflammatory responses and barrier function. Using hiPSCs derived from patients with specific disease genotypes presents opportunities to investigate the mechanisms of inflammatory responses in vascular diseases.

Funding Source: This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 812954.

Keywords: hiPSC-endothelial cells and monocytes, Vessels-on-a-Chip, Inflammatory responses

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HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED BONE MARROW ORGANOIDS TO MODEL HEMATOPOIETIC DEVELOPMENT AND DISEASE IN A COMPLEX HUMAN NICHE

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Organoids generated from induced pluripotent stem cells (iPSCs) are powerful tools to model human development and diseases in vitro. Three-dimensional model systems to study hematopoiesis in concert with vasculogenesis and osteogenesis are needed. Here we developed a method for the generation of complex human iPSC-derived bone marrow organoids (BMOs) consisting of a de novo vascular network, a multilineage hematopoietic compartment and a heterogeneous mesenchymal compartment. After

embryoid body (EB) formation mesoderm was induced and patterned by a sequential combination of Wnt activation and Activin/Nodal inhibition. EBs were embedded into a 3D matrix and stimulated with defined cytokine cocktails to generate hematopoietic progenitor cells while maintaining endothelial and mesenchymal cell generation. Sprouted EBs were then separated and transferred into a 96-well plate to promote full organoid maturation until day 17. Analysis of cellular composition by scRNA-seq revealed a hematopoietic compartment consisting of myeloid cells, such as GMPs, neutrophils, monocytes and macrophages, but also megakaryocyte-like cells, lymphoid progenitors and a cluster of cells expressing genes of HSPCs. The endothelial compartment was characterized by arterial gene expression. In the mesenchymal compartment we could identify pericyte-like cells, as well as smooth-muscle cells and osteochondrogenic precursors. These three main cellular compartments were confirmed by flow cytometry. Sorted HSPCs and MSPCs showed multilineage potential. Using confocal, two-photon and electron microscopy we found a complex spatial architecture of hematopoietic cells embedded into a lumen-forming vascular network covered by pericytes reminiscent of human bone marrow in vivo. Moreover, gene-edited BMOs modelled aspects of an inherited bone marrow failure syndrome. Finally, BMOs can be transplanted into immunodeficient mice and connect to the murine vasculature. In summary, human iPSC-derived BMOs may prove useful to study hematopoietic development and disease evolution in a complex three-dimensional environment. Since the lack of vasculature and immune cells is a common limitation of current organoid protocols, BMOs offer further potential for incorporation into other organoid systems.

Keywords: bone marrow organoid, hematopoiesis, human niche

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REPURPOSING OF FDA APPROVED DRUGS TO TARGET QKI-7 AND RESCUE DIABETIC VASCULOPATHY

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Vascular disease is a major cause of morbidity and mortality in diabetes mellitus. Whilst underlying mechanisms remain poorly defined, it is clear that endothelial cell (EC) dysfunction plays a central role, thereby representing a potential therapeutic target. Due to problems surrounding primary ECs, iPSC derived ECs (iPS-ECs) have emerged as valuable research models. We assessed the ability of diabetic derived iPS-ECs (dbiPS-ECs) to effectively recapitulate the disease phenotype. Sequencing and functional analysis revealed dbiPS-ECs to have reduced angiogenic capacity, mitochondrial dysfunction, impaired barrier function and increased ROS generation versus those from healthy donors; demonstrating dbiPS-ECs as clinically relevant models of diabetic vasculopathy that could help uncover pathogenic mechanisms and develop novel therapies. We previously highlighted QKI-7 to be a critical mediator of vascular dysfunction in experimental diabetes with knockdown in vivo following hindlimb ischemia to significantly restore blood flow and reperfusion. Further evaluation since has revealed QKI-7 to promote the degradation of downstream homeostatic genes, COL4A2, TMEM184A and PPP1R15A in dbiPS-ECs resulting in impaired tube formation, identifying three mediators of QKI-7 orchestrated angiogenic dysfunction and further highlighting QKI-7 as an attractive therapeutic target for diabetic vasculopathy. Connectivity mapping of QKI-7 gene signatures against FDA approved compounds was subsequently performed to identify drugs which may suppress its activity, with three drugs found to have potential to target QKI-7 expression. In vitro studies in dbiPS-ECs confirmed the ability of each drug to suppress QKI-7 signalling and restore vascular function, quantified by tube formation, barrier function and ROS production. Taken together, these findings validate the use of dbiPS-ECs as disease models, provide a key insight into the signalling pathways underlying diabetic vasculopathy, particularly identifying QKI-7 as a central mediator of EC dysfunction and highlights COL4A2, TMEM184A and PPP1R15A as key regulators of vascular function. Importantly, our results indicate repurposing of FDA approved drugs to specifically target QKI-7 as a promising therapeutic strategy for this debilitating condition.

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Keywords: iPS-ECs, Diabetic Vasculopathy, QKI-7

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TARGETED HESC DIFFERENTIATION IDENTIFIES USB1 AS A NOVEL MIRNA DEADENYLASE THAT REGULATES HEMATOPOIETIC DEVELOPMENT AND BONE MARROW FAILURE

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Mutations in the 3' to 5' RNA exonuclease USB1 (U6 Biogenesis 1) impair hematopoiesis, leading to bone marrow failure (BMF), and predisposition to hematologic cancer in patients with Poikiloderma with Neutropenia (PN). While USB1 is known to regulate U6 snRNA maturation, the molecular mechanism of PN remains unknown, as U6 levels are normal and RNA splicing is unaffected in patients. To understand the etiology of hematopoietic failure in PN, we utilized CRISPR/Cas9 to generate human embryonic stem cells harboring the PN-associated mutation c.531_delA in USB1, and subjected them to an in vitro differentiation protocol that recapitulates the major steps of blood development. These targeted hematopoietic differentiation experiments show that the USB1_ c.531_delA mutation severely impairs blood development, both of erythroid and myeloid lineages, similarly to what is observed in patients. We demonstrate that hematopoietic failure in USB1 mutants can be reverted by expression of WT USB1, and that this failure is not caused by reduced levels of U6 snRNA or defects in splicing. Rather, we show that dysregulated miRNA levels during hematopoiesis lead to impaired development in USB1 mutants. Our data demonstrates that impaired miRNA levels in USB1_ c.531_delA hematopoietic cells are caused by a failure to

remove destabilizing 3' end adenylated tails, establishing USB1 as a novel miRNA deadenylase. These results were confirmed when we purified recombinant human WT and mutant USB1 and tested its activity on 5'-FAM labeled miRNA substrates with different 3' end additions: WT, but not mutant USB1, removed adenosines from the 3' end of miRNAs in a time-dependent manner. We then show that the expression of impaired miRNAs in USB1 mutants restores hematopoietic development in these cells. Finally, we show that 3' end adenylation is performed by PAPD5/7 in vivo, and that the chemical inhibition of PAPD5/7 rescues hematopoietic development in USB1 mutants. This work shows that USB1 acts as a miRNA deadenylase and suggests PAPD5/7 inhibition as potential therapy for PN patients. The identification of USB1 as a deadenylase emphasizes the importance of adenylation and deadenylation as 3' end modifications that regulate decay of multiple ncRNAs, analogous to the regulation of protein stability by ubiquination and deubiquination.

Keywords: Hematopoiesis, Bone marrow failure, miRNAs

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DEFINING PUTATIVE EARLY MARKERS DURING DEFINITIVE MESODERM SPECIFICATION WITH NK/T CELL POTENTIAL

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We aim to identify early developmental gene markers that are up-regulated during the emergence of lateral plate and mesoderm formation using a platform of iPSCs differentiation towards T and NK cells. Identification of this early gene signature can help identify and isolate the earliest hematopoietic stem cell and provide access to an improve T cell differentiation protocol. We used a doxycycline inducible iPSC line to induce the Notch intracellular domain NICD1, which was instrumental in the emergence of definitive hematopoiesis. Cells from different time points during differentiation, including day 0-4, were used to map the gene signatures during the early stages of T cell differentiation via 10x genomics single-cell sequencing and Flow cytometry. Selected genes were validated via qRT-PCR and used to test isolation of progenitors with T/NK cell potential. Induction of the Notch 1 pathway immediately after exit from pluripotency yields robust mesodermal formation with potential for T and NK differentiation. In the early stages, as the cells leave pluripotency and enter the primitive streak, we found that dox-induced iPSCs and non-induced iPSCs navigate two different pathways of differentiation from day 0-2, which later converge back together into one population by day 4. This differential gene signature is associated with the emergence of nascent mesoderm strongly reminiscent of normal human embryonic development. Taking advantage of a novel protocol for the differentiation of human iPSC into T and NK cells, we have identified a gene set signature associated with the earliest emergence of nascent mesodermal cells with definitive hematopoietic potential.

Keywords: Notch activation, Mesoderm, T and NK differentiation

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A DERMAL FIBROBLAST DERIVED HUMAN INDUCED PLURIPOTENT STEM CELL (HIPSC) LINE THAT EFFICIENTLY DIFFERENTIATES INTO NK CELLS

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Natural killer (NK) cells are specialized cells of the immune system that play critical roles in innate and adaptive immunity. Their ability to target and kill tumor cells have made them a promising candidate for the next generation of cell-based immunotherapies. NK cells, like T cells, can be engineered to express chimeric antigen receptors (CARs) to improve targeting the cancer cell of interest. NK cell therapies have the potential to use as an allogenic therapeutic with main advantages being reduced cost and greater accessibility to patients. The use of primary NK cells to reach expansion requirements for manufacturing has proved difficult, and their genetic engineering is more challenging compared to other immune cell types. However, human induced pluripotent stem cells can be utilized to generate a nearly endless supply of NK cells and their genomes can be engineered easily. Here, we report the generation of a human induced pluripotent stem cell (hiPSC) lines with the intent to identify iPSC lines that are amenable to NK differentiation. We identified that one of the hiPSC lines derived from human dermal fibroblasts, which was normal in a variety of pluripotency and genomic stability assays, efficiently differentiates into NK cells. These NK cells express functional NK cell markers, and we also demonstrate that these hiPSC differentiated NK cells are functional by different cell- based assays like target cell killing and cytokine release assays. In summary, we have identified iPSC lines and a differentiation workflow that enable efficient generation of functional iPSC- derived NK cells.

Keywords: Natural Killer cells, human Induced Pluripotent Stem Cells, NK cell characterization

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INVESTIGATING THE ROLE OF APOPTOSIS REGULATION IN CANCER THERAPY-INDUCED VASCULAR TOXICITIES USING HUMAN IPSC-ENDOTHELIAL CELLS

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Advances in cancer treatments have improved overall survival rates of cancer patients. However, cytotoxic and targeted therapies can inflict collateral damage on healthy tissues and trigger apoptosis (programmed cell death), leading to long-term toxicities. Vascular toxicity is the second most prevalent clinical consequence for cancer survivors after chemotherapy or radiation therapy, and manifests itself as atherosclerosis, heart failure, acute thrombosis, or venous thromboembolism. The development of vascular toxicity is poorly understood, in part due to the lack of suitable in vitro model systems for mechanistic study. Using human pluripotent stem cells (hPSCs) for in vitro disease modeling, we investigated cancer therapy-induced vascular toxicities in hPSC-derived vascular endothelial (ECs) and smooth muscle cells (VSMCs). We hypothesized that high levels of apoptotic priming in these cell types would predispose them to dying in response to cancer therapies, resulting in a range of presentation and severity of vascular toxicity. Using BH3 profiling, we find that ECs and VSMCs are both primed for apoptosis, contributing to their high sensitivity to cancer therapies. We treated ECs and VSMCs with a panel of chemotherapeutic agents and radiation therapy and found divergent levels of sensitivity in ECs versus VSMCs to specific classes of agents, which were then linked to particular cardiovascular toxicities in patients based on pharmacovigilance data. We also found that radiation therapy preferentially induced apoptosis in vascular ECs and not parenchymal cells in vivo, indicating that ECs are the “weakest link” within most healthy tissues, with their loss driving cardiovascular and other tissue failure. Importantly, knockdown of pro-apoptotic BAX and BAK protects ECs from a panel of anti-cancer agents, therefore blocking apoptosis could potentially prevent toxicities in cancer patients. Altogether, we have developed an in vitro human platform to investigate vascular toxicity in response to chemotherapeutics, and show that vascular cells respond diversely to a variety of drugs. In future work we will investigate how age modifies vascular apoptotic regulation. These results will improve therapy regimens in the clinic, and ultimately decrease vascular toxicities in patients.

Funding Source: F31 NIH NCI Award #1F31CA275321-01

Keywords: apoptosis and cell death, cardiovascular toxicities and cancer therapy, hiPSC-Endothelial cells, vasculature

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VEGFR-3 IS A NOVEL MARKER FOR LEUKEMIC STEM CELLS

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Acute myeloid leukemia (AML) is a lethal cancer with high relapse and presents uncontrolled proliferation of immature cells. Refractory to chemotherapy and even hematopoietic stem cell transplantation (HSCT) is mainly caused by existence of leukemic stem cells (LSCs) under the leukemic microenvironments, which have multi-drug resistant features. Despite the apparent diversity and heterogeneity of LSCs and leukemic niches, cellular and molecular characteristics for LSCs are still very poorly understood and none of markers can clearly target the rare populations of repopulating leukemic cells. Vascular endothelial growth factor receptor-3 (VEGFR-3) is commonly expressed in mature lymphatic cells. Several papers showed VEGFR-3 as a marker for representing the progenitor itself or otherwise the supporter cells to maintain progenitor cells in pathophysiologic condition for AML. VEGFR-3 and its ligand VEGF-C are expressed in AML blasts and are closely involved in disease progression and used as an indicator for clinical outcomes, according to recent literatures including our previous reports. VEGF-C/VEGFR-3 axis can induce receptor phosphorylation and it occurs both in internally or externally in hematopoietic cells including AML as well as normal cells. We hypothesized that CD45dimCD34+CD38- LSCs include VEGFR-3+ leukemic blast cells and their protection against cancer drugs occurs by VEGRF-3 internalization under abundant VEGF-C ligand by PCR, immunostaining, colony forming assays, and FACS analyses. To prove it, we used VEGFR-3 antagonist and found that specific inhibition of VEGFR-3 in ALDHdimCD34+CD38- LSCs can decline leukemia homing and engraftment of bone marrow in repetitive studies of NSG AML mice in vivo experiments. Those results strongly suggested the pivotal role of VEGFR-3 as a LSCs marker. Furthermore, VEGFR-3+ LSCs are relevant for non-responsive to conventional therapies in the study. Lastly, findings of increased expression of Wilms’ tumor gene 1 and survivin by VEGFR-3+ stromal cells co-culturing suggest a protective role against apoptosis in leukemic niche and promote the leukemic cells proliferation throughout the mechanism of increasing cytosolic VEGFR-3. This study suggests the identity of LSCs expressing VEGFR-3+ as a prognostic and target therapeutic candidate for AML.

Keywords: acute myeloid leukemia, leukemic stem cell, VEGFR-3

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RAPID AND CONSISTENT GENERATION OF FUNCTIONAL MICROGLIA FROM REPROGRAMMED HIPSCS TO STUDY MECHANISMS IN NEURODEGENERATION AND NEUROINFLAMMATION

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Microglia are the tissue-resident macrophages of the brain, accounting for 75-80% of leukocytes and 10-15% of total cells within the central nervous system (CNS). They survey neuronal function, play roles in neurogenesis, synaptic remodelling, are the first responders to infection, and are thereby implicated in various CNS diseases. The life sciences sector relies predominantly on rodent models to mimic disease states for drug discovery. However, animal models do not always recapitulate human cell and disease phenotypes. To bridge this translational gap, several in vitro human models have been developed for the study of microglia, most typically primary microglia extracted directly from either embryonic, neonatal or adult tissue. However, primary cells are limited in supply, difficult to source, and often show donor-to-donor and user variability. There is a need for functional, consistent, scalable disease-relevant human microglia cells for neuroimmune research and the development of therapeutic or preventive strategies for neurodegeneration. We used transcription factor mediated precision cellular reprogramming technology, opti-ox™, to rapidly and consistently generate mature, functional, and physiologically relevant microglia, named ioMicroglia, from hiPSCs, at scale. ioMicroglia, 10 days post-revival, display typical morphology and express key phenotypic markers including TMEM119, P2RY12, and IBA1. RNA sequencing demonstrates that ioMicroglia have a transcriptomic signature similar to primary adult and foetal microglia. Consistent phagocytic and cytokine secretion functionality, with various stimuli, including amyloid beta, has been demonstrated for ioMicroglia, across multiple independent laboratories within industry and academia, highlighting the experimental reproducibility of ioMicroglia. Importantly, ioMicroglia can be co-cultured with neurons to more closely mimic in vivo brain function. In conclusion, with opti-ox precision cellular reprogramming, hiPSCs are rapidly converted into functional microglia offering a robust and scalable source of human microglia which can be used as a relevant in-vitro model to investigate the role of the CNS's immune system in health and disease, and to develop novel therapies for neuroinflammation.

Keywords: microglia, neurodegeneration, neuroinflammation

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MODELING THROMBOCYTOPENIA ABSENT RADIUS SYNDROME USING INDUCED PLURIPOTENT STEM CELLS

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Thrombocytopenia absent radius (TAR) syndrome is a rare congenital disorder primarily characterized by absence of the radii, severely reduced numbers of megakaryocytes (MKs), and thrombocytopenia. TAR is caused by mutations in the RBM8A gene, and although it has no known roles in MK biology, it may affect megakaryopoiesis through its role as a core factor in the exon-junction complex which is important for RNA splicing, particularly of genes regulating apoptosis and cell cycle. In vivo, MKs differentiate from hematopoietic progenitor cells (HPCs) through a process in which they increase RNA processing, undergo endomitosis, and depend upon the proper regulation of apoptosis factors to prevent premature death before packaging its cellular contents into platelets. To investigate whether TAR syndrome impairs the maturation of MKs due to changes in the splicing of relevant genes, we have generated two TAR patient induced pluripotent stem cell (iPSC) lines as well as isogenic corrected lines that contain a CD43-promoter-driven RBM8A construct to restore expression in HPCs and MKs. In our model, TAR iPSCs generate MKs with a 3-fold decrease in cell number and express significantly higher levels of MK surface markers. TAR MKs also display larger size, are more granular, and have similar in vitro proplatelet-forming potential compared to corrected MKs, suggesting TAR MKs may be maturing faster than the corrected lines. Transcriptome analysis of TAR and corrected MKs revealed significant changes in gene expression related to cell cycle and MK maturation. Splicing analysis also demonstrated differential splicing of genes in pathways related to proliferation such as E2F targets, G-2M checkpoint and Myc Targets (Hallmark). In addition to global changes, we found that TAR MKs displayed differential splicing of the mRNA-binding factor IGF2BP3, which is known to mediate MK morphogenesis during ontogeny. The TAR MKs preferentially express the short isoform of this gene which is only 30% the size of full length IGF2BP3 and likely does not have full functionally. Together, these data suggest that TAR syndrome alters MK development through the alternative splicing of genes in relevant pathways. These results expand our understanding of

how splicing may impact lineage-specific development and disease presentation.

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Keywords: induced pluripotent stem cells, disease model, megakaryopoiesis

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EXAMINING HEMATOPOIETIC STEM CELL FITNESS IN LOW EARTH ORBIT

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The International Space Station (ISS) offers a unique macroenvironment to study biology. The effects of microgravity in low earth orbit (LEO) can be leveraged to study inflammation and age-related diseases in an accelerated timeframe. As part of an ongoing study, we launched aged-normal CD34+ hematopoietic stem and progenitor cells (HSPC) into space to assess stem cell health and fitness in LEO over a mission duration of 30+ days. To study HSPCs in their microenvironment, we developed a nanobioreactor system capable of maintaining stem cell health and fitted for live cell imaging on orbit. To maximize information from these month-long missions, HSPCs are transduced with Fucci2BL, a cell cycle transit reporter. The bicistronic fluorescent reporter allows us to image and assess stem cell cycling in real-time. Fluorescence intensity quantification analysis shows that HSPCs cycle faster in LEO compared to their paired ground control, potentially a result of accelerated stem cell exhaustion. With the retrieval of greater than 60% viable cells, we performed whole genome sequencing (WGS) and whole transcriptome sequencing (RNA-seq) of flight and ground control samples. Preliminary WGS analysis shows that telomere length is shortened in flight. In summary, we have a system capable of studying the effects of microgravity on age-related cellular stress and inflammation of normal HSPCs.

Keywords: Microgravity, Hematopoietic stem cells, Nanobioreactor

TOPIC: KIDNEY

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VISCOELASTIC HYDROGELS REGULATE NEPHROGENESIS IN DIFFERENTIATING KIDNEY ORGANOIDS

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Kidney organoids with nephron segments can be generated from the directed differentiation of human pluripotent stem cells, but these organoids do not fully recapitulate the structure and function of adult kidneys. We hypothesize that scaffolds, which mimic the mechanical microenvironment in the developing kidney, may improve the physiological relevance of human kidney organoids. Here, we integrate differentiating kidney organoids into alginate hydrogels that are engineered to match the stiffness and viscoelasticity of the metanephric mesenchyme in the developing kidney. We observe that organoid differentiation efficiency is dependent on the timing of embedding within the alginate hydrogel. Furthermore, in contrast to low-concentration Matrigel or type I collagen, we find that non-degradable alginate supports the differentiation of kidney organoids over several weeks with proper nephron patterning. Organoid differentiation in alginate also decreases the ratio of proximal-to-distal nephron segments as compared to suspension culture, and the morphology of distal tubular nephron segments is significantly more convoluted in organoids differentiated in alginate relative to suspension culture. Finally, by tuning the viscoelasticity of the alginate hydrogel, we observe differences in the spatial organization of nephron segments in the resulting kidney organoids. Taken together, these results provide a fundamental understanding of how scaffold mechanics regulates nephrogenesis in three-dimensional culture and will inform new strategies for engineering nephrons.

Funding Source: NIH (NIBIB, T32EB016652; NIDDK, F32DK134115)

Keywords: extracellular matrix, biomaterials, human pluripotent stem cell differentiation

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ENGINEERING GLOMERULAR CAPILLARY WALL DEVELOPMENT AND FUNCTION WITH SMAD2 VARIANTS ASSOCIATED WITH CONGENITAL HEART DEFECTS

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Early cardiovascular and renal development involves extensive cell lineage diversification through distinct and shared molecular signaling networks. Recent clinical observations suggest that congenital heart defects (CHD) in the presence of SMAD2 variants are associated with global impairment in development, often resulting in a combination of heart defects and aberrant glomerulogenesis. Despite growing recognition that CHD can alter the predictive ability of risk factors involved in glomerular development and function, these hypotheses remain unproven. Here, we coupled human iPS cells and CRISPR to facilitate isogenic comparisons of loss-of-function SMAD2 variants in kidney podocyte differentiation. Our results show that abrogation of SMAD2 causes biased mesoderm lineage commitment. By harnessing the developmental programming, we differentiated mesoderm cells into intermediate mesoderm (IM) cells via temporal control of WNT-BMP7 signaling pathways. The mutant IM cells display epithelial-to-mesenchymal transition with a myofibroblast-like phenotype and necroptosis. Upon further lineage priming towards kidney podocytes, the mutants failed to develop characteristic morphological features such as the arborized cytoarchitecture. The mutant podocytes demonstrated mislocalization and altered expression of lineage markers along with expression of αSMA and NKD2, which are associated with fibrosis. Isogenic endothelial cells also exhibited a mesenchymal-like behavior. Our results indicate that SMAD2 variants of isogenic human iPS cell derivatives show a dysregulated TGF-ß/SMAD2 signaling axis causing aberrant changes in transcriptomic and proteomic profiles, consequently dysregulating cell fate decisions. We further investigated these SMAD2 LoF mutations' role in developing the glomerular filtration barrier, the kidneys' primary site for blood filtration. Reconstitution of the tissue-tissue interface with isogenic podocytes and endothelial cells in a glomerulus-on-a-chip platform corroborated significant protein loss through the barrier resembling proteinuria. Using CRISPR-based isogenic disease modeling experiments, we enabled a more precise interrogation of the impact of LoF SMAD2 variants on lineage specification and glomerular filtration barrier function.

Keywords: Glomerulus-on-a-chip, pediatric nephropathy, Cardiorenal syndrome

TOPIC: LIVER

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DEVELOPING A LIVER-ON-CHIP MODEL FOR SIMULATING LIVER INFLAMMATORY RESPONSE

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Inflammation is a common response seen in liver diseases and is usually managed by immunosuppressive therapies. Uncontrolled inflammation can lead to irreversible damage and liver failure that may require liver transplantation. Since human-based in vitro models can potentially mimic our biology more accurately than animal models, organ-on-chip platforms developed using cell and organoid culture have recently been employed to investigate disease mechanisms and perform drug testing. In this project, human embryonic stem cell (hESC) derived hepatocyte like cells (HLC) and HepG2 cells were cultured for modelling inflammatory conditions and screening potential therapeutic agents such as drugs and stem cell-derived products. To model inflammation, THP-1 monocytic cell line was polarized into pro-inflammatory macrophages using PMA and treated with LPS to obtain conditioned medium. We show that hESC successfully differentiated into HLCs and exhibited hepatic markers in 2D, while cultured HepG2 cells can form hepatic spheroids in 3D culture systems. We also uniquely developed a conditioned medium containing cytokines such as IL-6, TNF-α, IL-1β released from macrophages and used it for simulating the inflammatory disease microenvironment. HepG2 cells and the hepatocytes showed a significant drop in cell number in the CCK8 assay and in the albumin production measured by ELISA after treatment with conditioned medium, suggesting detrimental impact on the cultured cells. This approach has preliminarily demonstrated that inflammatory conditions can be recapitulated by administering conditioned medium containing cytokines to liver cells. This work has laid the foundation for developing more advanced hepatocyte cultures such as 3D organoid and organ-on-chip models to simulate inflammation in the liver, investigate disease mechanisms, and screen potential therapeutic agents. It would be of interest to investigate possible co-culturing of other liver cell types and integration of multiple organ-on-chips with biosensing monitoring and perfusion systems to model multi-organ diseases in the future.

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Keywords: Inflammatory cytokines, liver-on-a-chip, hepatic organoid

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IPSC-DERIVED HEPATOCYTES FOR INVESTIGATION OF LIVER-SPECIFIC THYROID HORMONE FUNCTION

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Thyroid hormones (TH) and their receptors (THRA/B) are critical for liver development and normal function. In hepatocytes, TH regulates de novo lipogenesis, beta-oxidation, and carbohydrate and cholesterol metabolisms. Low T3 levels, due to i.e. hypothyroidism, manifest in abnormal serum lipids and increased risk of non-alcoholic fatty liver disease (NAFLD). Existing knowledge regarding TH effects in healthy and diseased liver is predominantly derived from mouse studies, liver-cancer derived cell lines and primary human hepatocytes. Mouse models are not ideal, due to significant gene ortholog differences that hinder full understanding of TH axis components. Hepatic cancer cell lines cannot be used to investigate healthy cell phenotypes and primary human hepatocytes have limited availability and culture life span. Due to these limitations, there is an unaddressed need for a translationally relevant model system that allows for investigation of T3 role in healthy human-hepatocytes. To breach this gap, we adapted existing differentiation protocols to derive a robust and reproducible method for generation of iPSC-derived hepatocytes. Resulting cultures displayed functional characteristics of mature hepatocytes, including albumin secretion, cytochrome activity, and lipid uptake. IPSC-derived hepatocytes were able to internalize T3 as shown by radioactively labelled T3 uptake assay. Upon 24h stimulation of iPSC-hepatocytes with 50nM T3, we observed changes in expression of more than ~300 genes, consistent with known T3-target genes (KLF9, G6PC1, PKC1). Based on GOterm analysis T3-challenge resulted in upregulation of genes associated with fatty acid metabolism and gluconeogenesis. Additionally, we investigated the effects of persistent (7 days) exposure of iPSC-hepatocytes to 5nM T3. This treatment resulted in gene expression changes of ~2000 genes. Exposure to prolonged low level T3 improved hepatocyte maturation signature, as evident by upregulation of cytochrome C genes (CYP2B6, CYP3A4) and downregulation of AFP. GOterm analysis showed that continued T3 affected gene networks associated with lipid and steroid homeostasis, and alcohol and fatty acid metabolism. Taken together our results show that iPSC-derived hepatocytes are a new viable model for investigation of TH-action in liver.

Funding Source: LocoTact CRC/TR 296

Keywords: Hepatocytes, Thyroid hormones, Gene expression

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ENGINEERING OF THE LIVER-INTESTINE HYBRID TRANSCRIPTOME STATE OF HIPSC-DERIVED HEPATOCYTE-LIKE CELLS

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Pluripotent stem cell (PSC)-derived hepatocyte-like cells (HLC) are a promising future alternative to primary hepatocytes in pharma- and toxicological studies, however, some important limitations remain. We found that HLC are trapped in a hybrid transcriptome state of liver and intestinal gene transcription occurring in the same cells, preventing full lineage commitment of HLC and limiting the establishment of mature hepatocyte functionality. Bulk-level overrepresentation analysis, as well as regulon analysis at the single-cell level identified sets of regulatory factors discriminating HLC, fetal hepatocytes and PHH, indicating a central role for the nuclear receptor FXR and the intestine patterning transcription factor CDX2 in HLC hybrid state regulation. Combined FXR expression plus agonist exposure enhanced the expression of hepatocyte-associated genes and increased the ability of bile canalicular secretion as well as lipid droplet formation, thereby increasing HLCs' similarity to PHH. However, the hybrid features associated with intestinal differentiation remained largely unaffected. To address this, we knocked-out CDX2 in hiPSC which reduced gene expression associated with intestinal differentiation in HLC. Our data suggests that identification and subsequent manipulation of key regulators of the HLC hybrid state are required to overcome the hybrid state preventing complete hepatocyte differentiation of HLC.

Keywords: Hepatocyte-Like Cells, In-Vitro Differentiation, Hybrid State

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SCREENING PLATFORM FOR HUMAN ALPHA-1 ANTITRYPSIN DEFICIENCY USING IPSC-DERIVED HEPATOCYTE-LIKE CELLS

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Alpha-1 antitrypsin deficiency (A1ATD) is a clinically under-recognized genetic disorder that causes the defective production of alpha-1 antitrypsin protein (A1AT). The most prevalent cause of A1ATD is a homozygous missense mutation (p.E342K) in A1AT known as variant PiZZ. This mutation causes misfolding and promotes the intracellular accumulation of polymers of A1AT in hepatocytes. This accumulation can lead to severe liver damage, yet, no specific treatments are available for A1ATD-associated liver disease. Therefore, there is an urgent need to develop novel therapeutics capable of alleviating the disease phenotype. However, the lack of physiologically-relevant disease modelling platforms hinders their development. Human-induced pluripotent stem cell (iPSC)-derived hepatocyte-like cells (HLCs) provide a new approach to studying disease mechanisms. Using CRISPR/Cas9 we have studied pairs of A1TAD (PiZZ) and healthy (PiMM) HLCs derived both from healthy or A1ATD donors. In this study, we aim to develop a bioassay that mimics the accumulation of polymer in the A1ATD liver and that can reproducibly capture a response to a reference drug, carbamazepine (CBZ), known to reduce the accumulation of the polymeric A1AT. Four pairs of PiMM and PiZZ iPSC lines were successfully differentiated into HLCs and screened for disease markers using high-content imaging. The diseased lines displayed increased intracellular accumulation of polymeric A1AT compared to their isogenic controls. Upon treatment with CBZ, all diseased cell lines showed a decrease in intracellular accumulation of polymeric A1AT in a concentration-dependent manner. This dose-dependent phenotypic restoration was consistent across all the tested lines and could capture the differences in drug response expected in cells with a variety of genetic backgrounds. Taken together, we have developed a specific and robust in vitro model of A1ATD that recapitulates the disease pathophysiology and is responsive to small molecule-based treatments making it highly applicable to candidate drug screening in A1ATD.

Keywords: Alpha-1 Antitrypsin Deficiency, Liver Disease, Screening

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EN MASSE ORGANOID INTERROGATION REVEALS THE IMPACT OF METABOLIC STATUS ON GENETIC SUSCEPTIBILITY TO NASH

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Non-alcoholic fatty liver disease (NAFLD) in the U.S. has many unhealthy consequences and strongly contributes to the fact that approximately 10% of Americans have type 2 diabetes(T2D). While multiple genetic variants (>60) have been reported to be associated with NAFLD, disease-modifying alleles account for only a small percentage of the risk inheritance, especially because gene and environmental factors collaboratively contribute to the NAFLD phenotype. In this study, we have developed a pooled human organoid-panel (PoP) strategy using cryopreservable foregut progenitor cells that is able to differentiate into human liver organoids with NASH and T2D like symptoms en masse under optimized culturing conditions. This model was used for transcriptomic characterization of organoids derived from 24 pooled donors with minimal donor-dependent variations of hepatic gene expression. Live image based BODIPY analysis further showed significant lipid droplet accumulation, which was linked to SNP information from individual donors. Furthermore, the phenotypic profiling of our steatohepatitis PoP model captured known GWAS risk variants for hepatic steatosis, specifically PNPLA3-rs738409, GCKR-rs1260326 and rs780094 -risk alleles. Analysis of GCKR-rs1260326 carriers generated using CRISPR-Cas9 gene editing revealed that rs1260326 carriers have more prominent steatosis, GCK activity and increased de novo lipogenesis genes including SREBF1, FASN and ACC. To validate the organoid-based screening results, GCKR genotype associations in 1091 patients in the NASH clinical cohort were analyzed stratified by diabetes status. Consistent with PoP model analysis, GCKR-rs1260326-T allele elevates disease severity only under diabetic state. Transcriptomic, metabolomic, and pharmacological analyses revealed mitochondrial dysfunction caused by GCKR-rs1260326, and treatment of the GCKR-rs1260326 carriers steatohepatitis-organoid model with oxidative uncoupler, but not metformin, normalized mitochondrial function and suppressed fatty acid-induced inflammatory responses. In summary, the integration of in vitro PoP models with in vivo clinical data provided insights to improve capturing the highly variable in vivo pathogenesis of NAFLD/NASH risk variants.

Keywords: Human liver organoid, Non-alcoholic fatty liver disease, Glucokinase regulatory protein rs1260326

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REDEFINING PROGENITOR LANDSCAPE IN ANIMAL AND HUMAN LIVER DEVELOPMENT

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Comprehensive developmental roadmap of the liver is still unclear. Current developmental dogma is that ventral foregut endoderm that lacks posteriorized caudal type homeobox2 (Cdx2) expression is considered as a major source that drives the liver development. Contrasting to this prevailing notion, we and others reported the derivation of hepato-biliary-pancreatic precursors from posteriorized Cdx2 positive endoderm in human stem cell culture system. We herein combined rodent genetics and human organoid approach to define progenitor landscape during early hepatogenesis process. Utilizing the lineage tracing in mouse models, we demonstrated Foxa2+ Cdx2+ visceral endoderm (VE) derived hepatocyte located in the interzonal region at embryonic day 18.5. Consistently, single cell RNA sequencing of the developing mouse gut endoderm revealed competency of Foxa2+ Cdx2- definitive endoderm (DE) and Foxa2+ Cdx2+ VE in the differentiation of hepatoblasts. The human induced pluripotent stem cell (hiPSC) were differentiated into foregut and VE co-differentiating midgut endoderm to model the foregut-midgut-visceral tri-boundary interaction in human development. The epigenetic analysis showed activation of VE markers such as Dpp4 in the hiPSC derived Cdx2+ posterior gut endoderm and Dpp4 based magnetic-activated cell sorting enriched VE-like cells. In agreement with this, the hiPSC Cdx2+ posterior gut endoderm that includes Foxa2+ Cdx2+ Dpp4+ VE reproducibly emanate hepatic progenitors in our 3D foregut-midgut-visceral tri-boundary model. Finally, retinoic acid-dependent expression of the secreted Wnt antagonists Sfrp1/Sfrp2 in anterior gut mesenchyme precedes the activation of hepatic transcriptional program in the Cdx2+ endoderm and the overactivation of Wnt signaling abolishes hepatic potential both in mouse and human model systems. In conclusion, these experiments unfold the previously underappreciated endodermal progenitors of vertebrate liver organogenesis arising through interactions of regionalized endoderm and mesenchyme. We have established a novel hepatocyte progenitor with key molecular insights that involve the suppression of the Wnt pathway, resulting in a revised embryonic roadmap of hepatic formation and an improved recapitulation of the liver in vitro.

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Keywords: Liver, Organogenesis, Organoid

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DEVELOPMENT OF A LIBRARY OF NONHUMAN PRIMATE PLURIPOTENT STEM CELLS AND STEM CELL DERIVED HEPATOCYTE-LIKE CELLS TO STUDY SPECIES-SPECIFIC HOST RESPONSES AND RESTRICTIONS OF HEPATOTROPIC VIRUSES

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Infectious diseases contribute significantly to human morbidity and mortality. Many pathogens that cause disease in humans, exhibit a very narrow host range, which poses challenges both for study and for developing effective clinical therapies. In prior work we have shown that species-specific differences in the kinetics, diversity and magnitude of host dependency factors influence viral tropism and can be used to identify unique host susceptibility and resistance factors that can be used to target viral infection in humans. Here, we developed and utilized a novel platform to systematically define such species-specific host dependency factors contributing to infection outcome. We aimed to capitalize on a collection of dermal fibroblasts from several non-human primate species (chimpanzee, bonobo, gorilla, orangutan, olive baboon, rhesus macaque, and squirrel monkey) that we used to generate induced pluripotent stem cells (iPSCs) and differentiated in a stepwise manner to hepatocyte-like cells (HLCs). We then aimed to leverage this platform to characterize Hepatitis B Virus (HBV) infection. HBV currently infects over 250 million people worldwide and is the 10th leading cause of death. We compared HBV infections in HLCs derived from human, chimpanzee, bonobo, gorilla, orangutan, olive baboon, and rhesus macaque. Human, chimpanzee, and bonobo HLCs were permissive to HBV viral entry and had robust HBV pgRNA, DNA, and protein production. Orangutans HLCs were permissive to HBV viral entry but had less robust HBV pgRNA, DNA, and protein production. Rhesus macaque HLCs were not permissive to HBV viral entry or infection but transduction with the human sodium-taurocholate co-transporter polypeptide (NTCP) receptor restored HBV viral entry and infection. Unexpectedly olive baboon HLCs were not permissive to HBV viral entry or infection and this could not be rescued with expression of the human NTCP receptor. Orthologs of essential host factors critical for supporting the viral life-cycle are largely conserved across these species however the differential HBV susceptibility in the orangutan and olive baboon HLCs remains unclear and is being currently explored. Our goal is to leverage these species-specific differences to identify HBV host dependency factors that can then be used for targeted HBV therapy.

Keywords: nonhuman primate, pluripotent stem cell, hepatitis B virus

TOPIC: MESENCHYMAL STROMAL CELLS, ADIPOSE, AND CONNECTIVE TISSUE

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A SCALABLE APPROACH TO GENERATE PRE-VASCULARIZED AND FUNCTIONAL HUMAN BEIGE ADIPOSE ORGANOIDS

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Beige adipose tissues (beige AT) are key regulators of human physiology through their unique plasticity and ability to dissipate energy as heat according to the needs. Maladaptive plasticity of beige adipose tissues is known to be associated with metabolic disorders, aging and more recently massive burn and cancer associated cachexia extending its role beyond thermoregulation. Understanding beige AT emergence and maintenance in human is of great therapeutic interest but is hindered by the lack of suitable in vitro models recapitulating the complex microenvironment and functionality of native mature tissue. When cultured in adequate 3D culture conditions, stem cells can undergo in-vivo like morphogenesis and turn into self-organized structures called organoids. One limitation of their use in therapeutic applications including transplantation is the difficulty to grow them beyond millimetric scale. In this study a scalable approach is proposed to generate pre-vascularized and functional human beige adipose organoids using human stromal vascular fraction of white adipose tissue as a source of adipose-derived mesenchymal ste m cells (ASCs) and endothelial progenitors. To this aim, we engineered defined chemical and biomechanical environments, applicable at multiple tissue scales, to drive self-organization of ASC-derived spheroids into GelMA hydrogel towards beige adipogenesis and vascular formation. Resulting vascularized organoids display key features of native beige AT including inducible UCP1 expression, increased uncoupled mitochondrial respiration and batokines secretion. Guided assembly of spheroids allows the translation of organoid morphogenesis to the macroscopic scale with the generation of vascularized centimeter-scale beige adipose micro-tissues. Our approach represents a significant advance in the development of more physiological in vitro human beige AT models opening new ways for wide range applications from basic research to biotherapies. Besides, by exploiting the versatility of ASC functions and differentiation potential, we believed that this generation process of ASC-based microtissues could be easily transferred to other tissues and clinical applications.

Funding Source: French National Agency for Research (hiPSC-Adipospheres, Printiss Project), French RENATECH network, FEDER European Regional Funds, French Région Occitanie, CARe – Graduate School N°ANR-18-EURE-0003.

Keywords: Adipose derived stem cells, Organoid morphogenesis, Guided-assembly

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CHEMICALLY DEFINED BIOMATRICES FOR 3D MANUFACTURING OF STEM CELLS

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The application of stem cell-based therapies relies on the manufacturing of substantial amounts of stem cells for treating a large set of patients. Current 2D culture methods are incapable to meet this high demand for stem cells due to their limited scalability as a result of cost, space, and handling constraints. To upscale stem cell expansion processes cells can be encapsulated, grown in aggregates, or on microcarriers. Independent of the setup, the expansion phase ideally results in high cell yield with preserved stem cell quality. This can be achieved by optimizing the cell's microenvironment by reducing shear stress, manual handling, and mimicking the in vivo niche of the cells. An essential part of the in vivo niche is the extracellular matrix (ECM) which is composed of glycosaminoglycans (GAGs) and structural proteins with bind-

ing sites for specific integrins. To functionally recreate the ECM, yet simplify it to a minimal system, we designed biomatrices that combine GAG mimetics with synthetic biofunctional peptides, modular in composition and concentration. We further developed a method to apply these biomatrices onto 3D surfaces. With this method we were able to create ready-to-use microcarriers specifically tailored for mesenchymal stromal cells (MSCs) and induced pluripotent stem cells (iPSCs), cell types frequently used for cell-based therapies. The 3D culture on beadMATRIX microcarriers in a PBS Vertical-Wheel® bioreactor enabled MSC expansion in clinically relevant scales while maintaining MSC identity and potency. The microcarrier-based expansion of iPSCs in agitated culture on beadMATRIX+ microcarriers showed superior expansion rates and iPSC quality compared to aggregate culture. Thus, our biomatrix-coated microcarrier system contributes to meeting the goal of cost-effective and reliable manufacturing processes of high-quality MSCs and iPSCs for therapeutic applications.

Keywords: biomatrix, mesenchymal stromal cells, microcarrier culture

TOPIC: MUSCULOSKELETAL

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HUMAN PLURIPOTENT STEM CELL-BASED SYSTEMS RECAPITULATING INTRAMEMBRANOUS OSSIFICATION

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Vertebrates form their skeletal tissues through two modes of ossification: endochondral and intramembranous ossification. In endochondral ossification, cartilage is initially formed and gradually replaced by bone, whereas in intramembranous ossification, bone is directly formed by osteoblasts derived from mesenchyme. Especially in humans, the molecular and cellular mechanisms underlying intramembranous ossification are less known than those underlying endochondral ossification. Paraxial mesoderm- and neural crest-derived bones are developed by both modes of ossification depending on the site; however, determinants of the modes of ossification remain to be clarified. Therefore, we aimed to develop human pluripotent stem cell (hPSC)-based systems recapitulating paraxial mesoderm-derived intramembranous ossification, to better understand the human intramembranous ossification process. We first induced the sclerotome through the primitive streak, paraxial mesoderm, and somites by stepwise treatments of hPSCs with several small molecules in a two-dimensional culture. The sclerotome population was then cultured on spheroid plates for 15 days. The in vitro-induced spheroids showed significant upregulation of osteoblast-related genes with no upregulation of chondrocyte-related ones; immunohistochemical analysis revealed that the induced spheroids were enriched with cells expressing osteoblast-related factors. When the spheroids were implanted into renal capsules of immunodeficient mice, radio-opaque structures appeared around the implanted site in X-ray micro-computed tomography images at 8 weeks after implantation. Immunohistochemical analysis showed that cells expressing osteoblast-related factors were dominant in the structures, and no cartilage formation was detected; human nuclear antigen-positive cells composed the structures. These data suggest that the hPSC-derived spheroids include osteoblast progenitors and have potential to induce intramembranous ossification in vivo. Given that hPSC-derived spheroids generated by another culture protocol induced endochondral ossification in vivo, these two methods will contribute to our understanding of the process of human intramembranous ossification in comparison with that of endochondral ossification.

Keywords: bone, pluripotent stem cells, intramembranous ossification

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IN VIVO DELIVERY OF BASE EDITOR FOR GENE CORRECTION OF HUMAN DMD MUSCLE TISSUE IN MICE

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Precise gene correction owing to the recent advances in genome editing techniques, enables the establishment of isogenic pair of human pluripotent stem cells (hPSCs) from the patients haboring the pathogenic mutations, which would be useful for drug development and mechanism study for rare genetic diseases. Duchenne muscular dystrophy (DMD), occurring mutations in exons of X chromosome-linked DMD gene to encode the dystrophin protein, results from the loss of functional dystrophin expression. Thus, restoration of functional dystrophin with precise genome editing tools in vivo, would be a therapeutic approach for DMD patients. In order to examine the efficacy of in vivo therapeutic approach of gene correction in human muscle tissue harboring DMD mutation, we produced the muscle- enriched teratoma from DMD patient iPSCs (D-iPSCs) with inducible MyoD1 expression in mice model. The teratoma from D-iPSCs developed skeletal muscle tissue without dystrophin protein. Thus, the determination of dystrophin protein in teratoma from D-iPSCs after introduction of genome editing tools would be a valid approach to directly assess the functional recovery of the therapeutic gene editing. Then we injected the base editor mRNA-encapsulated lipid nanoparticles

into the DMD tissue in mice, which showed remarkable dystrophin recovery in teratoma muscle tissue.

Funding Source: Mistry of Food and Drug Saftey of South Korea (KFDA) (KDY0002259316-22202)

Keywords: Duchenne muscular dystrophy, hiPSC, base editor, in vivo gene editing, teratoma, Adenine base editor, MyoD1,

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DEFECTIVE AUTOPHAGY IN GNE MYOPATHY RESULTS FROM NON-CANONICAL MTOR ACTIVATION

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Autophagy, a key cellular event for tissue homeostasis, plays crucial roles in not only muscle development but also regeneration to affect the muscle stem cells. Thus, there are emerging evidence to prove that defects in autophagy contributes to the pathogenesis of diverse myopathies. GNE myopathy, a recessive autosomal disease by mutations in GNE (Glucosamine-(UDP-N-Acetyl)-2-Epimerase/N-Acetylmannosamine Kinase) is epitomized by deficient sialic acid production and the formation of rimmed vacuoles. However, both the mechanisms by which autophagy is altered and the causal link between autophagy and GNE myopathy remains undetermined. Along with multiple isogenic GNE myoblasts derived from human pluripotent stem cells (hPSCs) showing mutation-specific phenotypes, we established knockout of GNE (GNE KO) in the C2C12 myoblast model. Transcriptome analysis from two sets of GNE disease models, revealed that gene sets, associated with 'autophagy' were highly enriched in a mutation-dependent manner. Through phospho-proteome assay and successive biochemical analysis, we found that mTOR activity, of which activation inhibited Ulk1 complex dependent autophagy initiation, was increased in a non-canonical manner in GNE KO. Thus, inhibition of non-canonical mTOR activation with copanlisib, a newly FAD-approved PI3K inhibitor, rescued the defective autophagy in GNE KO, suggesting that copanlisib would be a feasible therapeutic option for GNE myopathy patients.

Keywords: Isogenic disease models, PI3K/AKT/mTOR; Autophagy, GNE myopathy

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DIRECT CONVERSION OF FIBROBLAST INTO SKELETAL MUSCLES BY TARGETING RECEPTOR SIGNALING PATHWAYS WITH AI-DESIGNED MINIPROTEINS

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A direct conversion of non-muscle cells into a skeletal muscle cell fate is a promising potential therapeutic avenue for tissue repair. Advanced skeletal muscle regenerative medicine is much needed to treat the muscle atrophy caused by trauma, surgery, aging, or congenital myopathies when the satellite cells lose their ability to repair. Here, our distinctive approach is to develop a therapeutically favorable strategy for direct conversion of human fibroblast into reprogrammed myotubes (iSKMs) by targeting the accurate signaling pathways using artificial intelligence (AI)-designed miniproteins. We are generating highly specific synthetic miniproteins against the receptors and utilizing various scaffolds to change valency and topology of miniproteins to screen for agonists. For instance, we have identified splice variant specific agonists with good biochemical properties (high stability and solubility) for FGFR, EGFR, PDGFR, TrkA, Insulin receptor etc. We have screened the agonist or antagonist miniproteins and identified the key targeted pathways in human fibroblast required for myogenic trans-differentiation. Further, we analyzed our single-cell sequencing data through a pathway analysis pipeline and identified these crucial pathways upregulated during myogenic trans-differentiation. Our strategy excludes stem cell transplantation, or genetic alterations, or lentiviral/ retroviral transduction that are likely to be tumorigenic. Exploiting the ability of receptor kinases to convert into myogenic fate has the potential for regenerative therapeutics and broad applications in research purposes, such as drug screening and disease modeling.

Keywords: miniproteins, Skeletal Muscle, transdifferentiation

TOPIC: NEURAL

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DEVELOPMENT OF AN OPEN SCIENCE COLLECTION OF PATIENT-DERIVED IPSCS FOR STUDYING DISEASE MECHANISMS IN NEURODEGENERATIVE DISEASES

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Central nervous system (CNS) diseases, such as Parkinson's Disease (PD) and Amyotrophic Lateral Sclerosis (ALS), are complex diseases with few treatments available to patients. In order to better study effective treatments of these diseases, more representative models of the human brain and its diverse cell types need to be made available to researchers. The advent of patient-derived induced pluripotent stem cell (iPSC) technology opens up the possibility of generating human brain cells on a dish, facilitating discovery and translational research. At the Montreal Neurological Institute (MNI), an open science collection of patient-derived iPSCs has been established through a collaboration between the Clinical, Biological, Imaging and Genetic repository (C-BIG) and the Early Drug Discovery Unit (EDDU). C-BIG is a collection of biologic samples (DNA, serum, PBMC, CSF, urine, stool or saliva), clinical information, imaging (de-faced MRI, CT scans, PET scans) and genetic data (RNA sequencing panels, SNV) from patients with neurologic disease as well as from health control subjects. The data and samples at C-BIG are accessible to research teams with scientifically and ethically valid proposal around the world, congruent with open science principles. Peripheral blood mononuclear cells (PBMCs) from consenting healthy and diseased donors are then reprogrammed by the EDDU and edited using CRISPR-Cas9 technology to create knock-outs, knock-ins, and isogenic controls. The EDDU has generated over 80 new IPSCs, 40 knock-out (KO) cell lines, 18 knock-in (KI) cell lines, and 24 disease corrected lines, reflecting many different diseases and disease-causing genetic variations. A rigorous QC process follows both reprogramming and gene editing where genome stability is consistently demonstrated as normal by analysis of karyotyping, qPCR for recurrent genetic abnormalities, and immunofluorescence of pluripotency markers Nanog, OCT3/4, Tra1-60, and SSEA4. IPSCs are differentiated into different neural subtypes for the study of neurodegenerative diseases and are demonstrated to show appropriate protein expression as determined by immunostaining and functionality through the detection of local electric impulses. Generated iPSCs are banked with C-BIG and are available for global distribution on a cost-recovery basis. Protocols and datasheets are available on EDDU Data Portal. So far, more than 45 collaborations with academic and industrial partners have taken place, aiming at expanding our research impact and speed up innovations in neuroscience that will benefit people affected by neurological disorders.

Funding Source: Brain Canada Foundation, Tanenbaum Open Science Institute.

Keywords: IPSC, open science, neurodegenerative disease

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MODELLING OF BILIRUBIN-INDUCED NEURO-INFLAMMATION USING HIPSC-DERIVED BRAIN ORGANOIDS

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Bilirubin-induced neurological damage (BIND) has been a subject of studies for decades, yet the core molecular mechanisms of the damage remain largely unknown. BIND, which is also known as kernicterus, occurs as a consequence of defects in the bilirubin conjugation machinery, resulting unconjugated bilirubin (UCB) to accumulate and cross the blood–brain barrier. Severe hyperbilirubinemia due to a mutation within the UGT1A1 encoding gene, has a direct contribution in bilirubin conjugation causing kernicterus as a symptom of Crigler Najjar Syndromes (CNS1, CNS2) and Gilbert syndrome, which may result in permanent neurological sequelae. Gunn rats and transgenic mice have been used as in vivo BIND models, however, these models cannot precisely recapitulate the human condition. Therefore, we are using human induced pluripotent stem cells (hiPSCs) derived 3D-brain organoids as a potential in vitro model system to unveil the breadth of complexities at the cellular and molecular levels (Gene ontologies and pathways) that accompany the detrimental effects of UCB to the developing human brain. In this comparative study, hiPSCs derived from healthy and CNS patients were differentiated into day-20 brain organoids, these were then stimulated with 200nM UCB and analyzed at 24 hours and 72 hours post-treatment. The results obtained so far point at UCB-induced neuro-inflammation at 72 hours post-stimulation in both the healthy and patient cell line. Transcriptome and associated KEGG and Gene

Ontology analyses revealed inflammation, neurodevelopmental, redox-status homeostasis and cell cycle repair-related pathways as perturbed upon UCB exposure. Distinct inflammatory pathways observed include MAPK signaling, calcium signaling, NFκ-ß activation, TNF signaling pathway, cytokine-cytokine receptor interaction, and upregulation of pro-inflammatory cytokines such as IL1, IL8, IL37 upon UCB stimulation. In summary, these initial results provide insights into how an iPSC-derived 3D-brain organoid model can serve as a prospective platform to study the etiology of BIND- Kernicterus.

Funding Source: medical faculty of Heinrich Heine University, Duesseldorf, Germany

Keywords: BIND, 3D-brain organoid, neuro-inflammation

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INVESTIGATING THE ROLE OF MICROGLIA MEDIATED NEUROINFLAMMATION IN AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic Lateral Sclerosis (ALS) or Motor Neurone Disease (MND) is a neurodegenerative disorder caused by the progressive loss of motor neurons in the motor cortex and spinal cord. The pathological mechanisms underlying ALS are unclear, although neuroinflammation is a major factor contributing to disease pathology. In ALS patients and animal models, resident macrophage cells called microglia demonstrate changes in morphology and function, including altered levels of pro-inflammatory cytokines. Although largely sporadic, a genetic cause underlies ~10% of ALS cases. A common genetic cause is the hexanucleotide repeat expansion (HRE) in C9ORF72, this accounts for ~40% of familial and ~10% of sporadic ALS. To investigate the role of microglia induced neuroinflammation in ALS, we generated microglia from induced pluripotent stem cells (iPSC) carrying mutations in C9ORF72 and isogenic controls. Human iPSCs were differentiated into microglia using an established yolk-sac mesoderm induction method with minor modifications. Generation of microglia was verified by expression of established cell lineage markers including TREM2, ITGAM, P2RY12, PU.1 and IBA1. The inflammatory profile of microglia upon lipopolysaccharide (LPS) stimulation was assessed via immunocytochemistry, flow cytometry, enzyme-linked immunosorbent assays and real time PCR. Microglia with C9ORF72 HRE or knockout showed reduced capacity for cytokine production compared to isogenic controls. More specifically, secreted levels of IL-6, IL-10 and TNFa are reduced, suggestive of an impaired LPS induced inflammatory response. In summary, we established iPSC derived models for ALS and investigated the inflammatory profile of microglia. Our preliminary microglia results confirm a complex loss and gain of function phenotype associated with C9ORF72 HRE ALS. Future work will broaden the phenotypic and functional profiling of these cell models using single cell RNAseq and explore disease phenotypes in motor neurons and microglia/motor neuron co-culture systems.

Keywords: Neuroinflammation, Microglia, C9ORF72

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HIPSC-DERIVED EXCITATORY-INHIBITORY NEURON CO-CULTURES OF SCHIZOPHRENIA PATIENTS SHOW ABERRANT NEURONAL CONNECTIVITY AND ACTIVITY

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Schizophrenia is a complex neuropsychiatric disorder often related to impairments in synaptic transmission and alterations of neuronal activity patterns. An imbalance of excitatory and inhibitory drive in the prefrontal cortex is suggested to contribute to some of the cognitive deficits observed in patients. However, there is still only little insight into the underlying morphological and functional mechanisms at play, especially in a human context. Here, we present a neuronal co-culture model derived from schizophrenia patient iPSC to study disease phenotypes in a humanized system in vitro. We demonstrate that iPSC-derived glutamatergic neurons and GABAergic interneurons robustly express lineage-specific synaptic markers and develop single-cell as wells as network activity when combined into co-cultures. Both glutamatergic neurons and interneurons derived from schizophrenia patients show a reduction of presynaptic terminals when cultured individually and in co-cultures. Interestingly, for excitatory as well as inhibitory neurons, formation of synapses onto glutamatergic neurons seems to be primarily affected. Single-cell calcium imaging in co-cultures demonstrates diverging activity patterns of glutamatergic and GABAergic neurons, stressing the need for cell type-specific activity analysis. Such subtype-specific examinations revealed significantly altered calcium peak parameters for both types of neurons when comparing schizophrenia patient cells with healthy control neurons. In conclusion, we generated a 2D in vitro human cellular test system to study morphological and functional signatures of glutamatergic and GABAergic neurons as well as their reciprocal interaction. We observed alterations of neuronal activity and synapse formation for both types of neurons derived from patients with schizophrenia. In the future, our model system could serve as a valuable in vitro platform for the mechanistic study of disease candidate drugs.

Keywords: Schizophrenia, neuronal co-culture, disease modeling

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MITOCHONIC ACID 5 IS A NOVEL THERAPY FOR GAUCHER DISEASE TARGETING MITOCHONDRIAL DYSFUNCTION AND THE NLRP3 INFLAMMASOME AXIS

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Gaucher Disease (GD) is an autosomal recessive disease and caused by glucocerebrosidase depletion. Type 2 and type 3 GD patients show neural symptoms induced by glucosylsphingosine accumulation in the brain which cause severe and lethal prognosis. Because the enzyme replacement therapy is not sufficient due to poor penetration across the blood-brain barrier (BBB), novel drugs for neuropathic GD are urgently needed. Because brain inflammation via mitochondrial dysfunction/NLRP3 inflammasome axis was reported in GD, drugs that improve mitochondrial function may play a therapeutic role. We recently established a mitochondrial homing drug, Mitochonic Acid (MA)-5 that can cross the BBB and is in Phase 1 clinical trial. Using microglia differentiated from GD patient-iPSCs, a key cell type in brain inflammation, we show that there is a significant increase in levels of mitochondrial DNA (mtDNA) released into the cytosol thus activating the NLRP3 complex, and consequentially releases IL-1b. However, all these phenotypes were mitigated upon treatment with MA-5. Single cell RNA-seq was performed, and the effects of MA-5 were analyzed. Furthermore, we generated a GD mouse model where we administered a glucocerebrosidase inhibitor and confirmed the therapeutic effects of MA-5 in vivo. We show that MA-5 significantly extended the lifespan in mice and improved motor function as measured by the rotarod performance test. We also observed an increase in microglial numbers and activation in GD mouse brains compared to healthy control mice. Upon treatment with MA-5, we also observe similar effects on the mitochondrial phenotype as in our human GD microglia model. Additionally, we wanted to see how MA-5 alters the metabolism of these cells using metabolomics. Our results show that the metabolome of GD mouse brains contain metabolites associated with inflammation and oxidative stress which were mitigated with MA-5 treatment. In conclusion, our studies have found that MA-5 ameliorates brain inflammation by regulating mitochondrial dysfunction / NLRP3 inflammasome axis in microglia and improve the neural symptoms of GD. Due to the involvement of the NLRP3 inflammasome complex in other inflammatory diseases, our drug is a potential therapeutic candidate to target this complex and thus have broad effects for incurable diseases.

Keywords: Gaucher Disease, NLRP3 inflammasome treatment, iPS-derived microglia

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MICROGLIA FERROPTOSIS CONTRIBUTES TO NEURODEGENERATION IN AN HIPSC-DERIVED TRI-CULTURE

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Ferroptosis is an iron-dependent form of regulated cell death. This pathway, as well as iron dysregulation, has been implicated in multiple neurodegenerative disorders, including Parkinson's disease (PD) and Amyotrophic Lateral Sclerosis. While neuron susceptibility has been well established, how iron overload influences microglia physiology and disease response is poorly understood. In a tri-culture of human iPSC-derived neurons, astrocytes, and microglia, the microglia were the most sensitive to ferroptosis at the transcriptomic, protein, and functional level. Single-cell RNA sequencing revealed a distinct ferroptotic signature in the microglia which correlated with marked increases in Ferritin at the protein level and increases in IL-8 production. These ferroptotic signatures were rescued with pharmacological inhibitors. Strikingly, removal of microglia from the tri-culture system, massively delayed and dampened neuronal ferroptosis, strongly suggesting the microglia sensitize the neurons to ferroptosis-dependent degeneration. Similar ferroptosis-associated signature (FAS) microglia were uncovered in PD, and the signature was also found in a large cohort of PD patient blood samples, raising the possibility that ferroptosis can be identified clinically. This work places microglial ferroptosis in the context of disease, reveals a potential role for this pathway in driving neuroinflammation, and suggests that further research into therapeutics that mitigate ferroptotic stress in not just neurons but also microglia may be of interest in treating neurodegenerative disorders.

Keywords: Ferroptosis, tri-culture, microglia

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REPRODUCIBLE GENERATION OF FUNCTIONAL HUMAN STEM CELL-DERIVED NEURAL CIRCUITS AT SINGLE CELL RESOLUTION

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Application of in vitro neuronal networks to model neurological diseases is largely based on randomly formed networks. Such arbitrary structures cannot be reproduced among samples and do not reflect the in vivo network architecture like hierarchy and modularity. In order to create more realistic in vitro models of neuronal networks, a tight control over network morphology is necessary, ideally down to the single cell and sub-cellular levels. As a result, this would improve the robustness of electrophysiology data in reproducibly engineered in vitro networks, unlike the random networks that exhibit uncontrollable network effects (noise) on electrophysiology data. Creating in vitro neural networks with

an accurate structure is not a trivial task since different aspects like placement of neuronal cells, guidance of neurite growth, synaptic connections, readout efficiency etc. need to be considered. With our novel approach, we can create defined 2D neural networks by placing neurons in a microchannel scaffold atop a multielectrode array (MEA). Human induced pluripotent stem cell (hiPSC)-derived neurons are printed with µm precision using a custom-built cell-jet printer. Neuronal outgrowth is guided using microfluidics. Neurons grow along a predefined topography of channels and connect to other cells. Different cell types are used for circuit assembly. HiPSC-derived neurons offer the flexibility, scalability and diversity needed to build circuits bottom-up. Single neuronal cells start to show activity seven days after placement and can be studied over several weeks. Our MEA setup also enables evoking neuronal activity using optogenetic or electrical stimulation. Here, we are able to engineer network motifs that are found in biological neural systems like retinal circuits. Our neural circuit system will be used for fundamental neuroscience and translational approaches such as modelling neurological diseases on-chip.

Funding Source: Volkswagen Foundation, Joachim Herz Foundation

Keywords: hiPSC-derived neurons, microelectrode array electrophysiology, circuit engineering

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INVESTIGATING THE MECHANISM OF PTEN MUTATION-RELATED NEURODEVELOPMENTAL DISORDERS IN GENETICALLY ENGINEERED HUMAN PLURIPOTENT STEM CELLS-DERIVED 2D AND 3D NEURAL CULTURES

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Mutations in the PTEN gene are strongly associated with a variety of severe neurodevelopmental disorders including autism, macrocephaly, and epilepsy. Currently, treatment options for these patients are extremely limited. In the present study, we used human pluripotent stem cells (hPSCs) based culture systems to study the mechanism of PTEN-related neurodevelopmental disorders. To investigate the role of PTEN in human cortical neurons, we engineered a dual-targeted hPSC line (H1-iCas9-iNgn2) that contains doxycycline-inducible transgenes for Cas9 and Ngn2 in the AAVS1 “safe-harbor” loci. Transduction with sgRNA-containing lentivirus and doxycycline treatment can rapidly generate edited cortical human neurons in as little as a week. We found that PTEN mutant neurons are significantly hypertrophic and electrically hyperactive. Because PTEN is known to inhibit both the mTORC1 and mTORC2 pathways, we next investigated the molecular mechanisms of these cellular phenotypes by genetically manipulating individual mTOR complexes in the PTEN mutant human neural cells. Contrary to previous mouse model studies, we found that restoration of either mTORC1 or mTORC2 was sufficient to rescue the electrical hyperactivity and hypertrophic phenotypes in the PTEN mutant human cortical neurons. Consistent with our lab’s previous findings, we showed PTEN-KO human brain organoids were larger in size and hPSCs-derived PTEN-KO human neural precursors (NPs) displayed significantly increased proliferation compared to their isogenic controls. Next, we showed that genetic intervention of either mTORC1 or mTORC2 reverts the increased proliferation of PTEN-KO human NPs and the enlarged size of PTEN-KO human brain organoids. Together, this study demonstrates the molecular mechanisms that underlie PTEN-related human neurodevelopmental disorders and highlights the novel therapeutic targets.

Keywords: Engineered human pluripotent cell line, Cas9-Ngn2 neuronal differentiation, neurodevelopmental disorder modelling

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FRAGILE X SYNDROME: CHARACTERIZATION OF THE NEURODEVELOPMENTAL DEFECTS USING INDUCED PLURIPOTENT STEM CELLS DERIVED FROM PATIENTS

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Fragile X Syndrome (FXS) is the most common hereditary cause of intellectual disability and autism spectrum disorder. FXS is caused by the lack of expression of the Fragile X Mental Retardation Protein (FMRP), a widely expressed RNA-binding protein that plays a key role in the regulation of protein synthesis. Loss of FMRP-related translational regulation has been linked to abnormal synaptic transmission and improper dendritic spines morphogenesis. However, our current understanding of FXS physiopathology remains limited, since most of the research that has looked into the subject has been conducted with animal models or human subjects. To overcome this issue, we decided to study FXS etiology using induced pluripotent stem cells (iPSCs) derived from FXS patients. We investigated the neurodevelopmental characteristics of the disease by i) evaluating the commitment of iPSCs towards neuronal differentiation in absence of FMRP expression. ii) assessing the neural progenitor cell (NPC) proliferation and differentiation. We showed that FXS NPC exhibited dysregulated cell signaling, protein synthesis, proliferation, and cell fate upon differentiation. Multi-omics analyses of FXS NPC and neurons also revealed key mechanistic insights into how the loss of FMRP expression leads to these phenotypes. We seek

to further investigate the potential contribution of these mechanisms to FXS pathophysiology and evaluate their modulation as a potential therapeutic avenue for FXS.

Keywords: Fragile X Syndrome, Neurodevelopmental Disorders, Intellectual Disability

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PHENOTYPIC HIGH-CONTENT SCREENING FOR THE IDENTIFICATION OF DRUG CANDIDATES TARGETING LEIGH SYNDROME IN PATIENT-DERIVED NEURONAL PRECURSOR CELLS

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Leigh syndrome (LS) is a severe genetic neurodevelopmental disorder affecting 1 in 36,000 live births and classified as a rare disease. Most individuals with LS have defects in mitochondrial energy production, resulting from deficiency in enzymes of the mitochondrial respiratory chain, such as mitochondrially encoded MT-ATP6. At present, there is no cure for this disease. Development of novel treatment modalities to ameliorate rare diseases is challenging for numerous reasons. Drug repurposing is a time- and cost effective drug development strategy for the identification of known drugs for alternative targets and indications. Previously, it was shown that induced pluripotent stem cell (iPSC)-derived neuronal precursor cells (NPCs) from patients could serve as an effective disease model for LS. NPCs are proliferative, retain the parental mitochondrial DNA and depend primarily on oxidative phosphorylation. Patient-derived NPCs carrying the deleterious homoplasmic mutation (m.9185T>C) in MT-ATP6 showed blunted ATP production and an increased mitochondrial membrane potential (ΔΨm). We established an automation-compatible image-based assay for ΔΨm quantification in live cells using the mitochondrial membrane potential indicator dye TMRM, which accumulates proportional to ΔΨm. Using this assay, we screened a highly annotated small molecule repurposing library with 5.632 compounds including (pre-)clinical, marketed and withdrawn drugs to identify novel therapeutic candidates that normalize mitochondrial metabolism for LS patients. We found a hit population of 187 drug candidates that reduced the ΔΨm in m.9185T>C NPCs by at least 32.5%. We confirmed this effect for 100 compounds, tested 64 most active in dose-response titration experiments and validated their toxicity profile. Currently, drug candidates with dose-dependent effects are further profiled in secondary assays that are independent from ΔΨm. The drug-mediated effect will also be validated in other LS patient-derived NPCs and healthy controls. In summary, we have established a hit identification and validation pipeline using patient-derived NPCs enabling phenotypic screening for rare neurological diseases and identified potential drug candidates that have the potential to become treatment options for patients suffering from LS.

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Keywords: High-content screen, Patient-derived neuronal precursor cells, Drug repurposing

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EXAMINING SELECTIVE VULNERABILITY TO TAU PATHOLOGY USING HUMAN IPSC-DERIVED NEURONS

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Alzheimer's disease (AD) is characterized by a stereotypical progression of tau pathology throughout the brain. Tau alterations typically appear first in the hippocampus and cortex, while midbrain and hindbrain regions are relatively spared. These neuropathological observations suggest certain regions have a selective vulnerability to the disease processes, but the molecular underpin-

nings of selective vulnerability have yet to be well defined. Here, we took advantage of human induced pluripotent stem cell (hiPSC) technology to derive neuronal identities predicted to have different propensities to form tau aggregates following seeding using a recent in-house, well-established tau aggregation high content imaging assay. We differentiated commercially available hiPSC lines derived from apparently healthy donors (3 female, 2 male, ages 15-64) into five neuronal populations resembling AD vulnerable and resilient neuronal subtypes from the forebrain, midbrain, and hindbrain. Protein and RNA markers specific to each neuronal subtype were measured via immunofluorescence, single cell RNA-seq and qPCR, which confirmed distinct expression of brain region- and neuronal subtype-specific markers for each hiPSC-derived neuronal population. When subjected to our tau seeding assay, the unique neuronal subtypes showed differential vulnerability to tau aggregation following seeding with either sarkosyl-soluble or -insoluble AD brain-derived pathological tau with high concordance between donor lines and across seed types. Importantly, the differential vulnerability observed mirrors aspects of selective vulnerability to tau pathology observed in AD brains. These results build on previous in vitro studies by applying a recently developed hiPSC tau seeding model to hiPSC-derived neuronal populations with distinct regional identities, further validating the physiological relevance of this model to Alzheimer's disease. Disclosures: IW, CP, GS, LG, MB, LR, OM, KN, SC, TK, HL, PR, NR, KY, AW, JW, XL, and JM are employees of AbbVie. JT and PP are AbbVie contractors. The design, study conduct, and financial support for this research were provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.

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Keywords: Alzheimer's disease, Selective vulnerability, Tau

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MODELING THE EMERGENCE OF NEURAL NETWORK DYSFUNCTION IN RETT SYNDROME WITH MOSAIC ORGANOIDS

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Rett syndrome (RTT) is an X-linked neurodevelopmental disorder primarily caused by heterozygous mutations in the MECP2 gene with symptoms including developmental delays, stereotyped movements, and seizures. The expression of dysfunctional and functional MECP2 in RTT is mosaic due to randomized X-chromosome inactivation. Both the proportion of dysfunctional cells and the specific mutation impact symptom severity. MECP2 dysfunction appears to impact neural network development by affecting the process of creating the excitation-inhibition balance required for normal neural circuit function. Prior organoid models relied on the use of RTT-patient derived induced pluripotent stem cells that either completely contain or lack MECP2 function to create organoids with homogenous cell populations. We have created and validated a mosaic organoid model for RTT and will present our progress using this system to understand the cell-autonomous and non-cell autonomous impacts of MECP2 dysfunction on human neurodevelopment. Our analysis is based on a multi-faceted approach including immunohistochemistry and electrophysiological approaches.

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Keywords: Rett Syndrome, Mosaic Organoids, Neural Network Development

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NOVEL MODULAR IN VITRO PLATFORM FOR THE STUDY OF EXTERNAL FORCE ON TISSUE

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One of the biggest challenges of cell cultures in vitro is mimicking the mechano-physiological forces applied to cells including applying external forces. While different approaches were demonstrated, none of them were able to induce external force without touching the cells which can be tuned and changed over time. In this work, we developed a modular platform that enables the induction of external forces on cells, without direct contact, with different forces, durations, and that can be applied to multiple cell types. In this work, we demonstrate the strength and versatility of the system by two examples: preload-and afterload: we demonstrate how applying an external force on cardiomyocytes enables us to better assess drug response and cardio-physiology; and traumatic brain injuries (TBI): here we demonstrate how such a system can decouple the forces which apply on the brain during TBI and assist us to better understand the neuronal response to injury. It is important to note that this platform is very versatile and allows us to apply it to various tissues for better mimicking the forces which are applied to tissues in vivo.

Keywords: traumatic brain injury, cardiomyocytes preload and afterload, mechano-physiological forces

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IN VIVO EXAMINATION OF THE TREM2-DEPENDENT HUMAN MICROGLIAL RESPONSES TO ALZHEIMER'S DISEASE PATHOLOGY USING PROTEOMICS IN CHIMERIC MICE

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Alzheimer's disease (AD) is the most common neurodegenerative disease. Recent genome-wide association studies identified many risk genes expressed in microglia, suggesting that microglia play a central role in AD. One of the strongest risk genes, TREM2, is exclusively expressed by microglia. Loss of function mutations of TREM2 was showed to increase AD risk dramatically. TREM2 knockout in mice was shown to impair the ability of microglia to respond to beta-amyloid plaques. However, it remains unclear whether human TREM2 knockout microglia exhibit similar or perhaps additional functional deficits. To further examine the impact of TREM2 deletion on human microglia, we used CRISPR to generate TREM-knockout (TREM2-KO) induced pluripotent stem cells (iPSCs). Isogenic wildtype and TREM2-KO iPSCs were differentiated into hematopoietic progenitors (HPCs) and transplanted into postnatal immunodeficient AD mice (hCSF1-5xFAD). Six months later, human microglia were isolated from chimeric mice and examined via bulk proteomic analysis. Analysis of proteomic dataset revealed significant and novel impacts of TREM2 deletion on the response of human microglia to beta-amyloid pathology. The protein abundance of a set of AD risk genes showed TREM2-dependence in their response to beta-amyloid pathology. Immune-related pathways in human microglia were altered between TREM2 genotypes in AD mice. The engulfment and phagocytosis of mouse synapses by human microglia were also altered by the loss of TREM2. These data reveal important new information about changes occurring within human microglia in response to amyloid pathology and loss of TREM2 expression.

Keywords: Microglia, In vivo model, TREM2

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LABEL-FREE FUNCTIONAL CHARACTERIZATION OF IPSC-DERIVED NEURONS AT SUBCELLULAR RESOLUTION

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In recent years, brain models derived from pluripotent stem cells have become a fundamental tool for studying common neurological disorders, such as epilepsy, Alzheimer's disease, and Parkinson's disease. The ability to measure the electrical activity of human iPSC-derived neurons in real time and label-free can provide much needed insights into the complexity of the neuronal networks. Nowadays, combining single cell resolution with high-throughput physiological assays, which can potentially deepen our understanding of subtype-specific neuronal activity, is especially valuable and yet difficult to achieve. In this study, the MaxTwo System (MaxWell Biosystems, Switzerland), a multi-well high-density (HD)-MEA platform was used. MaxTwo HD-MEA System allows in vitro extracellular recordings of action potentials at different scales, ranging from network through single-neuron to subcellular features. Moreover, we showed the advantages of having an HD-MEA system featuring 26,400 electrodes per well, which are key to increase the statistical power of the data collected from iPSC-derived neurons over multiple days/weeks and to capture the smallest neuronal signals. Finally, we present the Axon Tracking Assay, a tool for automated recording and analysis of individual axonal arbors of many neurons in parallel. The Axon Tracking Assay enables to measure action potential conduction velocity, axonal length, and number of axonal branches. With this unique method, we characterized the function and axonal structure of different iPSC-derived neuronal cell lines. Our HD-MEA platforms and the extracted metrics, such as firing rate, spike amplitude, and network burst profile among several others, provide an extremely powerful and user-friendly approach for in vitro drug screening and disease modelling.

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Keywords: HD-MEA, iPSC-derived neurons, axons

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TARGETING TCF4 TRIPLET EXPANSION REPEAT IN FUCHS ENDOTHELIAL CORNEAL DYSTROPHY: TOWARDS A CELL PLATFORM FOR DISEASE MODELING

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The CTG18.1 repeat expansion in the transcription factor 4 (TCF4) intron 3 is the main cause of Fuchs endothelial corneal dystrophy (FECD), a complex degenerative condition underlying cornea-related blindness. The prevailing paradigm is that the expanded repeat exerts toxic effects, resulting in dysfunction and loss of corneal endothelial cells. However, due to the lack of in vivo models and multiple limitations related to the available in vitro platforms, the mechanisms fueling the disease are not fully understood. We explored the use of CRISPR/Cas9 for disease modelling by excising the CTG18.1 expansion in three FECD patient-derived induced pluripotent stem cell lines (iPSCs) and one healthy control. Using a dual-plasmid-based approach, we generated four ΔCTG18.1 lines characterized by the complete excision of the CTG18.1 expansion, as confirmed by Sanger sequencing. The pluripotency was confirmed by immunocytochemistry of Oct4, Nanog, SSEA4 and TRA-1-81 markers and tri-lineage differentiation. The Tcf4 protein level was validated by Western blot analysis and potential CRISPR/Cas9 cleavage activity was investigated by PCR and Sanger sequencing of the predicted off-target sites. To produce feeder-free iPSC-derived corneal endothelium cells (iCEnCs), we used small molecule induction including transforming growth factor beta receptor inhibitor SB431542, GSK-3-specific inhibitor CHIR99021 and retinoic acid according to recently published work. As a proof of concept, we differentiated the healthy control line towards iCEnCs, characterized by the morphology and positivity of human periocular mesenchyme (e.g. Pitx1, Foxc1) and corneal endothelium markers (e.g. Z0-1, Na+/K+, CD166) through immunocytochemistry at multiple time points. With this study, we aim to provide a valuable cell platform for investigating the contribution of the CTG18.1 expansion to FECD pathogenesis, paving the way to a comprehensive characterization of the disease and validation of innovative therapies against FECD.

Keywords: Fuchs endothelial corneal dystrophy, CRISPR/Cas9 gene editing, Corneal endothelium

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CAS9-MEDIATED KNOCKINS OFTEN ASSOCIATED WITH MULTICOPY INSERTIONS OF THE PLASMID DONOR

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We developed a highly efficient CRISPR-based method to generate homozygous HaloTag knockins in human induced pluripotent stem cells (iPSCs). Following co-delivery of Cas9 RNP and plasmid donor DNA, a majority of single cell-derived clones appear to be correctly targeted events in both copies of the target gene. However, loss of HaloTag expression was observed in some homozygous clones prompting us to sequence knockin alleles by Cas9 nanopore targeted sequencing. Surprisingly, the loss of HaloTag expression is correlated with the presence of tandem arrays of the entire plasmid donor. We then designed a PCR assay detect these aberrant events and found that 40-80% of knockin clones contained multicopy insertions. Further nanopore targeted sequencing of clones edited with a plasmid containing degenerate bases supports a rolling circle DNA replication mechanism for multicopy integration. Thus, our results reveal a frequent and undesirable 'on-target' effect associated with the use of circular plasmid donor templates for Cas9-mediated knockin experiments.

Funding Source: NIH (NIA, NINDS)

Keywords: Genome editing, 'on-target' effects, induced pluripotent stem cells

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MODELLING INNER EAR DEVELOPMENT IN HUMAN IPSC DERIVED ORGANOIDS

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The sense of hearing allows awareness of the surroundings and orientation in the environment, and it is arguably the most important sense for human communication. Globally, hearing loss affects over 400 million people, and it is associated with environmental causes, aging and with congenital mutations that lead to defects in ear development and function. Thus, understanding

the molecular mechanisms that drive the formation of a functional inner ear during normal development is key to uncover the reasons behind otic impairment, and to identify potential therapies. The inner ear is responsible for sound perception and balance, and it arises from a relatively simple embryonic structure known as the otic vesicle, which is gradually transformed into the highly specialised adult inner ear. Patterning of the otic vesicle, directed by signals from surrounding tissues, is critical for normal development: any patterning defects cause ear malformation. How patterning is integrated with cell differentiation and ear morphogenesis remains an open question. Here, we explore the patterning events that shape the human ear taking advantage of inner ear organoids, 3D structures derived from human induced pluripotent stem cells (iPSCs) that mimic ear development in vitro. Extensive immunohistochemical characterization while organoids accurately recapitulate early stages of ear development, the vesicles are poorly patterned. These findings provide novel insight into their apparent lack of cochlea-associated cells and spatial organization. We are currently investigating the competency of iPSC-derived otic vesicles to respond to patterning signals, which will in turn enlighten future signal modulation studies in organoid cultures. Creating models that better replicate normal ear formation will allow us to address the fundamental principles underlying early ear development in humans and has the potential to ultimately model disease states.

Keywords: Inner ear development, organoids, patterning

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MODELLING HUMAN EPILEPSY IN A DISH: EXPLORING THE ROLE OF SCN1A LOSS OF FUNCTION IN PRENATAL BRAIN DEVELOPMENT

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The sodium channel subunit, SCN1A, is detectably expressed postnatally and increases through to adulthood. Its expression on inhibitory interneurons demonstrates its role in synaptic inhibition, thus, SCN1A dysfunction upsets a fine balance in brain circuitry, giving rise to epileptic disorders. SCN1A variants resulting in loss of protein function are responsible for the rare but severe Dravet Syndrome (DS). Most human cases of this infantile-onset epilepsy are attributed to SCN1A haploinsufficiency, resulting in pharmaco-resistant seizures, developmental delays, cognitive dysfunction, and high incidence of epilepsy-associated death. It is hypothesised that complete loss of function causes embryonic lethality, though the role of SCN1A in pre-natal brain development remains unclear. Though mouse models of this disorder have been studied extensively, the disparity between the mouse and human developing brain prompts the need for clinically relevant models. To explore its role during human embryonic development, CRISPR-Cas9 technology was used to target the endogenous start codon of SCN1A, generating homozygous and heterozygous SCN1A knockouts in the human embryonic stem cell line, H9. These were validated by genotyping and next generation sequencing, confirming a 200bp deleted region. All knockout clones were karyotypically normal and immunocytochemistry verified expression of OCT4, SOX2 and TRA-181 within stem cell colonies, and lack of germ-layer marker expression, confirming their retained pluripotency. SCN1A knockout clones and unedited H9 cells then underwent targeted differentiation into 3D forebrain organoids of dorsal and ventral lineages to study the developing telencephalon in vitro. Preliminary data revealed early transcriptional alterations in markers of the medial ganglionic eminence between knockout and control organoids, suggesting novel implications for SCN1A in ventral forebrain induction during pre-natal brain development. Further investigation will involve functional readouts of neuronal activity and a deeper exploration into cell-type specific deficits associated with depleted SCN1A activity. Hence, our work sheds light on early DS-associated mechanisms during pre-natal neurodevelopment, directing future studies into therapeutic strategies.

Keywords: Neurodevelopment, Gene editing, Organoids

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INVESTIGATING THE IMPACT OF SPATA5 DEFICIENCY DURING HUMAN CORTICOGENESIS USING CEREBRAL ORGANOIDS

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Variants of Spermatogenesis-associated protein 5 (SPATA5) are associated with several neurodevelopmental deficits, including microcephaly, intellectual disability, and epilepsy. SPATA5 belongs to the AAA ATPase family of proteins and has been proposed to play a role in early spermatogenesis and in ribosome assembly. Recent evidence suggests a role for SPATA5 during neuronal axonal growth and in mitochondrial dynamics. However, the role for SPATA5 during human corticogenesis has not been studied and how SPATA5 deficiency causes neurodevelopmental deficits in patients is not known. Further, no study to date has performed neuropathological examinations in a patient containing SPATA5 variants. Here we report clinical and neuropathological findings in a post-mortem NCH patient with compound heterozygous SPATA5 variants (c.554G>A; p.Gly185Glu and c.1877G>C; p.Trp626Ser). The patient exhibited intellectual disability, microcephaly, epilepsy, and progressive leukoencephalopathy. Immunostaining analysis showed a reduction in the number of cortical neurons, particularly in layers 2/3. Astrogliosis was also prominent in superficial cortical zones. Western blot analysis revealed a reduction in SPATA5 protein levels, suggesting the variants contribute to decreased protein stability and/or degradation. Currently, we are investigating whether mitochondrial morphology and ribosomal biogenesis are compromised using post-mortem brain tissue and in vitro models. We have established SPATA5 knockout

induced pluripotent stem cell (iPSC) lines using CRIPSR-Cas9-mediated non homology end joining and are generating a cerebral organoid model to further investigate the impact of SPATA5 deficiency during human corticogenesis. Results will be validated using post-mortem patient brain specimens. These findings are the first to establish a critical role for SPATA5 during human brain development and future studies will provide novel mechanistic insights into understanding how SPATA5 deficiency causes neurodevelopmental deficits in patients.

Keywords: SPATA5, Cerebral Organoids, Neurodevelopment

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GENOME-WIDE EFFECTS OF SEX CHROMOSOME DOSAGE IN ISOGENIC NEURAL AND CARDIOMYOCYTE MODELS OF X CHROMOSOME ANEUPLOIDY

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Sex chromosome dosage (SCD) is a fundamental genetic difference between females (XX) and males (XY). X chromosome inactivation (XCI), which silences most genes on one X chromosome in females, balances X expression between the sexes. However, ~15-25% of genes escape XCI and remain expressed from the inactive X (Xi) in humans. Aberrant expression of escape genes has been implicated in genome-wide gene dysregulation and clinical phenotypes of sex chromosome aneuploidy (SCA) syndromes, including Klinefelter (XXY), Turner (X), and Triple X (XXX), which are associated with cognitive and cardiovascular defects. Studying the impact of SCA in humans is complicated both by confounding variables (i.e. genetic variation or environmental and hormonal influences) and by inaccessibility of clinically relevant cell types. To address this, we generated isogenic sets of hiPSCs with different sex chromosome content on the same genetic background. XXY/XY, XY/X, XX/X, and XXX/X isogenic pairs were derived by reprogramming cells from mosaic individuals or by selective removal of one X chromosome. These isogenic hiPSC lines are uniquely suited to address epigenetic and transcriptomic effects of SCD - dosage of the X chromosome and/or presence of a Y chromosome - while minimizing individual variation. To examine the effects of SCD in clinically relevant cell types, we differentiated our hiPSC lines to neural precursor cells and cardiomyocytes. Increased SCD in XXY/XY and XXX/X isogenic pairs resulted in genome-wide changes in autosomal gene expression and in distinct DNA methylation patterns, which were more prevalent in comparing XXX to X than XXY to XY. Gene expression and DNA methylation changes were noticeably less prominent for isogenic samples than among independent samples. Our data are consistent with trans-acting effects of SCD on autosomal gene regulation. In addition, we observed SCD-dependent changes in expression of escape genes including genes located in the pseudoautosomal region. Cell lines with a single X chromosome showed the largest number of differentially expressed genes, consistent with severe developmental anomalies in Turner syndrome. Subsets of genes were differentially affected by SCD depending on cell type, stressing the importance of examining cell types relevant to SCA conditions.

Keywords: Sex Chromosome Dosage, X Chromosome Aneuploidy, Isogenic hiPSC Models

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NEUROIMMUNE CORTICAL ORGANOIDS OVEREXPRESSING C4A EXHIBIT MULTIPLE SCHIZOPHRENIA ENDOPHENOTYPES

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Elevated expression of the complement component 4A (C4A) protein has been linked to an increased risk of schizophrenia (SCZ). However, there are few human models available to study the mechanisms by which C4A contributes to the development of SCZ. In this study, we established a C4A overexpressing neuroimmune cortical organoid (NICO) model, which includes mature neuronal cells, astrocytes, and functional microglia. The C4A NICO model recapitulated several neuroimmune endophenotypes observed in SCZ patients, including modulation of inflammatory genes and increased cytokine secretion. C4A expression also increased microglia-mediated synaptic uptake in the NICO

model, supporting the hypothesis that synapse and brain volume loss in SCZ patients may be due to excessive microglial pruning. Our results highlight the role of C4A in the immunogenetic risk factors for SCZ and provide a human model for phenotypic discovery and validation of immunomodulating therapies.

Keywords: C4A, Neuroimmune Cortical Organoids, Schizophrenia

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SERUM-FREE DIFFERENTIATION AND CULTURE OF IPSC-DERIVED ASTROCYTES

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Astrocytes play a key role in synapse formation, neural network maturation, and neurodegenerative disease phenotypes. Next-generation cellular models that combine induced pluripotent stem cell (iPSC)-derived neurons and iPSC-derived astrocytes as 2D or 3D cultures are increasingly recognized as predictive models for compound screening and preclinical drug discovery. Many iPSC-derived astrocyte differentiation and culture protocols require serum, which has been shown to activate astrocytes and introduces unknown variables into the culture. In this study, we characterize the purity and performance of cryopreserved human iPSC-derived astrocytes differentiated and maintained in a serum-free medium. First, we show that this differentiation process consistently yields a highly pure population of human astrocytes, as determined by flow cytometry or immunostaining for CD44, CD49f, S100β and GFAP. Gene expression analysis showed expression of relevant astrocytes markers (Aquaporin, EAAT2, ALDH1L1) further confirming an astrocyte phenotype. Functional analysis of these iPSC-derived astrocytes following 7 days of serum-free culture revealed robust and consistent glutamate uptake across multiple lots. To examine the capacity of these iPSC-derived astrocytes to promote neural network formation, they were co-cultured with iPSC-derived glutamatergic and dopaminergic neurons. Astrocyte-containing cultures show enhanced neural network formation using multielectrode array (MEA) platforms. Additionally, serum-free iPSC-derived astrocytes in complex multi-cultures can form 3D neurospheres and support neural activity, as evaluated by formation of calcium oscillations. Lastly, these astrocytes successfully supported barrier function when co-cultured with iPSC-derived brain microvascular endothelial cells (BMECs) and pericytes. Together, these data demonstrate the highly consistent manufacturing of serum-free iPSC-derived astrocytes that display characteristic functions of astrocytes in both monoculture and multicellular 2D and 3D cultures.

Keywords: Neuroinflammation, Spheroid, Blood Brain Barrier (BBB)

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OPTIMIZATION OF THE DIFFERENTIATION AND PURIFICATION OF SATB2+ HUMAN CORTICAL NEURONS

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Generating specific neuronal cell types from human pluripotent stem cells (hPSCs) facilitates targeted model systems for studying human brain development and disease. Special AT-rich binding protein 2 (SATB2) expression in the brain is a hallmark of upper layer neocortical excitatory neurons and is required for the formation of the corpus callosum. Further, the SATB2 gene is associated by multiple genome-wide associated studies with autism spectrum disorder and schizophrenia. Despite the importance of SATB2-expressing neurons in brain development, we currently lack an efficient method for generating this cell type from hPSCs. In this study, we aim to improve the differentiation efficiency of SATB2+ neurons using a lentiviral reporter for the SATB2+ expression and small molecule screening. Achieving this goal will open new opportunities for in vitro modeling of SATB2+ neurons and their role in neurodevelopment.

Keywords: Neocortical Development, SATB2, Neuronal Differentiation

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DOSE DEPENDENT EFFECTS OF KETAMINE ON ELECTRICAL ACTIVITY IN HUMAN CORTICAL ORGANOIDS

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Human induced pluripotent cell-derived organoids directed towards cortical lineages have shown periodic and regular oscillatory electrical events from spontaneously developed neural network formation in months-old cultures. These network events are dependent on glutamatergic and GABA-ergic signaling and when compared to neonatal EEG forms show resemblance to early delta waves in utero. Delta-band activity is relevant in several psychiatric disorders including decreased delta activity in sleep as a known-predictor of depression symptoms. Ketamine is thought to increase delta activity during sleep in addition to

its rapid antidepressant and dissociative anesthetic effects. 3D human cortical organoid cultures are likely to be useful surrogates to determine electrical activity responses for mechanistic understanding of rapid-acting psychiatric medications. To investigate the effect of ketamine at physiologically relevant concentrations we fine-tuned previous protocols in our lab where cortical organoids were differentiated for 3-6 months from iPSCs. We used two modalities to investigate neural cell function: calcium imaging, and electrical activity analysis from multi—electrode arrays (MEAs). Calcium fluxes with Zeiss microscopy and Axion Biosystems MEAs were recorded in live imaging conditions with baseline states were recorded prior to treatment. Ketamine was added to treat cells at physiologically relevant concentrations in three varying doses starting with 2.5 µM modeling sub-dissociation anti-depressant action and up to 100µM modeling deep anesthesia. Control-treated organoids were given isotonic solution and active treatment wells were randomized to condition. Initial results with three biological replicates and at least four technical replicates suggested ketamine had decreased total network activity in spontaneously firing organic cultures. Further results suggest that there is a dose-dependency to decreasing spiking rate with ketamine, and possibly a thresholding effect whereby after 3D neural cultures reach a certain concentration below 100µM there is a rapid decrease in firing rate. We hope these 3D organoid models will prove useful for determining electrophysiological patterns of response to ketamine which may eventually be translated from cells to patients.

Keywords: ketamine, organoid, MEA

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IMPAIRED CA2+ NEURONAL ACTIVITY AND DIFFERENTIAL GENE EXPRESSION OF NEURONS IN RETT SYNDROME PATIENT-DERIVED HUMAN CEREBRAL ORGANOIDS

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Human cerebral organoids from induced pluripotent stem cells are powerful experimental systems for analyzing human brain developmental processes in vitro. Patient-derived brain organoids, furthermore, recapitulate disease processes based not only on differential gene expression but also subsequent structural changes. Most brain organoid research to date has capitalized on the anatomical characteristics of organoids to model disease phenotypes that impact human brain growth. Mutations in the X-linked methyl-CpG binding protein 2 (MECP2) gene, which are associated with Rett syndrome (RTT), are widely believed to downregulate and upregulate numerous downstream genes, causing unorganized and/or abnormal neuronal activity. However, the early developmental activity-dependent changes associated with MeCP2 mutation typically proceed alongside or even in the absence of measurable structural changes, raising the question of whether organoids from patient-derived cells possess sufficient complexity to model disease processes. Here, we cultured and characterized Rett syndrome patient-derived human cerebral organoids which had two specific MeCP2 mutations (V247X and R306C mutations), and measured neuronal activity in live organoids using a calcium sensor with two-photon and three-photon microscopy. We found abnormalities in the amount and pattern of neuronal activity in RTT organoids compared to isogenic controls. Treatment with NMDA and AMPA receptor antagonists suppressed this activity, indicating that the neuronal activity is associated with synapse formation and synaptic transmission in the organoids. Transcriptome analysis further revealed differential gene expression related to inhibitory neurotransmission such as GABAA receptors and excitatory transmission between RTT organoids and isogenic controls. Furthermore, optogenetic stimulation performed under two-photon imaging with red-shifted channelrhodopsin and GCaMP6, to study synaptic transmission and potentiation in organoids, revealed clear responses of neurons in organoids upon optical stimulation. Together, these findings provide fundamental new information on neuronal and circuit mechanisms that influence electrical activity in brain organoids and their early developmental changes in Rett syndrome.

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Keywords: Organoid, Rett syndrome, Stem cell

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HUMAN-SPECIFIC FMRP REGULATION OF RACK1 IS CRITICAL FOR PRENATAL CORTICAL DEVELOPMENT

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Fragile X messenger ribonucleoprotein 1 protein (FMRP) deficiency leads to fragile X syndrome (FXS), an autism spectrum disorder. The role of FMRP in prenatal human brain development remains unclear. Here we show that FMRP is important for human and macaque prenatal brain development. Both FMRP-deficient neurons in human fetal cortical slices and FXS patient stem cell-derived neurons exhibit mitochondrial dysfunctions and hyperexcitability. Using multiomics analyses, we have identified both FMRP-bound mRNAs and FMRP-interacting proteins in human neurons and unveiled a previously unknown role of FMRP in regulating essential genes during human prenatal development. We demonstrate that FMRP interaction with CNOT1 maintains the levels of receptor for activated C kinase 1 (RACK1), a human-specific FMRP target. Genetic reduction of RACK1 leads to both mitochondrial dysfunctions and hyperexcitability, resembling FXS neurons. Finally, enhancing mitochondrial functions rescues deficits of FMRP-deficient cortical neurons during prenatal development, demonstrating targeting mitochondrial dysfunction as a potential treatment.

Keywords: fragile X syndrome, FMRP, mitochondria

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CHARACTERIZATION OF 16P11.2 DELETION EFFECTS ON CORTICAL DEVELOPMENT AND NEURONAL NETWORK FUNCTION IN PATIENT-SPECIFIC HUMAN MODELS OF DISEASE

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A variety of different neurodevelopmental disorders, including autism spectrum disorder, schizophrenia, bipolar disorder, deficits in social communication and delays in speech development have been associated with copy number variations of the 16p11.2 region. 16p11.2 deletion also causes macrocephaly and increased body mass index. Recently, a number of studies with human iPSC derived neurons have been conducted in vitro to study the disease phenotypes of these neuronal cells. Despite of these efforts, the molecular mechanisms underlying these disorders have remained largely unresolved. In this study we aimed to investigate how cortical neuron development and function is altered in the setting of 16p11.2 deletion. To answer this question, we transduced iPSCs with human NGN2-lentiviral vector for making of glutamatergic neurons and human ASCL1/DLX2-lentiviral vector for making of GABAergic neurons. We collected samples from these neuronal cultures for RNA sequencing, metabolomics, and for functional assays on low-density micro-electrode array (MEA) and high-density MEA platforms. Our preliminary functional data demonstrate that the 16p11.2 deletion in glutamatergic neuron networks leads to increased weighted mean firing rate, number of bursts, neurite length and neuron conduction velocity compared to control neurons. Furthermore, the expression of different lipids was altered in these neurons compared to control neurons. These results indicate that the 16p11.2 deletion affects negatively the development of cortical neurons in vitro. In the future, these network deficits may be utilized for testing of different drugs in cell culture and for the development of new therapies for the 16p11.2 CNV disorders.

Funding Source: Rosamund Stone Zander Translational Neuroscience Center Boston Children's Hospital, The Rothberg Family Fund for Cognitive Science Boston Children's Hospital.

Keywords: Copy number variations in neurodevelopmental disorders, High density multi-electrode array, cortical neuron development in vitro

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MICROGLIA GENE KNOCKDOWN OF DDX39B ALTERS VIABILITY OF HUMAN ALS/FTD NEURONS

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive loss of motor neurons and shares pathological and genetic etiology with frontotemporal dementia (FTD). Recent discoveries have elucidated the influence microglia have over disease progression in ALS/FTD, as with many other neurodegenerative disorders, revealing an appealing target for therapeutic interventions. However, the capability to harness the neuroprotective properties of the inflammatory response while diminishing the exacerbation of disease pathology, remains a source of debate. To investigate this, we targeted recently identified key genetic regulators of microglia functional states to examine the characteristic properties and each states' capacity to conserve neuronal integrity in an in vitro model of ALS/FTD neurodegeneration. We found that suppression of the microglia DEAD-box-helicase gene, DDX39B, had the most potent effect on neighboring neuron viability. Using transcriptomics and proteomics, we classified the induced microglia state from knockdown of DDX39B, as with an array of other genetic regulators. Ultimately, our screen identifies potential microglia states that would impede in vivo ALS disease pathology and the key genetic regulators that can induce each functional state.

Keywords: ALS, Microglia, Neurons

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USING STEM-CELL DERIVED BRAIN ORGANOIDS TO INVESTIGATE THE ROLE OF KDM6B IN AUTISM SPECTRUM DISORDER AND EARLY BRAIN DEVELOPMENT

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Autism Spectrum Disorders (ASDs) are neurodevelopmental conditions that affect over 50 million individuals worldwide. ASDs often present as changes in motor/social skills, altered sensory processing ability, and restricted behaviors/interests. ASDs are associated with changes in neuronal connectivity and synaptic function, however, the mechanisms underlying these changes remain unclear. Lysine Demethylase 6B (KDM6B), which enables gene expression by promoting removal of repressive H3K27me3 chromatin marks on promoters, is highly associated with ASDs with heterozygous loss. In developing mice, homozygous loss of Kdm6b gene function leads to thinning of the cerebral cortex. Additionally, mice lacking one copy of Kdm6b display autistic-like behaviors. To assess the roles that KDM6B plays in human brain development we modeled the effects of disrupting its function using a selective inhibitor of KDM6B demethylase function along with CRISPR/Cas9-mediated inactivation of KDM6B gene function. KDM6B disruption resulted in changes in the size of neural progenitor rosettes and neuronal production, without noteworthy effects on neuronal migration. In my presentation, I will present our ongoing work exploring the consequences of KDM6B loss on progenitor maintenance, neurogenesis, and synaptogenesis in greater detail. In determining KDM6B's role in neural development, we seek to understand how ASDs arises and identify new therapeutic targets to improve patient quality of life.

Funding Source: The Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research at UCLA, Rose Hills Foundation and CSUN CIRM Bridges 3.0 Stem Cell Research & Therapy Training Program (EDUC2-12718).

Keywords: Brain Organoids, 3D Culture, Lysine Demethylase 6B

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APPLYING A NOVEL IPSC-DERIVED MICROGLIA PROTOCOL TO INVESTIGATE CELL-AUTONOMOUS PHENOTYPES IN PARKINSON'S DISEASE

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Microglia are the tissue-resident macrophages of the central nervous system and have important roles in brain development, homeostasis, and disease. The concentration of microglial cells is particularly high in the substantia nigra pars compacta of the midbrain, where dopaminergic neurons die in Parkinson's Disease (PD), and microglial activation and neuroinflammation is a hallmark of the disease. Since microglial development is intertwined with that of the neural tissue, we applied a protocol that considers their ontogeny and maturational context to generate iPSC-derived microglia. In this study, we used isogenic PD-model iPSC lines carrying SNCA (alpha-synuclein) triplication and LRRK2 G2019S mutation to evaluate microglia-intrinsic PD phenotypes. After small molecule-mediated differentiation, we assayed the iPSC-derived cells for microglial identity markers and functionality. The protocol generated CD11c-positive erythromyeloid progenitors (EMPs) that infiltrated neurospheres. EMPs matured when co-cultured with neuronal cells, exhibiting strong Iba-1 signal and characteristic microglial morphodynamics. We adapted the protocol to allow the cryopreservation of early EMPs (day 15) and older maturation stages (day 24 and 30), verifying their viability after thawing in both mono- and co-culture. Early EMPs exhibited the highest viability and proliferation potential after thaw, while older stages performed better when thawed on neuronal cultures. Finally, cells generated with this protocol, including thawed cells, were able to phagocytose zymosan, demonstrating a key microglial function and the potential of these cells to model microglia in both health and disease. Our next steps include applying this protocol to investigate cell-autonomous disease phenotypes through the evaluation of neuroinflammatory responses and cell-to-cell interactions.

Funding Source: This project is part of Aligning Science Across Parkinson's (ASAP), a global basic research initiative.

Keywords: iPSC-derived microglia, Parkinson's Disease, Differentiation protocol

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MULTIDONOR HUMAN CORTICAL CHIMEROIDS REVEAL INDIVIDUAL SUSCEPTIBILITY TO NEUROTOXIC TRIGGERS

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Inter-individual genetic variation has been identified as an important modulator of disease susceptibility, such that different individuals show heterogeneous responses to disease risk factors. Modelling the contribution of human genetic background to phenotypes is limited by the necessity of using human models, and the difficulty of scaling these systems to represent many individuals. Here, we present human brain “Chimeroids”, a highly reproducible, multi-donor brain organoid model that allows co-development of human cerebral cortex from a panel of individuals in a single organoid, while maintaining developmental features similar to endogenous tissue. By re-aggregating single-donor organoids at the neural progenitor stage, Chimeroids maintain balanced donor contribution even when using hPSC lines with different biases for growth and differentiation. We leveraged this Chimeroid system to investigate susceptibility across multiple individuals to developmental exposure to neurotoxic stressors: ethanol and the anti-epileptic drug valproic acid. We show that individual donors vary in both the penetrance of the effect on target cell types, and the molecular phenotype within each affected cell type. Our results show that human genetic background is an important mediator of neurotoxin susceptibility, and introduce Chimeroids as a scalable system for high-throughput investigation of the contribution of diverse genetic backgrounds to brain function and disease.

Keywords: Brain-Organoids, Neurodevelopment, Disease-modeling

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DYNAMIC CLONAL MIGRATION OF GLIOBLASTOMA STEM CELLS IN HUMAN CEREBRAL ORGANOIDS

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Glioblastoma (GBM) is the most common and deadly primary central nervous system tumor. It is associated with extremely poor prognosis and quality of life with very limited treatment options. It is known that continued GBM migration into healthy neuronal tissue drives its rapid spread throughout the brain and consequently, mortality. These genetic and molecular mechanisms, however, are poorly understood. To define the migratory pattern and biological interactions of glioblastoma stem cells and normal tissue we established a method of microinjection of fluorescently tagged GBM stem cells into human iPSC-derived cerebral organoids. We injected 50cells/ul GBM Red fluorescent protein GBM stem cells loaded into a custom-made micropipette and precisely dispensed into organoids to micrometer precision. We have shown following injection, GBM stem cells rapidly adapt to the organoid microenvironment and establish proliferative niches of cancer cells in discrete clonal distribution in the organoids. After clonal expansion mass is achieved, GBM stem cell migration progresses in a radial fashion from the bulk tumor to establish additional niches in tumor naïve areas within the organoid. This process propagates until the organoid has been completely enveloped by tumor. Experiments are ongoing to define the migratory and proliferation potential of cluster of GBM stem cells and apoptosis of both normal and tumor tissue. Two-photon microscopy will be utilized to capture time series images of single cell migration and bulk RNAseq to measure changes at the gene expression of heterotopic interactions between GBM stem cells and normal tissue and to explore reciprocal molecular adaptation between growing tumor and surrounding normal tissue.

Keywords: Glioblastoma, Organoid, Migration

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MODELING ALZHEIMER’S DISEASE BY HUMAN PATIENT IPSC DERIVED CEREBRAL ORGANOIDS

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Cerebral organoids are 3D neural tissues derived from human pluripotent cells via organoid culture techniques providing a better platform for researches in human brain development and brain-related diseases. Alzheimer’s disease (AD) is a common neurodegenerative disease in the elders, resulting in heavy burdens to society and families. AD disease mechanism is the key to new drugs of AD, yet lacking proper AD disease models bringing a lot obstacle. Human induced pluripotent cells (iPSCs) obtain human specific gene backgrounds, which is closer to human beings rather than AD mice model. In our research, we established AD patient iPS cell lines and differentiated the iPSCs into cerebral organoids. We found that AD brain organoids had similar AD hall

markers such as Aβ plaques, neurofillbrillary tangles (NFT) and neuronal death. With our optimized brain organoid culture system, we established a better AD brain organoid disease model with functional electrophysiology by MAX Well MEA. Our AD cerebral organoids provide a promising way for drug screening in the future.

Keywords: Alzheimer's Disease, Cerebral Organoid, Disease Model

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MODELING FAMILIAL ALZHEIMER'S DISEASE USING INDUCED PLURIPOTENT STEM CELLS: A TOOL FOR INVESTIGATING THE DISEASE PATHOGENESIS AND DEVELOPING TREATMENTS

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Alzheimer's Disease (AD) is a neurological disorder, leading to memory loss, behavioral change, and degeneration of all cortical areas. Pathological hallmarks are the presence of amyloid plaques, phospho-Tau tangles, synaptic atrophy, and neuronal death, Yet, the exact origin of age-related AD remains unknown. Early-onset Familial Alzheimer's Disease (FAD), either of sporadic or familial origin, represents less than 2-3% of cases, and is genetically linked to dominant point mutations in Amyloid Precursor Protein (APP), Presenilin 1 (PSEN1) or Presenilin 2 (PSEN2) genes. Despite this knowledge and the identification of FAD-associated mutations decades ago, there are still no treatment to prevent or slow FAD progression, since it is challenging to obtain tissue from the patient to study the disease before death. Induced pluripotent stem cells (iPSCs) hold a great potential for studying AD. They are generated by reprogramming mature adult cells into a pluripotent state, allowing them to differentiate into any cell type, including neurons. In this study, we have reprogrammed human fibroblasts from 2 control and 4 FAD patients into iPSCs. All iPSC lines were showing the classical morphology of human iPSC colonies, and expressing the pluripotency marker. Using the default model of neural induction and Noggin, control and FAD iPSC lines could be differentiated into cortical neurons 60 days in vitro. Using confocal immuno-fluorescence, we confirmed that FAD neurons from the FAD cell lines presented accumulation of p-Tau and amyloid. We plan to use it as screening platform in order to identify new drugs that are mutation specific. Our FAD model can provide a tool to investigate the effect of specific PSEN1 or PSEN2 mutations on signaling pathways, and cellular function. This is a cost-effective and less time-consuming platform to discover and test new drugs, reducing the risk of adverse effects in patients during the clinical trial stage.

Keywords: iPSCs-derived neurones, Familial Alzheimer's Disease (FAD), Disease modelling

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INVESTIGATING THE FUNCTIONAL CONSEQUENCES OF STRUCTURAL VARIATION MUTATIONS CAUSING INHERITED PERIPHERAL NEUROPATHIES USING PATIENT-DERIVED IPSCS AND SIMPLE MODEL ORGANISMS

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Inherited peripheral neuropathies (IPN) are a group of rare Mendelian diseases affecting the motor and/or sensory neurons of the peripheral nervous system. The key pathophysiological hallmark of IPN is length-dependent axonal degeneration beginning with the longest nerves. Patients present with wasting and weakness of distal limb muscles resulting in lifelong chronic disability. Currently there is no cure. Since up to 40% of IPN families remain genetically unsolved after excluding genome-wide gene coding mutations, our focus has been to screen unsolved IPN families for structural variation (SV) mutations. Using whole genome sequencing we have identified SVs causing distal hereditary motor neuropathy (DHMN1) and X-Linked Charcot Marie Tooth disease (CMTX3). However, the underlying pathogenic mechanisms of how the SV events cause degeneration of peripheral nerves in these families remains poorly understood. Our use of iPSC-derived disease relevant neural tissues has facilitated unravelling the precise molecular events resulting from the genomic re-arrangements in the respective families. We have shown the DHMN1 1.35 Mb complex insertion creates a novel gene-intergenic fusion transcript (UBE3C-IF) involving ubiquitin E3 ligase UBE3C. The presence of this aberrant transcript down regulates wild-type UBE3C protein expression in DHMN iPSC-derived motor neurons (MN). Overexpression of the UBE3C-IF transcript in C. elegans causes synaptic transmission deficits and heat stress susceptibility. The CMTX3 78-kb insertion results in temporally restricted down regulation of SOX3 in CMTX3 iPSC-derived neuroepithelial cells. SOX3 is a crucial developmental transcription factor and nearest gene to the SV breakpoint. Our results have expanded the spectrum of known disease mechanisms causing IPN and provided a disease relevant pre-clinical model for future therapy development. In addition, we have shown the growing importance of recognising SVs are an impactful class of mutation to consider in genetically undiagnosed IPN families.

Keywords: motor neuron degeneration, inherited peripheral neuropathies, transcriptome

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PHOX2B-PARMS PERTURB HINDBRAIN PATTERNING IN HUMAN PLURIPOTENT STEM CELL-DERIVED BRAINSTEM ORGANOIDS OF CONGENITAL CENTRAL HYPOVENTILATION SYNDROME

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Congenital central hypoventilation syndrome (CCHS) is caused by the loss of retrotrapezoid nucleus (RTN) neurons in the brainstem, leading to an impaired ventilatory response to hypercarbia. PHOX2B-polyalanine repeat mutations (PHOX2B-PARMs) are associated with CCHS and it often occurs with Hirschsprung disease (HSCR) which affects the enteric nervous system (ENS) of the colon. However, it is still unclear how PHOX2B-PARMs contribute to the pathogenesis of CCHS and HSCR and the underlying disease mechanisms. In this study, using human pluripotent stem cells (hPSCs), we generated brainstem (HBSO) and cerebral (HCO) organoids, which contain hypercapnia-responsive neurons and resemble the RTN-respiratory center. HCOs and HBSOs derived from PHOX2B-PARM mutant hPSCs lacked PHOX2B+V-GLUT2+ RTN neurons, recapitulating the CCHS phenotypes. Single-cell transcriptomic analyses of the organoids further revealed that PHOX2B+7Ala PARM interrupts the progenitor-to-neuronal transition of PHOX2B-expressing neurons and also the differentiation of other hindbrain neurons. Dysregulations of Hedgehog pathway and HOX genes were observed in the neurons expressing PHOX2B-7Ala, suggesting that PHOX2B-PARMs disrupt the pattern specification process. A CCHS-HSCR hPSC line carrying a PHOX2B+7Ala PARM displayed severe defects in the production of PHOX2B+ glutamatergic CNS neurons and dopaminergic ENS neurons. The correction of PARM could rescue most of the CCHS-associated CNS phenotypes, but only some of the HSCR-associated ENS defects. This implies the clinical phenotypes associated with CCHS-HSCR are likely contributed by the sensitizing genetic background of the patients. In sum, using the hPSC-derived brain organoid models, we defined the molecular mechanisms underlying the detrimental effects of PHOX2B-PARMs in hindbrain development and demonstrated the different vulnerability of the CNS and ENS neurons to the PHOX2B-PARMs.

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Keywords: PHOX2B-polyalanine repeat mutations, Congenital central hypoventilation syndrome, Human brainstem organoids

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ZIKA VIRUS INFECTION WORSEN THE PATHOLOGICAL PHENOTYPES IN NEURAL PROGENITORS DERIVED FROM HUNTINGTON'S DISEASE IPSC

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Zika virus (ZIKV) preferentially infects human neural progenitor cells (hNPCs) and causes microcephaly in neonatal. However, mostly ZIKV studies focus on the healthy case, the impact of ZIKV infection in neurodegenerative disease, such as Huntington's disease (HD) has been largely ignored. The progresses of HD could perturb by not only mutant huntingtin (muHTT) genetic contributions but also by the environmental factors. We infer the cross interaction of endogenous genetic mutations and exogenous viral protein make the disease phenotypes more sever. In the present study, we demonstrated that HD-NPCs were more susceptible to ZIKV and exhibited a higher DNA damage and apoptotic cell death which could cause from the impairment of Non-homologous end joining (NHEJ) DNA repair pathway through Ku70 downregulation. In addition, ZIKV enhanced the muHTT expression causing muHTT-induced genotoxic stress and increased the interaction between muHTT and Ku70 which would promote the ubiquitination of Ku70 protein and further disrupt the integrity of NHEJ DNA repair complex and then leads to impairment of the DNA repair activity. These finding support our hypothesis that ZIKV infection deteriorated HD pathological changes at neurodevelopmental process and could further influence the progress of Huntington's disease at the presymptomatic stage.

Keywords: HUNTINGTON'S DISEASE, IPSCs, ZIKA VIRUS

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MODELING PTSD USING AN AUTOMATED, HIGH-THROUGHPUT PLATFORM FOR STUDYING HUMAN DISEASE

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Modeling complex human diseases in vitro has long been hindered by the inability to analyze large numbers of patient samples in parallel. The intrinsic line-to-line variability in stem cells exacerbates the technical challenges of defining phenotypes in large-scale studies, highlighting the need for inter- and intra-batch control. Variables such as incubation times, seeding densities, and cell handling all impact any given experiment and can swamp true signals with experimental noise, especially in biologically heterogeneous cohorts. To overcome these challenges, we have developed robust, dynamic, high-throughput automated cell culture pipelines as part of the NYSCF Global Stem Cell Array®. This platform can differentiate large batches of cell lines (tens to hundreds per batch), with multiple batches running in parallel. Experiments can span a variety of plate formats from large (12/24 well) to small (96/384). The platform can run a variety of protocols, including media-based protocols and lentiviral or piggyBac-driven differentiations, resulting in a range of downstream cell types, including astrocytes, glutamatergic/GABAergic neurons, hepatocyte-like cells, and cardiomyocytes, along with 3D neural and pancreatic organoids. Here we present our recent work establishing the first hiPSC models of post-traumatic stress disorder (PTSD) and examining the transcriptional response to stress mediators in hiPSC-glutamatergic neurons from a well-controlled, entirely combat-exposed cohort. We found PTSD-specific glucocorticoid-induced transcriptomic changes with pronounced glucocorticoid hypersensitivity in human neurons. We also revealed a co-regulated network of transcription factors with shared effector regulation capable of mediating glucocorticoid hyper-responsiveness in PTSD. These findings underscore the power of iPSC-derived NGN2-neurons as a model for mechanistic investigations of PTSD and of our automated platform for defining robust disease features across patient cohorts. We are thus applying automated platform to population-scale disease modeling toward therapeutic discovery across various disease areas, including diabetes, rare diseases, and neurological conditions such as autism spectrum disorder, schizophrenia, Alzheimer's, and Parkinson's Disease.

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Keywords: Post Traumatic Stress Disorder, Automation, Neuropsychiatric

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MULTI-LEVEL 3D GENOME REORGANIZATION DURING NEURONAL DIFFERENTIATION IN HUMAN RETINAL ORGANOIDS

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Retinal development involves orderly generation of multiple cell types, with distinct gene expression patterns resulting from interaction of specific transcription factors with genomic DNA. Retinal organoids derived from human pluripotent stem cells recapitulate many developmental features, as revealed by bulk and single-cell RNA-seq data. To elucidate the spatiotemporal dynamics of the chromatin landscape and its relationship with coordinated gene expression changes during the specification of distinct neuronal cell types, we performed high-resolution in situ Hi-C analysis of human retinal organoids in chronological order during differentiation. We demonstrate progressive changes in multi-level chromatin structures that can be correlated to specific stages of retinal development. By integrating these data with corresponding transcriptomes, we show that genomic regions shifting towards A compartment are enriched for protein-coding retinal genes and higher expression whereas regions shifting towards B compartment include non-coding genes and exhibit low expression. Genes associated with retinal development are enriched near lost boundaries of topologically associated domains (TADs).

Moreover, higher-order assemblages of TADs (i.e., "TAD cliques") tend to localize in the active chromatin regions and are enriched for expressed transcription factors. Additionally, regions gaining chromatin loops are enriched for genes related to neuronal differentiation. We suggest that changes in chromatin organization fine-tune the commitment of distinct cell lineages in the retina by influencing the expression of specific transcription factors at different time periods. Our studies provide new insights into genomic remodeling and gene regulation during cell type specification in the human retina and highlight the paradigm of using organoids as a model for investigating human development.

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Keywords: Retinal Organoids, 3D Genome, Human Retina Development

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MODELLING NEURODEGENERATION USING A HUMAN ISOGENIC SYSTEM: A NEXT GENERATION APPROACH TO STUDY FRONTOTEMPORAL DEMENTIA AND AMYOTROPHIC LATERAL SCLEROSIS

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Development of therapies to treat neurodegenerative diseases is hampered because less than 10% of findings derived from preclinical animal models can be translated to humans. Patient-derived induced pluripotent stem cells (iPSCs) enable generation of in vitro models that can recapitulate human disease phenotypes. However, conventional human iPSC differentiation protocols are often lengthy, inconsistent, and difficult to scale. The lack of genetically matched controls for patient-derived models further complicates the investigation of disease phenotypes. bit.bio has developed opti-ox™, a robust iPSC reprogramming technology that overcomes these limitations and enables generation of mature cell types and isogenic disease models. Our objective was to generate disease models for frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) for use with isogenic, wild type ioGlutamatergic Neurons to improve screening specificity and accelerate drug discovery for these neurodegenerative disorders. We used CRISPR/Cas-9 gene editing to introduce the disease-relevant mutation M337V in the TARDBP gene, encoding TDP-43, and the mutations P301S or N279K in the MAPT gene, encoding Tau. During the pathogenesis of FTD and ALS, mutant TDP-43 and Tau proteins are prone to misfolding, aggregation and mislocalisation, and have been reported to affect a range of neuronal subtypes, including cortical glutamatergic neurons. Characterisation of the FTD and ALS disease models showed that the expression profiles of TDP-43, Tau, and pan-neuronal and glutamatergic markers are highly similar to the ioGlutamatergic Neurons. We demonstrate characterisation of these disease models and the genetically matched control to show the differences in their transcriptome, neuronal activity and proteinopathy. Using opti-ox technology to produce hiPSC-derived isogenic disease models provides physiologically-relevant, robust, standardised tools for neurodegenerative research and drug discovery.

Keywords: Disease Model, Neuroscience, Reprogramming

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GLIA-INDUCED NEURONAL SYNAPTIC MATURATION IS ASSOCIATED WITH CHANGES IN EXPRESSION OF GENES IN TGFB- AND CHOLESTEROL BIOSYNTHESIS PATHWAYS

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The maturation of neurons and the development of synapses – while emblematic of neurons – also rely on interactions with astrocytes and other glia. Although glial cells are necessary for the functional maturation of neurons, many gaps remain in our understanding of the specific cellular and molecular programs that mediate these processes. We recently found that the presence of astrocytes enhanced synaptic gene-expression programs in neurons when in physical contact with astrocytes. These changes in neurons correlated with increased expression in the cocultured glia of genes that encode synaptic cell adhesion molecules, which were greatly enhanced in the glia in coculture. Here, to further investigate the molecular pathways underlying the glia-induced neuronal maturation, we performed RNA sequencing (RNAseq) of human pluripotent stem cell (hPSC)-derived excitatory neurons that were cocultured with mouse glial cells and in monocultures at multiple stages (from 6 hours to 28 days) during differentiations. We reasoned that cellular responses to regulatory interactions between neurons and glia would be in part mediated through changes in cell states that could be detected in gene expression differences between the culture conditions. We found that the changes in synaptic gene programs in neurons were preceded by induction of TGFB pathway genes in neurons within only hours (6 hours) after contact with astrocytes. This was quickly (24 hours) followed by a downregulation of genes in the cholesterol biosynthesis and in a reciprocal induction of cholesterol biosynthesis genes in astrocytes in coculture. The most significantly

induced genes in astrocytes included Apoe and Clu (ApoJ) which encode for lipoproteins that shuttle cholesterol from astrocytes to neurons. Overall, our findings suggest that astrocyte-expressed genes with synaptic functions are associated with stronger expression of synaptic genetic programs in neurons and suggest a potential role for astrocyte-neuron interactions in schizophrenia, along with a central role of the TGFB and cholesterol biosynthesis pathway in regulating these programs.

Keywords: neuron, glial cells, cholesterol

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HUMAN INDUCED SENSORY NEURONS ADOPT ADULT MORPHOLOGY IN CO-CULTURE WITH RODENT SATELLITE GLIA

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Chemotherapy Induced Peripheral Neuropathy (CIPN) is a neurodegenerative disorder impacting the distal axons of peripheral sensory nerves, leading to debilitating symptoms including allodynia, paresthesia, temperature sensitivity, and numbness. More than half of patients receiving chemotherapy develop CIPN, and in severe cases, symptoms lead to discontinuation of treatment and may persist thereafter. Recently, induced sensory neurons (iSNs) generated from pluripotent stem cells have been used to model human CIPN in vitro to better understand its pathogenesis, identify molecular targets of intervention, and test potential therapeutics. However, current differentiation protocols produce sensory neurons with an immature phenotype, mimicking the morphology and physiology of embryonic sensory neurons. Since CIPN and other peripheral neuropathies occur postnatally, it is crucial to develop a differentiation protocol that will produce sensory neurons with adult functionality. Current evidence suggests that peripheral glial cells play an integral role in the development of mature sensory neurons in vivo, leading to the development of their hallmark pseudo-unipolar morphology. Here, we have tested the hypothesis that differentiation of induced human neural crest stem cells in co-culture with rodent E15 dorsal-root peripheral glia (rDRG), will quickly and efficiently produce mature hiSNs. Our results indicate that iSNs differentiated in co-culture with rDRGs transition to pseudounipolar morphology significantly more frequently than iSNs differentiated alone. Our data suggest that this transition requires physical contact between rDRG satellite glial cells and developing iSNs. Pseudounipolar morphology is an essential component to sensory neuron cell signaling, allowing for efficient relay of information from the peripheral to the central nervous system. These new methods for generating iSNs with adult morphology will allow for targeted studies of degenerating peripheral axons, as these are the projections that are specifically impacted in CIPN and in other neuropathies. Moreover, these studies provide a way to identify the molecular mechanisms underlying pseudounipolarization, and could eventually provide new methods for generating adult iSNs without the need for glial co-culture.

Keywords: induced Sensory Neurons, CIPN, Neurodevelopment

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INTERROGATION OF NEURAL DIFFERENTIATION DEFICITS IN CEREBRAL ORGANOIDS DERIVED FROM BIPOLAR DISORDER PATIENTS

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Bipolar Disorder (BD) is a psychiatric disorder characterized by extreme fluctuations in mood, with a lifetime prevalence rate of more than 1% worldwide regardless of ethnicity or socioeconomic background. New methods to study this disease in vitro are needed as human brain tissue from patients are not readily accessible and animal models are not optimal for studying uniquely human diseases that involve neuroanatomical features that are significantly different in humans. A promising experimental model is patient-specific 3D cerebral organoids – self-organizing tissues derived from human induced pluripotent stem cells (iPSCs) – which are able to emulate key aspects of brain development, while displaying many important functional features and neuroanatomical structures present within a developing human fetal brain. Through analysis of iPSC-derived cerebral organoids from bipolar patients stained with a number of neuronal and cortical plate markers, we found that the area and perimeter of neural rosettes was decreased in bipolar organoids compared to healthy control organoids. Analysis of neuronal distribution in the organoids showed that there were relatively fewer MAP2+ neurons in the preplate layer of bipolar organoids. Ventricular zone thickness was greater in bipolar organoids, while the preplate layer thickness was greater in healthy control organoids. The decreased ratio of MAP2+ neurons in the preplate layer and decreased preplate layer thickness in bipolar organoids is suggestive of aberrant neural differentiation trajectories in BD organoids. Characterization of neural differentiation deficits in organoids derived from BD patients will provide a platform for investigation of mechanisms of pharmacological agents used in treatment of BD. Studies of BD using cerebral organoid models are limited, so this research can contribute to a better understanding of the neurobiology of BD and provide a path for personalized medicine approaches using patient-specific cerebral organoids.

Keywords: Bipolar Disorder, Cerebral Organoid, Neural Differentiation

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MECHANISMS AND CONSEQUENCES OF POST-MITOTIC NEURONAL SENESCENCE IN ALZHEIMER'S DISEASE

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The concept of senescence as a phenomenon limited to proliferating cells has been challenged by growing evidence of senescence-like features in terminally differentiated cells, including neurons. The persistence of senescent cells late in life is associated with tissue dysfunction and increased risk of age-related disease. We found that Alzheimer's disease (AD) brains have significantly higher proportions of neurons that express senescence markers, and their distribution indicates bystander effects. AD patient-derived directly induced neurons (iNs) exhibit strong transcriptomic, epigenetic, and molecular biomarker signatures that illuminate a specific human neuronal senescence-like state. AD iN single-cell transcriptomics revealed that senescent-like neurons face oncogenic challenges, metabolic dysfunction, and display a proinflammatory signature. Integrative profiling of the inflammatory secretome of AD iNs and patient cerebral spinal fluid revealed a neuronal senescence-associated-secretory-phenotype (SASP), that could trigger astrogliosis in human astrocytes. A neuronal SASP thus represents a late-life proinflammatory gain-of-function that could trigger a chronic paracrine response in reactive cell types in the aged human brain. The mechanisms promoting a SASP in senescent cells has been largely elucidated in proliferating cells, and little is known how such a response could be mediated in aged human neurons. Here, we provide an evaluation of neuronal SASP mechanisms in aged human neurons from AD and healthy age matched control patients, and strategies to silence or attenuate this response. Although we show that senescent neurons can be removed by a senolytic approach, these results point to the possibility of a functional senomorphic that could be employed to silence a neuronal SASP while leaving the cells intact.

Keywords: Senescence, Alzheimer's Disease, Induced Neurons

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MODELING EARLY ONSET EPILEPSY WITH IPSCS DERIVED NEURONS

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Epilepsy is one of the most prevalent neurological disorders. Pathogenic bi-allelic variants in the SLC13A5 gene inactivate the sodium citrate transporter (NaCT) causing neonatal epilepsy refractory to treatment. Early onset of seizures impairs brain development, leading to detrimental cognitive and behavioral impairments that worsen over time. NaCT is expressed in the brain, and SLC13A5 patients have elevated citrate levels, however, the role of variants and their impact on citrate levels and early seizures is unknown. Induced pluripotent stem cells (iPSCs) are an excellent model system to study cellular and molecular mechanisms in the brain because they capture the genetic and epigenetic background of each patient and enable the generation of brain cells directly from patients. This system allows the analysis of the basic signaling mechanisms and cell-type specific measurements of gene and protein expression and function. In collaboration with the Tess Foundation, we generated seven iPSC lines from SLC13A5 variants obtained from affected probands and unaffected parents and corrected the mutations using the CRISPR/Cas9 system. The mutants and corrected iPSCs were differentiated into neurons using a 3D neuronal differentiation method and the differences in action potentials were measured with and without citrate using multielectrode array (MEA) technology. Our data shows that SLC13A5 iPSCs derived cortical neurons from different variants undergo normal neuronal differentiation but display differences in action potentials. Neurons harvesting mutant SLC13A5 display premature bursting, delay in neuronal synchronization and differences in activity and synchrony recovery after treatment with citrate. These studies provide an innovative functional human model to test prospective therapeutics for safety and efficacy, including AAV gene therapy for the functional rescue of SLC13A5 function to prevent epilepsy in children. Furthermore, this study indicates precision therapeutics can benefit a significant number of people who suffers from epilepsy.

Keywords: EPILEPSY, NEURONS, CITRATE TRANSPORT

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ELECTROPHYSIOLOGICAL NOCICEPTIVE AND ITCH ASSESSMENT OF HUMAN IPSC-DERIVED SENSORY NEURONS USING MULTI-ELECTRODE ARRAY (MEA) AND THEIR POTENTIAL AS AN ALTERNATIVE MODEL FOR DORSAL ROOT GANGLIA

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Sensory neurons are afferent neurons in sensory systems that transmit stimuli to the central nervous system as electrical signals. Primary afferent neurons that are affected by non-noxious and noxious stimuli are present in the dorsal root ganglia (DRG),

and the DRG sensory neurons are used as an in vitro model of the nociceptive response. However, DRG derived from mouse or rat give a low yield of neurons, and they are difficult to culture. In addition, DRG from rodents sometimes show a different response from human neurons. To help alleviate this problem, we differentiated hiPSCs into sensory neurons. Previously, we measured the expression of sensory neuron-related genes and drug responses induced by noxious stimuli. In this study, we compare a mixed population of iPSC-derived cortical neurons to these sensory neurons for responses to capsaicin, menthol, and temperature by Multi-Electrode Array (MEA) analysis to confirm that the responses are sensory neuron specific. iPSC-derived cortical neurons did not respond to capsaicin and menthol at the same concentration as iPSC-derived sensory neurons showed a response to these stimuli. When temperature increased from 37 °C to 46 °C the firing rate of iPSC-derived sensory neurons gradually increased. In contrast, iPSC-derived cortical neuron firing rates gradually decreased. These data suggest that the sensory response is specific to sensory neurons. We measured the response of iPSC-derived sensory neurons to histamine and its receptor inhibitor pyrilamine. in the presence or absence of pyrilamine. Increased levels of histamine caused an increased firing rate, which was blocked by pyrilamine. In another experiment, iPSC-derived sensory neurons responded to chloroquine, an itch-inducing compound, and they reached their firing rate peak at 5-10 min, whereas histamine-evoked activity reached its peak at 25-30 min. These data suggest iPSC-derived sensory neurons respond to itch stimuli. In conclusion, we have demonstrated our iPSC-derived sensory neurons show sensory neuron-specific reactions and may be able to substitute for rodent-derived DRG in in vitro models of sensory neurons. We believe that these iPSC-derived sensory neurons will be valuable for drug discovery research.

Funding Source: This research was funded by REPROCELL, Yokohama, Japan.

Keywords: Disease modeling, Neurons, Drug Discovery

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MODELING FRAGILE X SYNDROME WITH HUMAN HIPPOCAMPAL ORGANOIDS

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Fragile X syndrome (FXS) is a leading genetic cause of intellectual disability and autism spectrum disorder. It is an X-linked dominant disorder, typically caused by CGG trinucleotide repeat expansion within the 5' UTR of the FMR1 gene that leads to a complete loss of its encoding protein – Fragile X Messenger Ribonucleoprotein (FMRP). FMRP is a selective RNA-binding protein forming a messenger ribonucleoprotein complex with polyribosomes in the regulation of protein synthesis. Years of studies have showed that hippocampus, one of the brain regions that plays critical roles in mood and cognition, is significantly impacted by the loss of FMRP. It is enlarged in individuals with FXS at younger ages, suggesting the presence of an atypical developmental trajectory. In Fmr1 KO mouse, global protein synthesis in the hippocampus is elevated by 15–20%, and electrophysiological studies also revealed prolonged epileptiform discharges as well as impaired synaptic plasticity in the hippocampus. Despite the remarkable findings researchers have made over the past decades, how loss of FMRP affects human hippocampus development is still largely unknown. To address this question, we developed human hippocampal organoids (HOs) from iPSCs. Interestingly, we observed increased NPC proliferation and decreased generation of PROX1+ dentate gyrus granule cells in FXS HOs. To determine how loss of FMRP disrupts the trajectory of gene regulatory network, we profiled the dynamic of transcriptome of control and FXS HOs at different developmental stages using bulk RNA-seq. We found that genes involved in neuron development and synaptic transmission were upregulated in FXS during hippocampus development. To better understand the specificity and complexity of the effect of FMRP deficiency on human hippocampus development, we performed single-cell RNA-seq on day 150 HOs and found that both cell type composition and developmental trajectory were altered in FXS. Lastly, using eCLIP-seq, we identified a number of mRNAs that are specific targets to FMRP in the hippocampus. Together, our study not only delineated the molecular and cellular impacts of FMRP deficiency in hippocampus development, but also identified hippocampus-specific mRNA targets of FMRP, which have the potential to serve as therapeutic targets for FXS and autism in general.

Keywords: fragile X syndrome, hippocampal organoids, neurodevelopment

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EARLY NEURODEVELOPMENT AND CYTOARCHITECTURE IS ALTERED IN TUBEROUS SCLEROSIS

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Tuberous Sclerosis Complex (TSC) is a debilitating developmental disorder characterized by a variety of clinical manifestations. While benign tumors in the lungs, kidney, and brain are all hallmarks of the disease, often the most severe symptoms of TSC are neurological, including seizures, autism, psychiatric disorders, and intellectual disabilities. TSC is caused by a heterozygous loss

of function mutation in the TSC1 or TSC2 genes, which encode the hamartin/tuberin proteins respectively. Hamartin/tuberin function as a heterodimer that negatively regulates mechanistic Target of Rapamycin Complex 1 (mTORC1). While TSC neurological phenotypes are well-documented, it is not yet known how early in neural development TSC1/2-mutant cells diverge from the typical developmental trajectory, and whether such phenotypes are seen in the heterozygous-mutant populations comprising the majority of cells in patients. To examine early neurodevelopmental phenotypes, we utilized TSC patient-derived induced pluripotent stem cells (iPSCs) with a heterozygous microdeletion mutation in TSC2. Within the field, it is debated whether second hits are required. To model this state, CRISPR was used to create a similar deletion mutation in the other TSC2 allele, producing a homozygous mutant line. The heterozygous mutant was also corrected to wild type. This isogenic series was compared to another allelic series with TSC2 deleted. Using immunofluorescent microscopy and flow cytometry, we observed aberrant early neurodevelopment in both sets of TSC2 mutant iPSCs. Homozygous mutant neural progenitors exhibit altered behavior as in vitro differentiation proceeds, including changes in multicellular structures within the first 10 days with misexpression of key transcription factors associated with lineage commitment. Collectively, these data suggest that mutation/loss of TSC2 has early effects on gene expression in proper neural development. Indeed, our preliminary studies have found that DNA methylation is altered with some key genes in neurodevelopment being hypermethylated in the wild type cells and hypomethylated in TSC2 mutant cells. Understanding precisely when development is disrupted in TSC1/2- mutant brain will be essential to tailoring treatment and determining whether prenatal treatment should be pursued.

Keywords: Tuberous Sclerosis, Neurodevelopment, stem cell

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VASCULARISATION OF HUMAN STEM CELL DERIVED 3D RETINAL ORGANOIDS

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Retinal degeneration is the leading cause of irreversible blindness in current population. Sudden photoreceptor cell loss initiates a cascade of pathways causing structural and functional disintegration of the retina. To investigate retinal diseases and explore respective targeted therapies, exclusive human-based models are cardinal. Retinal organoids (RO) are 3D in vitro structures that attempt to recapitulate the development, structure, and physiological functioning of in vivo human retina. The RO field has come a long way meticulously regulating features of organoid protocol like structural complexity, scalability and reproducibility. However, the lack of efficient distribution of oxygen and nutrients result in loss of functional output at an early stage causing premature cell death and aggressive necrosis, leading to growth arrest. Self-organizing vascular systems within the developing organoids have shown to combat above mentioned drawbacks. In this project we make use of an efficient human induced pluripotent stem cells (hiPSC)-derived inducible endothelial cell differentiation protocol that gives rise to an endogenous, self-organising vascular system sustaining the long-term functioning of the RO. Using these hiPSCs, we successfully generated RO and tracked their development over the course of 32 weeks. hiPSC-derived endothelial cells were successfully characterised followed by integration into the organoid protocol to generate vasculature-like network. The aim here is to understand and explore the impact of this vascularization in the growing organoid. This incorporation indicated an improvement in the expanding size of the organoid when compared to non-vascularised ones. At later time points, the vasculature also reduced the apoptotic cell rate thereby improving the stability of the 3D structure. Our results also indicate increased proliferation rate of early photoreceptors. How it preserves the functionality of ganglion cells is ongoing. This significant step in the RO field will therefore help in generating long-term viable cultures that will help investigating age-related retinal degeneration. Lastly, increment in photoreceptor cell number invites the opportunity to re-access patient-targeted cell transplantation therapy for curing irreversible blindness.

Keywords: hiPSC, Retinal organoid, Vascularization

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PHYSIOXIA CONDITION GOVERN NEURAL CELL FATE IN HUMAN BRAIN ORGANOIDS THROUGH MODULATION OF CELL METABOLISM AND CELL DEATH PATHWAYS

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Low oxygen condition (5% O2) referred as “physioxia” is typical for neural stem cells endogenous niches. On the other hand, the advent of brain organoids (BO) technology enabled to obtain the only existing 3D biomimetic in vitro model for early human neurodevelopment. Physioxia conditions are crucial to mimic natural niche of brain development, however most in vitro studies with BO are conducted in atmospheric (21%) level of oxygen. In this study we focus on the influence of physioxia on the metabolic and cell death pathways with regard to the ability for neural differentiation in human BO. Up to date such studies in this model have not been reported. BO grown 44-days (44D) in different oxygen conditions (21% and 5% O2) were used to decipher the role of the physioxia on neuronal differentiation and to uncover the molecular mechanism behind it. To reveal a modulatory mechanism of physioxia on neuronal differentiation and cell metabolism, analysis of RNA-seq data was conducted. Gene ontology enrichment analysis of transcriptomics data showed the upregulation of pathways involved in response to oxygen level, NAD and carbohydrate metabolism, whereas downregulated were processes including neuronal differentiation, dorsal/ventral pattern formation, apoptosis and positive regulation of glial cell proliferation in BO grown 44D in physioxia. Investigation of network prediction between genes with known functions revealed interactions between neuronal and glial genes as well as those involved in cell metabolism and apoptosis pathways. Further RT-qPCR gene expression analysis in physioxia conditions showed decreased expression of: EMX1, SYP (mature neurons); GFAP, ALDH1L1 (glial cells); MT-ATP6, MT-CO1, MT-ND5 (OXPHOS genes) and increased level of MAP2, DCX (early neuronal markers) and LDHA, HK2, PGK1 (glycolytic genes). This suggests that physioxia at the stage of 44D BO prevails glycolysis over oxidative phosphory-

lation and promotes early neurogenesis, while suppressing gliogenesis and further maturation of neurons. This is accompanied with significant decrease in expression of BNIP3, BAX, BCL2L1, BAK1, BAD (apoptosis), which may indicate neuroprotective role of physioxia. This study is a first insight into a modulatory role of physioxia on neural lineage specification in brain organoid model.

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Keywords: Brain Organoids, Physiological Oxygen Condition, Neural Differentiation

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DISEASE MODELING AND INVESTIGATION OF NMDA RECEPTOR MODULATORS IN NOVEL 2D AND 3D HUMAN IPSC-DERIVED NEURAL CELL SYSTEMS

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N-Methyl-D-aspartate receptors (NMDARs) in the brain are essential for information processing and learning. Malfunctions of NMDARs are associated with pathophysiological processes in neurodegeneration and diseases like Alzheimer's, Parkinson's, and drug addiction. A promising therapeutic approach to these diseases is the development of selective NMDAR modulators. Ifenprodil and several derivatives are known to be potent NMDAR selective antagonists. Using human induced pluripotent stem cells (hiPSCs) combined with electrophysiological techniques, we developed 2D and 3D screening systems for NMDAR modulators and their effect on human neurons.

Keywords: NMDAR, glutamatergic neurons, pharmacology

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ENABLING THE LONG-TERM MAINTENANCE OF ADHERENT NEURAL CELLS CULTURES IN A STABLE MICROENVIRONMENT VIA A MULTICOMPONENT PERFUSION CIRCUIT PLATFORM

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Advancements in synthetic biology for the development of cells differentiated from stem cell sources, coupled with recognition of stable microenvironments as critical for the maturation of these cultures, has created a need for a system capable of long-term maintenance and monitoring of adherent cultures. Current methods require cells to be removed from stable environments, such as incubators, and have media changes which have been shown to increase markers of stress in cell cultures and disrupt function. Here we showcase research into the development of a perfusion circuit system which is capable of maintaining adherent cell cultures intervention free for extended periods of time. System viability was tested over 4 weeks by using functioning neural progenitor cells derived from human induced pluripotent stem cells. By plating these cells on a high-density multielectrode array (MaxWell Biosystems) along with traditional transparent dishes, the functional activity of these cells could also be monitored in real time. The system was perfused with Brainphys media (STEMCELL Technologies; 0.25% Penicillin/Streptomycin) and gas (air and 5% CO2) and then monitored. Results strongly support the viability of this approach, with an efficient gas exchange of 73.3±2% dissolved oxygen, stable pH of 7.4±0.4, and temperature of 37±0.5℃. Likewise, microscopy assessment of neural cells revealed the maintenance of dendrites and axons continued through the 4-week assessment. Electrophysiological assessment also revealed that neural activity was able to be maintained, even when cells were subjected to high density stimulation and recording through the closed-loop DishBrain pong environment for up to 16 hours. This represents that viability was maintained over significantly longer stimulatory and recording periods, as previously cell degradation would begin on sessions that exceeded 2 hours. While further research is still required, these results support the viability of this approach in the long-term maintenance of adherent cells with minimal intervention compared to regular methods. Ultimately it is proposed that future developments will be helpful in studying both development of these cell cultures and for long-term testing of pharmacological agents or disease models.

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Keywords: cortical neurons, cell culture maintenance, perfusion circuit

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DERIVATION OF HUMAN FETAL-DERIVED EARLY NEUROEPITHELIAL PROGENITORS MIRRORING THE DEVELOPING HUMAN BRAIN

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The isolation of long-term self-renewing neural stem cells (NSC) from human primary tissue mirroring the early neurodevelopment is a long-standing aim. Here, we present the stabilization of neurulation-stage stem cells from human fetal brain tissue employing a chemically defined culture medium. We obtained highly proliferative, homogenous neural precursor cells, designated as fNPCs, being able to maintain NSC properties and normal karyotype > 50 passages. fNPCs can grow monoclonally and exhibit a naïve, non-polarized morphology expressing early neuroepithelial markers including SOX1, PAX6, NESTIN, SOX2 and ZO-1 judged by immunofluorescence and RT-qPCR. Flow cytometry analysis revealed the presence of bona fide NSC surface markers CD133, CXCR4 and PSA-NCAM in >90% of fNPCs. In vitro differentiation of fNPCs yields progenies of central and peripheral lineages corroborating a naïve state of neuroepithelial multipotency with high neurodevelopmental potential. In vitro and in vivo, fNPCs exhibit strong neurogenic potential and show astroglial differentiation capacity in vitro. Electrophysiological analyses of fNPC-derived neurons revealed spontaneous action potentials and evident synapse formation is demonstrated by immunostainings and ultrastructural examination. Systematic comparative analysis of single cell RNA sequencing data confirmed a predicted cell type of early neuroepithelial stage and developmental age correlating with 5.5 to 6 weeks post conception as judged by reference atlases. In particular, fNPCs express not only neuroepithelial (TJP1, HES1), but also radial glia (VIM, CDH) and neural plate border genes (SOX3, ZIC1). Furthermore, patterning towards ventral regional identity is indicated by upregulated NKX6-1, SFRP2 and downregulated NKX2.2. Together, our data suggest a novel early NSC population with broad central and peripheral nervous system differentiation capacity and virtually unlimited proliferation. fNPCs will be instrumental to elucidate early neurodevelopmental processes and provide a novel source for cell replacement and drug screening applications.

Keywords: fetal neural progenitors, early neurodevelopment, single cell RNA sequencing

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ASSESSMENT OF MICROGLIAL ACTIVITY IN AN INDUCED PLURIPOTENT STEM CELL-DERIVED NEURAL ORGANOID ON SYNTHETIC HYDROGELS TO STUDY NEUROINFLAMMATION

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Cerebral organoids derived from induced pluripotent stem cells (iPSCs) offer a promising platform for modeling neuroinflammation. A critical component of neuroinflammation modeling is recapitulation of the physiological environment, including responses to a variety of insults from disease states, infection or injury. The interplay between neurons, microglia, endothelial cells, and other cell types following brain insults gives rise to an immune response and release of inflammatory mediators. There is a critical need to model this dynamic interplay in vitro given the difficulties of interrogating neuroinflammation in vivo. To this end, Stem Pharm has used its proprietary hydrogel technology as the basis to build a robust, human iPSC-derived neural organoid model for studying neuroinflammation. The neural organoids are formed in 96-well plates and ready for screening in as early as 21 days. Single-cell transcriptional analysis indicates that the organoids are comprised of diverse cell types, including neuronal subtypes, astrocytes, microglia, and endothelial cells. Synchronous calcium oscillations were recorded in organoids that were cultured for >40 days, consistent with physiologic establishment of signaling between neurons. Microglia integrate into the organoid at physiologic levels (approximately 5% of total cell composition), distribute throughout the organoid structure, portray ramified morphology at homeostasis, and respond appropriately to pro- and anti-inflammatory stimulants. We have investigated the effect of lipopolysaccharide (LPS) and interferon gamma (IFN-γ), amyloid beta (Aβ), and isolated apoptotic neurons. Aβ stimulation of organoids with and without microglia demonstrates the necessary role of microglia in components of the proinflammatory response.

The addition of apoptotic neurons into the organoids increased the phagocytic marker CD-68 but not the proinflammatory marker TNF-α, suggesting microglia in our organoids perform basal phagocytic activity without provoking a TNF- α transcriptional response. Collectively, these data suggest that Stem Pharm's neural organoid platform is a robust platform for studying neuroinflammation in vitro and demonstrates a promising tool for prediction of translation between preclinical and clinical outcomes.

Funding Source: This work has been supported by NIH NIEHS SBIR grants 1R43ES029898-01A1, 1R43ES029897-01, and 1R43ES029897-01S1 to support participation in the NIH I Corps program.

Keywords: Cerebral organoids, Hydrogels, Neuroinflammation

TOPIC: PANCREAS

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A 3D-PRINTED GELMA SCAFFOLD ASSEMBLY FOR THE CO-CULTURE OF HUMAN ENDOTHELIAL CELLS AND BETA CELLS

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A significant hallmark of Type 1 diabetes is the extensive diminution of β cell mass in the pancreas, which accounts for the impairment of glucose homeostasis. Despite the success observed with the transplantation of islets into diabetic patients (such as with the Edmonton protocol), a critical complication still persists: the transplanted islets suffer from decreased survival due to a lack of functional blood vessels and oxygen supply. As such, a more effective support system for isolated islets that better replicates the interactions between β cells and vascular endothelial cells in the Islets of Langerhans is required. To achieve this, we conducted a systematic evaluation of the compatibility of various 3D-bioprinted scaffold materials to co-culture human β cells with human umbilical vein endothelial cells (HUVECs) through the creation of different bioink formulations. Gelatine Methacryloyl (GelMA)-based scaffolds enable the attachment of HUVECs and β cells that remain viable after ten days of culture. The assembled co-culture scaffold also demonstrated functional glucose-stimulated insulin secretion from the β cells. This exploration provides a working basis for the development of a human islet delivery system, that is amenable to bioprinting, in the effort to restore insulin production in diabetes patients.

Keywords: 3D Printing, Co-Culture, Bioartificial pancreas

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MODELLING CONGENITAL HYPERINSULINISM USING PATIENT DERIVED INDUCED PLURIPOTENT STEM CELLS

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Congenital Hyperinsulinism (CHI) is a severe monogenic disorder associated with enhanced insulin secretion and persistent hypoglycaemia. Symptoms firstly appear in new-borns and infants and all current non-invasive therapeutical treatments are mostly ineffective. Moreover, multiple questions are still unanswered in CHI research, with 50% of cases remaining genetically unsolved and the molecular mechanisms underlying CHI pathophysiology not fully understood. In this study, we turned to patient derived induced pluripotent stem cells to investigate functional and developmental changes in hyperinsulinemic β cells. We firstly identified deleterious disease-causing genomic variants from a cohort of 5 CHI iPSC lines. Exome-sequencing analysis revealed mutations in key pancreatic transcription factors (HNF1A, HNF4A), in the SUR1 subunit of KATP dependent channels (ABCC8) and in a key epigenetic regulator (EP300). Then, we developed an experimental pipeline to characterise the differentiation of iPSC lines to β-like cells and to test the effects of CHI-causing mutations. We selected a homozygous mutation in the KATP dependent channel SUR1 for CRISPR-Cas9 genome editing to model the most common and severe form of this disorder. These cells are currently being investigated for transcriptional, morphological, and functional defects. Altogether, these data will provide us with a deeper understanding of the functional and developmental differences observed in CHI β-cells, will offer a valuable preclinical model to explore alternative treatments for hyperinsulinemic patients while also providing valuable insights into β-cell biology.

Keywords: Congenital Hyperinsulinism, Pancreatic Beta Cells, Disease Modelling

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A METHOD FOR THE GENERATION OF HUMAN STEM CELL-DERIVED PANCREATIC DELTA CELLS

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Cell-based therapies hold great promise in treating the increasing burden of Type 1 diabetes. These therapies require human pancreatic beta cells that can properly respond to and secrete insulin in response to glucose levels in the body. Although islet transplantation has been effective, a shortage of donor islets restricts its use in cell therapy. An alternative approach is the use of stem cell-derived beta cells (SC-β) for Type 1 diabetes treatment. However, for proper functioning within islets, endocrine cells experience both paracrine and autocrine regulation, which is not present in single cells generated using stem cells. To improve treatment efficacy to that of donor islets, SC-β cells can be combined with other stem cell-derived pancreatic endocrine cells, but this is limited by a lack of precise methods for generating these cell types. While SC-β and stem cell-derived alpha (SC-α) cells have been successfully generated, other endocrine cell

types are not yet accomplished. Here we report the first protocol for generating human stem cell derived delta cells (SC-δ) from pluripotent cell sources. Using several small molecule screening approaches, we identified small molecules that could efficiently convert human embryonic stem cells into pancreatic delta cells. The protocol results in somatostatin expression in nearly 60% of the cell population, with 17% of the cells expressing only somatostatin as monohormonal SC-δ cells. The resulting SC-δ cells expressed key delta cell-specific markers such as Hhex, Ptch1, and Pax6, and could secrete somatostatin in response to glucose challenge. Furthermore, we demonstrate the ability of these SC-delta cells to inhibit the secretion of glucagon in SC-alpha cells. The identification of these small molecules has led to the development of a novel protocol that produces functional human SC-δ cells. This not only advances our understanding of human delta cells but also provides a basis for drug screening, disease modeling, and the development of designer islets, which has the potential to refine and improve current cell therapeutic regimens.

Keywords: Type 1 Diabetes, Stem cell-derived pancreatic delta cells, cell therapy

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UNRAVELING ISLET STRESS RESPONSE THROUGH CELL-TYPE-SPECIFIC TRANSCRIPTIONAL AND CHROMATIN ANALYSIS

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Diabetes occurs when pancreatic beta cells fail to properly function or die, resulting in the onset and progression of the disease. As this occurs, the islets experience an increase in endoplasmic reticulum (ER) and inflammatory stress. This phenomenon has primarily been studied using murine models, yet this approach is limited by the species-specific differences between humans and mice. Furthermore, beta cells reside within islets of Langerhans, which are comprised of multiple cell types. However, previous studies have not explored the cell-type-specific responses to ER and inflammatory stress in the islet. Stem cell-derived islets (SC-islets) can serve as a viable model system to address this gap. Using single-cell sequencing, we have examined the cell-type-specific transcriptional and chromatin responses to ER and inflammatory stress in human islets. To simulate stress in islets, we used thapsigargin, a mixture of cytokines (IL1B+IFNG+TNFa), and Brefeldin A (BFA). Through pairwise comparisons between control and stress conditions, we found that beta, alpha, and ductal cells are more susceptible to ER and inflammatory stress than other cell types in islets. Additionally, our analysis revealed candidate genes that can modulate stress response in SC-islets. By genetically editing SC-islets using lentiviral short-hairpin and open-reading-frame constructs, we have explored how these genes impact islet function and stress response. These results provide cell-type-specific index of islet stress response, enhancing our understanding of islet health during diabetes.

Keywords: Stem cell derived islets, multiomic single cell sequencing, genetic editing

TOPIC: NO TISSUE SPECIFICITY

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THE EFFECT OF THE VARIANT MTHFR 677TT GENOTYPE AND MODULATION BY RIBOFLAVIN ON PHENOTYPE AND EPIGENETIC PROFILE IN IPSC TO VSMC DIFFERENTIATION

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The B vitamin riboflavin is required as flavin adenine dinucleotide (FAD) by methylenetetrahydrofolate reductase (MTHFR), a key enzyme that generates the folate cofactors to supply methyl groups for DNA methylation. The common MTHFR 677C➔T polymorphism reduces enzyme activity in vivo and increases risk of hypertension and cardiovascular disease. Our previous trials demonstrated that riboflavin intervention lowers blood pressure specifically in hypertensive patients with MTHFR 677TT genotype. This modulatory effect of riboflavin on blood pressure may be mediated via DNA methylation of hypertension-related genes. This study aims to determine the contribution of the MTHFR genotype to altered DNA methylation profiles at loci involved in the development of hypertension, and how riboflavin supplementation may alter the aberrant methylation profile of MTHFR 677TT cell lines. The fibroblasts from two donors with MTHFR 677CT genotype have been reprogrammed into iPSCs using non-integrating Sendai Viruses. The morphology of three iPSC lines from each donor resembled that of human embryonic stem cells, and no exogenous reprogramming factors were undetected after passage 10. The pluripotency of iPSC lines was verified both at mRNA and protein levels by qRT-PCR and immunostaining. The MTHFR 677CT genotype in the iPSC lines was confirmed by sanger sequencing. The differentiation capacity of the iPSC lines and their karyotype will be evaluated soon. Next-generation CRISPR technology, prime editing will then be used to create isogenic iPSC lines from iPSC lines bearing the MTHFR 677CT genotype. These techniques will enable us to create isogenic iPSCs with either the CC or TT genotype and allowing us an ex-vivo model for mechanistic research to study the impacts of the MTHFR 677C➔T polymorphism. To identify new riboflavin-modifiable

epigenetic targets for the treatment of hypertension, iPSCs will be differentiated into a hypertension-relevant cell type, vascular smooth muscle cells (VSMCs) under riboflavin deplete and replete conditions followed by epigenome-wide sequencing. The study will enable a better understanding of the biological mechanisms linking the MTHFR 677CT polymorphism with hypertension and provide a valuable resource for future large-scale initiatives aimed at advancing patient care.

Funding Source: The EpiHyper Project is funded by the Health Education Authority (HEA) North-South Research Programme, a collaborative scheme funded through the Irish Government's Shared Island Fund.

Keywords: MTHFR, Riboflavin, VSMC differentiation

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A HUMAN IPSC-ARRAY-BASED GWAS IDENTIFIES A VIRUS SUSCEPTIBILITY LOCUS IN THE NDUFA4 GENE AND FUNCTIONAL VARIANTS

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Genome-wide association studies (GWAS) have been broadly applied to identify genetic variants associated with human diseases. However, population-based studies to identify disease-associated risk alleles typically require samples obtained from large number of individuals. Here, we developed a human induced pluripotent stem cell (hiPSC) array-based screening strategy to link human genetics with viral infectivity. We screened an array containing 77 hiPSC lines with diverse genetic backgrounds for sensitivity to Zika virus (ZIKV) infection. A GWAS identified a cluster of single-nucleotide polymorphisms (SNPs) in a cis-regulatory region of the NDUFA4 gene, which was associated with susceptibility to ZIKV infection. Loss of NDUFA4 led to decreased sensitivity to ZIKV infection and reduced viral replication. Isogenic hiPSC

lines carrying non-risk alleles of SNPs (rs917172 and rs12386620) or deletion of the cis-regulatory region exhibited decreased NDUFA4 expression and lower sensitivity to ZIKV infection. In addition, loss/reduction of NDUFA4 was associated with resistance to other viruses, including dengue virus and SARS-CoV-2. Mechanistic studies indicated that loss/reduction of NDUFA4 causes mitochondrial stress, which leads to the leakage of mitochondrial DNA and thereby upregulation of type I-interferon signaling. This tightly controlled platform provides a rapid and cost-efficient system using a relatively small sample size to systematically evaluate association of genetic variants with certain diseases, a paradigm shift for population based GWAS, and identifies NDUFA4 as a previously unknown susceptibility locus for viral infection.

Keywords: GWAS, iPSC, ZIKV

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DOES VAPING DURING PREGNANCY HARM HUMAN EMBRYOS?

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Exposure of pregnant women to electronic cigarettes (ECs) may influence human prenatal development. It is unknown what effect individual flavor chemicals in ECs have on human embryos in pregnant women who vape. Human embryonic stem cells (hESCs), which model the epiblast, were used to study the effects of menthol on early postimplantation development. Menthol, a commonly used flavor chemical in ECs, activates TRP (transient-receptor-potential) channels, which are present in hESCs. A Ca2+ influx assay was performed to determine if menthol activated the TRPA1 and TRPM8 channels in hESCs. Ca2+ was measured in Fluo-8 loaded H9 hESCs growing in 96-well plates, and fluorescence was analyzed using a Synergy multi-mode plate reader. Nanomolar concentrations of menthol rapidly increased intracellular Ca2+, which was blocked by a TRPA1 inhibitor (AM0902). TRPM8 channels did not respond to menthol at nM concentrations. However, µM concentrations of menthol increased in intracellular Ca2+, which was blocked by a TRPM8 inhibitor (TC-I-2014). To determine if activation of TRP channels by menthol affected hESCs growth, time-lapse data were collected in a BioStation CT every 4 hours using 3×3 tiling, and growth features were extracted using StemCellQC software. Menthol at µM concentrations inhibited the increase in colony area during 48 hours of in-vitro incubation. The TRPA1 and the TRPM8 inhibitors reversed the effect of µM menthol and restored the colony area to that of the untreated controls. hESCs treated with µM menthol for 48 hours were evaluated for apoptosis using Magic Red® Caspase-3/7 Assay Kit. Magic red fluorescence was elevated in menthol-treated colonies indicating an increase in apoptosis. Apoptosis was confirmed by StemCellQC software (Brightness/Total Area ratio). The proliferation of hESCs treated with µM menthol was not affected when evaluated with a Ki-67 antibody. Given the high concentrations of menthol in ECs and the low concentrations that induce Ca2+ influx and apoptosis through TRP channels, it is likely that human embryos would be affected by menthol in pregnant women who vape. These data could help prevent alterations in normal development leading to birth defects. The use of ECs by pregnant women should be discouraged until the effects of flavor chemicals on their embryos are fully understood.

Funding Source: Supported by a Training Grant EDUC4-12752 from the California Institute for Regenerative Medicine.

Keywords: Human Prenatal Development, Pregnancy, Human Embryonic Stem Cells, Embryos, Birth Defects, Transient Receptor Potential Channels, Intracellular Calcium, H9 Growth, H9 Apoptosis, H9 Proliferation, Vaping, Electronic Cigarettes, Menthol

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HUMAN PLURIPOTENT STEM CELLS-DERIVED SERTOLI CELLS (PSC-SC) AS AN IMMUNE MODULATOR OF THE CELL TRANSPLANTATION THERAPHY IN A DIABETIC MICE MODEL

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Sertoli cells (SCs) are somatic cells that are a part of seminiferous tubules in testes and support germ cell development and maturation. Also, SCs play a main role to protect male germ cells from immune destruction by the formation of blood-testis-barrier and the secretion of several immunoregulatory factors. Based on these characteristics, it has been reported that SCs have been utilized to create a tolerogenic environment, to protect co-grafted tissue and cells. However, the clinical application of SCs would be very difficult because mature SCs are quiescent somatic cells in the testis and show lower proliferation activity in vitro. In this study, we reported that human pluripotent stem cells (PSCs) have been successfully differentiated into Sertoli cells (SCs) by stepwise differentiation protocol. Also, human PSC-SCs have well expressed marker genes of SCs, such as GATA4, SOX9, CLDN11, and AR, as well as have shown immune-modulation activity similar to human bone marrow-mesenchymal stem cells. In diabetic-induced mice co-transplanted into subcutaneous tissue with EndoC-βH1 cells (insulin-secreting cells) and PSC-SCs, lower blood glucose levels were maintained for 6 months than those transplanted EndoC-βH1 cells alone. From these results, we suggest that human PSC-SCs could be a useful cell sources in cell transfer therapy for treatment of many diseases, as an immune modulator.

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Keywords: human pluripotent stem cells, Sertoli cells, Immune modulation

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TARGETING LIPID HANDELING DEFECTS- A POTENTIAL THERAPEUTIC STRATEGY FOR STARGARDT DISEASE

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Stargardt disease (STGD1) is a rare inherited retinal degeneration affecting 1 in 10,000 children in the U.S. and is currently untreatable. Progressive photoreceptor (PR) cell death induced by atrophied retinal pigment epithelium (RPE) leads to vision loss in patients. Mutations in gene ABCA4 primarily cause the disease. Earlier stages of Stargardt disease are characterized by the accumulation of lipid-rich lipofuscin deposits in the RPE, suggesting a defect in lipid homeostasis in the eye. Recently, others and we have identified ABCA4 on the apical surface of RPE cells, challenging the current dogma that ABCA4 is a PR-specific protein. To discover the role of ABCA4 in RPE cells, we developed an in vitro model for Stargardt disease using ABCA4 mutant induced pluripotent stem cell (iPSC)-derived RPE. Fully characterized ABCA4-/- iPSCs were differentiated into RPE using a developmentally guided protocol (S-iRPE). S-iRPE was cultured on semi-permeable membranes for 6 weeks to obtain a functionally mature and polarized monolayer tissue. Cells were collected after exposure to the photoreceptor outer segment regimen (8 days) and complement competent human serum (CC-HS) treatment to induce lipid deposits. Disease phenotype was evaluated using BODIPY and ceramide lipid staining. S-iRPE demonstrated the disease phenotype of lipid accumulation and progressive RPE atrophy compared to healthy iPSC-RPE. Our ability to recapitulate Stargardt disease phenotype in ABCA4 mutant iPSC-RPE without the use of Stargardt POS suggests a cell-autonomous lipid metabolism defect in these cells. To discover if lipid lowering drugs could be used as therapeutic candidates for Stargardt's diseases, we used the following drugs: ABCA1 activator (GW3965: LXR agonist), and metformin known to improve cellular lipid metabolism by activating the AMPK pathway. All drugs lowered lipid accumulation in S-iRPE cells. In conclusion, our data suggest the autonomous role of ABCA4 in RPE lipid metabolism contributes to Stargardt disease pathology, and improvement of the activity of the lipid metabolism pathway will likely change the disease course. Our results indicate that strategies for reducing lipid accumulation in the RPE provide promising therapeutic approaches for STGD1.

Keywords: Stargradt, RPE, Lipid accumulation

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PRECLINICAL MODELS USING ANTISENSE OLIGONUCLEOTIDE (ASO) THERAPY TO TARGET DISEASE CAUSING MUTATIONS IN AUTISM SPECTRUM DISORDER

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Autism Spectrum Disorder (ASD) is a neurodevelopmental condition involving social and communication impairments and repetitive behavior. Recent studies implicate de-novo single-nucleotide mutations as causes of ASD in approximately 60%, many of which are gain-of-function (GOF), predicting they may respond to ASOs. Here we assess patient-induced pluripotent stem cells (iPSCs) directed to neural differentiation the monogenetic syndromic forms of ASD genes: ASXL3 and PACS1, causing Bainbridge-Roper syndrome and Schuurs-Hoeijmakers syndrome, respectively. We generate induced neurons (iNs) from patient iPSC lines by lentiviral transduction of Doxycyline-inducible Neurogenin2 (NGN2), and profiled the transcriptome with RNAseq at weekly intervals. We found NGN2 led to reduced expression of IPSC marker genes and increased expression of neural genes over a 3-week time course. We also performed optimization of ASO dosing using qRT-PCR to assess expression of the positive control of the 5-10-5 2'-O-methoxyethyl (MOE) gapmer ASOs targeting alpha-actinin, achieving 80-85% knockdown of targeted genes in patient iNs. In the future, we will assess the effects of ASOs targeting ASXL3 and PACS1 on expression of these GOF mutations and on genes dysregulated in patient iNs. Our approach will contribute to the development of RNA therapeutics for ASD patients. The study will also aide our understanding of the reversibility of cellular markers of ASD, which will be correlated with clinical benefit observed in patients treated with these same ASOs.

Funding Source: CIRM Bridges To Stem Cell Research Training Grant Program San Diego State University

Keywords: Autism Spectrum Disorder, Antisense oligonucleotide therapy, Neuronal Differentiation

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DEVELOPING NOVEL MODELS TO CHARACTERIZE ADAR1 ACTIVITY IN METASTATIC BREAST CANCER

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Approximately 10 to 20% of patient diagnosed with breast cancer (BC) will develop metastatic disease which remains the leading cause of death. Breast is the second organ, after the lung, at high risk of developing brain metastases (BM). The incidence of BM is increasing due to improved treatments and detection of metastatic sites. Adenosine deaminase acting on dsRNA (ADAR1) is known to drive transcriptome remodeling through a plethora of mechanisms (including changes in amino acid sequence, mRNA splicing and/or canonical polyadenylation sites) which can also promote cancer stem cells progression. Our laboratory demonstrated that ADAR1 p150, the interferon-inducible isoform, favors the alternative splicing of proteins with invasive potential in leukemia stem cells. Moreover, in telomerase positive cancer, ADAR1 was found to promote telomerase activity by resolving R-loop formation in RNA:DNA hybrids of aberrant telomeric repeats. In BC, ADAR1 plays an essential role in cell survival due to increased apoptosis and reduced proliferation found in multiple BC cell lines engineered to express reduced levels of ADAR1. High levels of ADAR1 have been associated with shorter survival and disease progression in BC patients, however, the role of ADAR1 is only partially understood in the context of metastasis. We aim to establish orthotopic and organoid models to better characterize ADAR1 activity in BC metastatic niches, with a special focus on the less studied BM. We are now in the process of collecting tumor biopsies from metastatic BC patients at the University of California, San Diego. Overall, this study will provide new insights into the mechanistic role of ADAR1 in breast metastatic disease and establish novel models for the development of new therapeutics.

Funding Source: Beasley Award

Keywords: Metastasis, Breast Cancer, ADAR1

822

OPTIMIZATION OF A 2D MONOLAYER LARGE SCALE PRODUCTION METHOD FOR THE EXPANSION OF HIGH-QUALITY HUMAN PLURIPOTENT STEM CELLS IN CGMP HPSC CULTURE MEDIUM

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Efficient expansion of human pluripotent stem cells (hPSCs) is critical for applications requiring large numbers of high-quality cells for production of cell banks and directed differentiation. Traditionally, large-scale production in 2D monolayer culture has been performed using a large number of single-layer flasks. The development of large-scale multilayer tissue-culture plastics and stabilized hPSC maintenance media have led to improved methods for 2D expansion of hPSCs. Here, we demonstrate an optimized protocol for the 2D expansion of hPSCs in a 10-layer cell factory designed to maximize cell yields, while reducing consumption of expensive reagents such as media and matrix. hPSCs were seeded as single cells in mTeSR™ Plus, supplemented with CloneR™2 and ES-Qualified Matrigel® to coat the cell factory at 4.5 µg/cm2 (n = 3 cell lines, n = ≥ 2 biological replicates). Using human embryonic (H9) and induced pluripotent (STiPS-003A and WLS-1C) stem cell lines, an average of 6.7 x 10^5 ± 7.6 x 10^4 (mean ± SD) viable cells were generated per mL of medium consumed. Using this optimized workflow, 5.6 x 10^5 ± 5.0 x 10^4 (mean ± SD) cells per cm2 are generated in 10 days, equivalent to 3.6 x 10^9 viable cells in a 10-layer cell factory, when starting from one well of a 6-well plate, with an intermediate expansion step in a T-175 cm2 flask. When harvesting single cells from a cell factory and single-layer companion T-175 cm2 flask, and re-plating in CloneR™2-supplemented mTeSR™ Plus, the recovery of viable cells after 24 hours is 108.8 ± 9.3% from the cell factory, compared to 122.6 ± 15.2% (mean ± SD) from the single-layer companion T-175 cm2 flask. hPSCs harvested as single cells from the cell factory retain > 90% viability, > 90% expression of markers of the undifferentiated state (OCT4 and TRA-1-60), and the capacity to differentiate to the three germ layers. We have developed a simple 2D hPSC expansion protocol, which has been optimized to reduce costs and time for users seeking to generate large numbers of high-quality hPSCs.

Keywords: scale-up, GMP, iPS Cells

824

DEVELOPMENT OF A REAL-TIME ASSAY FOR TRACKING THE PROLIFERATION OF IPSCS AND IPSC-DERIVED ORGANOIDS

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The flexibility and accessibility of induced pluripotent stem cell technology has allowed complex human biology to be reproduced in vitro at high throughput scales. Indeed, rapid advances in stem cell technology have led to widespread adoption for the development of in vitro models of human physiology to be used in screening applications in drug discovery and safety. Furthermore, advanced cell preparations, such as organoids, are under investigation with aims toward establishing mature human phenotypes in vitro. For the development and validation of relevant in vitro iPSC-derived organoid models, it is critical to develop a label-free assay to track organoid growth and expansion. The objective of this work was to develop and validate a real-time, label-free imaging assay as an efficient approach for tracking proliferation of iPSCs and iPSC-derived organoids in vitro. An automated, scanning microscope was used to acquire whole-vessel brightfield images of multiwell plates within an incubator, and image analysis algorithms computed parameters including confluency, colony size, and organoid cross-sectional area. Here, we characterized and validated this assay across multiple use-cases for iPSCs and iPSC-derived organoids. For iPSC-derived organoids, patient-derived tumor organoids (PDTOs) were developed to model head and neck squamous cell carcinoma (HBSCC), with CRISPR editing to create specific mutations. Cross-sectional area was used to quantify the growth and expansion of wild-type and mutant organoids, with mutant organoids growing faster than wild-type in both patient models (fold change over 5 days: Patient 1 Mutant = 2.3 +/-0.3, Patient 1 WT = 1.6 +/-0.3, Patient 2 Mutant = 2.4 +/- 0.5, Patient 2 WT = 1.8 +/- 0.6). A CellTiter-Glo Assay was performed on the same models yielding similar results. These results support the continued development and use of real-time, label-free imaging assays to efficiently track the growth and expansion of iPSCs and iPSC-derived organoid models.

Keywords: organoid, live-cell imaging, iPSC

826

ECLIPSE, AN AUTOMATED CRISPR PLATFORM FOR THE LARGE-SCALE GENERATION OF CELL MODELS FOR THE IPSC NEURODEGENERATIVE DISEASE INITIATIVE (INDI)

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Major advances in gene editing using CRISPR have improved accessibility for the creation of disease relevant cell models, allowing researchers to readily interrogate genetic drivers for disease and elucidate novel targets for previously untreatable disorders. The National Institutes of Health led iPSC Neurodegenerative Disease Initiative (iNDI) is the largest iPSC genome engineering project attempted with the goal of generating a widely available and standardized set of diseased cell models for over 100 single nucleotide variants (SNV) mutations associated with Alzheimer's disease and related dementias (ADRD) in isogenic iPSC lines. The standardization of cell models is of vital importance for the generation of reproducible and actionable data in therapeutic development. Despite this, the rapid generation of disease-relevant iPSCs at the scale needed for successful and efficient therapeutic development remains challenging due to the technical challenges of using traditional manual gene editing approaches. As part of a multi-institution collaboration, Synthego was selected for the generation of 25 SNVs in the candidate KOLF2.1 iPSC line. Toward these goals, we describe the use of our automated, high throughput CRISPR editing platform, ECLIPSE, for the rapid generation of knock-in iPSC models of ADRD. We leveraged our state-of-the-art knock-in methods and automated pipelines for the design, experimental optimization, and clonal isolation of 23 of the candidate target mutations in iPSCs. For each SNV target, at least 3 clonal homozygous and 6 clonal heterozygous mutation lines were generated for a total of 264 clonal cell lines over a 6-month period. The utilization of automated systems such as our ECLIPSE platform are critical catalysts for the rapid development of relevant cell models in large scale disease initiatives such as iNDI.

Keywords: CRISPR, iPSC, Alzheimers

828

DNA DAMAGE AND CELL DEATH IN AN IN VITRO MODEL OF FUCHS ENDOTHELIAL CORNEAL DYSTROPHY

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Fuchs endothelial corneal dystrophy (FECD) is one of the leading causes of corneal transplantation caused by progressive loss of corneal endothelial cells. Corneal endothelial cells in FECD are vulnerable to oxidative stress resulting in mitochondrial dysfunction, cell degeneration and death. While cell death is an essential aspect of FECD, previous reports have often analyzed immortalized cell lines, thus making cell death evaluation difficult. We, therefore, established a new in vitro FECD model to evaluate the pathophysiology of FECD. Induced pluripotent stem cells (iPSC) were derived from monocytes of FECD patients, which were then induced into corneal endothelial cells. Following treatment with H2O2, a cell viability assay using Sytox Green staining revealed that the cell death ratio was higher in FECD than control. Additionally, the Sytox Green assay elucidated that induced cells were more prone to cell death than immortalized cells. We then examined the underlying mechanisms of cell death. Immunofluorescence analysis revealed higher levels of DNA double-strand breaks marker, γH2AX, in FECD compared to control cells. Next, we assessed PARP expression. PARP plays vital role in genomic stability and double-strand break repair. PARP expression level was higher in FECD than in control cells. In conclusion, this in vitro model of FECD could be suitable for assessing cell death phenotype in FECD and could be used for future studies on the pathophysiology of FECD and drug development.

Funding Source: This work was supported by Grants-in-Aid for Scientific Research from Japan Society for the Promotion of Science to Shigeto Shimmura (Grant Number: KAKENHI 19K09978), Japan Cornea Society (Novartis Pharma Grants 2020) to Emi Inagaki.

Keywords: Fuchs endothelial corneal dystrophy, cell death, oxidative stress

THURSDAY, 15 JUNE 2023

TRACK: TISSUE STEM CELLS AND REGENERATION (TSC)

Session 2: Odd

10:00 AM – 10:45 AM

TOPIC: CARDIAC

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HEART MIMETIC MICROENVIRONMENT IMPROVES CHEMICALLY INDUCED DIRECT CARDIAC CONVERSION

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Direct reprogramming via genetic modification is efficient but has potential risks such as genetic abnormalities and tumorigenicity. Using chemically defined cardiomyocyte conversion without the requirement of gene delivery may be more suitable for application in cardiac regenerative medicine because it overcomes safety problem associated with genetic modifications. However, maturation of reprogrammed cardiomyocytes and efficiency of cardiac induction, which are important for regenerative potential, still need to be improved. Here we report that three-dimensional heart mimetic microenvironment formed with cardiac decellularized extracellular matrix enhance efficiency of primary fibroblasts to chemically induced cardiomyocytes (CiCMs) and cardiac maturation. The CiCMs encapsulated with decellularized heart extracellular matrix-based hydrogel indicate further promoted cardiomyocyte-specific marker gene expression, sarcomeric organization and electrophysiological properties. Our platform mimicking heart-like microenvironment may expand the possible application of CiCMs for regenerative medicine.

Funding Source: This work was supported by grants from the National Research Foundation of Korea (NRF) (2021R1C1C2009131, 2022M3A9B6082675) funded by the Ministry of Science and ICT (MSIT).

Keywords: Direct reprogramming, Cardiomyocyte, Decellularized extracellular matrix

971

CARDIAC STEM CELLS SECRETE SPARC AND EXPRESS DESMIN AND REDUCED EXPRESSION OF SPARC OR DESMIN DISRUPTS MYOCARDIAL DIFFERENTIATION OF CARDIAC STEM CELLS

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Mammalian hearts contain cardiac stem cells throughout life; however, these cells could not be harnessed to repair damaged myocardium in vivo so far. Assuming physiological relevance of these cells which had evolved and had been maintained during evolution, we seek after their function using murine cardiac stem cell lines as am in vitro model system. We want to contribute to the understanding of the transcriptional and signaling network which directs myocardial differentiation, maturation and homeostasis in adult cardiac stem cells. Here we focus on the identification of cell autonomous and autocrine signals. We found that cardiac stem cells express and secrete SPARC and their differentiating progeny take up SPARC which promotes cardiomyogenesis in an autocrine manner. SPARC expression in cardiac progenitor cells is regulated by desmin expression and reduced expression of one or the other gene partially disrupts cardiomyogenesis. Preliminary data suggest an influence of SPARC and desmin on canonical and non-canonical TGF-ß signaling during myocardial differentiation of cardiac stem cells. Demonstration of SPARC secretion and its influence on signal transduction and differentiation in cardiac stem cells opens the possibility that cardiac stem cells might fulfill their physiological function in homeostasis and ageing of the heart by a gland like secretion of factors which modulate the environmental influences for the benefit of a longer life.

Funding Source: Austrian Science Fund (FWF)

Keywords: Cardiac Stem Cells, SPARC, Desmin

973

SCALING UP HUMAN ENGINEERED MYOCARDIUM FOR HEART REGENERATION

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As the field of heart regeneration aims to replace cardiomyocytes (CM) lost to injury or disease, scaling up cell numbers in engineered tissues is required to meet the demands of dosing and delivery. It is estimated that up to 1 billion CMs die during a myocardial infarction, necessitating delivery of a comparable number of cells to the region of cell loss. Engineered tissues overcome many limitations of cell injections by providing a 3D environment for maturation and syncytium formation that reduces arrhythmia risk, improves engraftment and localization of hiPSC-derived CMs, and provides increased mechanical support of the cardiac wall. To date, a lack of rigorous studies exploring the biomanufacturing design space for clinical translation of human engineered myocardium (hEM) poses a significant hurdle to their use in translational applications. To this end, we are focused on understanding how scaling up hEM in size, CM number and density, and matrix density impact the electromechanical function and surgical handling of the tissue. We fabricate hEM by mixing hiPSC-CMs (>90% pure) with 5% human cardiac fibroblasts in a collagen-1 hydrogel and assess electromechanical function through optical mapping of action potentials and tensile mechanical testing. With an increase in CM density from 5 M/mL (standard in the field) to 15, 30, or 50 M/mL, compaction decreased up to 6-fold, stiffness (measured by elastic modulus) decreased up to 20-fold, and active stress generation decreased up to 6.5-fold. Despite this apparent setback in hEM in vitro formation and function, a single hEM of 7 cm x 8 cm with 1 mm thickness was able to support 1 billion cells with dynamic culture up to 7 days without inducing significant necrosis in the core. Further, geometric redesign to uniformly distribute tension during compaction and use of culture medium rich in fatty acids induce uniform AP propagation with a quiescent diastolic phase and no reentrant arrhythmia patterns . By understanding the design space and functional changes, this work supports the translational feasibility of using highly dense hiPSC-CMs within hEM as a regenerative therapy to remuscularize the failing heart.

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Keywords: cardiac tissue engineering, heart regeneration, hiPSC-derived cardiomyocytes

975

A NOVEL APPROACH TO DISCOVER KEY LIGAND-RECEPTOR INTERACTIONS REGULATING ZEBRAFISH HEART REGENERATION

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Myocardial infarction remains a major cause of human death worldwide due to the inability to remove the fibrotic scar and reduced cell proliferation. While the zebrafish heart retains its ability to regenerate throughout adulthood, the interaction and regulation of critical cell populations involved in this process are not fully understood. To investigate cell-to-cell interactions we re-analyzed transcriptomics data from previously identified critical cell populations during zebrafish heart regeneration: periostinb+ fibroblasts, sox10+ cardiomyocytes and kdrl+ endocardial cells in a cryoinjury versus sham injury model. We evaluated intercellular interactions through Ligand Receptor (LR) based signaling creating a network of communication. Employing elements of network theory such as PageRank and hierarchical interacting partners we identified central nodes that regulate cell -cell interactions during heart regeneration. To further assist in novel target prediction, we performed an automated text mining on PubMed to determine potential LR candidates not studied in the context of heart regeneration. Our analysis pointed to a preponderant role of Fibroblasts in orchestrating regeneration. We integrated and validated our network by assembling a broader transcriptomics meta-analysis with data from other zebrafish heart regeneration studies and across species from regenerating neonatal mouse hearts. Next, we selected 5 candidate interactors for biological validation. To do so we will generate full body knockouts (KO) and cell population-specific KO using CRISPR-Cas9 approaches. Moreover, we will use Lipid Nanoparticles to deliver mRNA of our candidate genes to the regenerating heart, to induce their overexpression. With our bioinformatics approach and its functional validation, our project aims to find important novel players involved in zebrafish heart regeneration. Our findings will be made accessible in a user-friendly web app interface to the wider community.

Funding Source: Funded by European Union's Framework Programme for Research and Innovation "Horizon 2020". Reanima 2020. Grant agreement ID: 874764

Keywords: Heart regeneration, Ligand receptor, Meta analysis

977

HUMAN CARDIOMYOCYTE DIFFERENTIATION FROM HIPSC ACCORDING TO MACHINE-LEARNING

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Cardiomyocytes derived from human induced pluripotent stem (iPS) cells are a promising cell source for heart tissue regeneration and enable the study of cardiac physiology and the developmental testing of new therapeutic drugs in a human setting. However, finding potential compounds for differentiation and maturation among many candidates is a resource and time-intensive process, often requiring much trial and error. In this presentation, we describe the use of machine learning to predict potential compounds for cardiomyocyte differentiation. Our machine-learning algorithms use three-dimensional molecular shape information as input and can predict whether the molecule has a cardiac differentiation effect on the basis of a simple neural network model. The trained algorithms achieved a high-level accuracy (true positive rate of over 75 %), despite the small amount of training data used. By applying this model to a small selection of computer-generated compounds, we could then identify ones that are predicted to show a cardiac differentiation effect. To demonstrate the utility of this model, we then assessed the differentiation and maturation markers expression profiles of cardiomyocytes after introducing predicted compounds into real hiPSC. These results demonstrated that the machine-learning approach could be taken as an effective in-silico approach for accelerating the screening of compounds for stem cell research.

Keywords: Regenerative Medicine, Human Induced Pluripotent Stem Cell, Cardiomyocyte

TOPIC: EPITHELIAL INCLUDES SKIN GUT LUNG

979

TNF SIGNALING DEFICIENCY PROMOTES UVB-INDUCED MELANOCYTE STEM CELL ACTIVATION AND MIGRATION

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Cutaneous melanoma is one of the deadest skin cancers worldwide, and the activation of melanocyte stem cells (McSCs) has been identified as a crucial step in melanoma initiation. Previous studies have found that ultraviolet B (UVB) induced inflammation increases the risk of melanoma initiation by elevating McSC activation. In this study, we aimed to investigate the effect of inhibiting tumor necrosis factor (TNF) signaling, a major pro-inflammatory pathway, on UVB induced McSC activation by globally knocking out Tnfrsf1a and Tnfrsf1b. Surprising, we found that the Tnfrsf1a/b knockout mice exhibited significantly higher McSC activation following UVB irradiation. Further analysis revealed that

this increase in McSC activation was associated with altered macrophage phenotype and heighted inflammatory response in Tnfrsf1a/b knockout mice. This study suggests that blockage of TNF signaling may cause controversial effect on melanoma initiation, and calls for further investigation of TNF signaling in inflammatory response.

Funding Source: NIAMS NIH 5R01AR075755-03 RRID: SCR_021740 RRID: SCR_021727

Keywords: melanocyte stem cell, UVB, tumor necrosis factor

981

ADULT STEM CELL BEHAVIOUR IS CONTROLLED BY SNORNA HOST GENE LONG NON-CODING RNAS UNDERGOING RAPID EVOLUTION

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A large portion of mammalian genomes is transcribed but not translated. Genes containing intronic snoRNA have recently emerged as a source of non-canonical transcripts that can regulate gene expression and in several cases can generate abundant long non-coding RNA species. Actively transcribed, non-coding parts of the genome provide a potential platform for the dynamic evolution of new functional sequences, but their biological and evolutionary roles remain largely unexplored. Here we show that long non-coding RNAs arising from snoRNA Host Genes (SNHGs), are a group of fast evolving, highly expressed transcripts that can profoundly impact adult stem cell biology. Using SNHG7 and epithelial stem cells as a model, we describe a mechanism by which these RNAs can increase the proliferation and inhibit the differentiation of stem cells through sponging of specific microRNAs. SNHG7 lncRNA sequence is conserved only among primates and its function is lost whenever the short sequence required for microRNA binding is absent. Taken together, our results highlight the importance of fast-evolving genetic elements in the regulation of adult stem cell function and pave the way for further investigation into the biology and evolutionary dynamics of poorly conserved, actively transcribed non-coding sequences.

Keywords: Long non-coding RNA, Epidermal stem cells, Evolution

983

UNCOVERING THE GENE REGULATORY NETWORK THAT MAINTAINS THE MULTIPOTENCY OF HUMAN AIRWAY BASAL CELLS

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Basal cells (BCs) are the primary stem cell population of the human trachea and intrapulmonary airway epithelium, possessing the ability to regenerate the airways under both steady state and injury conditions. BC dysfunction, contributes to airway remodeling in several chronic diseases. BC subpopulations, that differ in their proliferative capacity and differentiation ability, have been described in human airways. Despite the significance to human health, we lack a detailed understanding of the mechanisms that regulate the BC stem cell program and contribute to aberrant BC behavior during disease. Our overall goal is to define the core gene regulatory network that governs the stem cell potential of BCs. First, to assess for heterogeneity amongst human BCs, we fluorescently labelled subsets of early passage (P1) primary human BCs to track individual clones. BCs were transduced with a lentivirus with constitutive expression of GFP or tdTomato and tracked as they differentiated in-vitro in standard air liquid interface (ALI) culture conditions. Next, we utilized the CellTag lentiviral barcoding library paired with time series single-cell RNA-Sequencing to lineage trace the progeny of individual BC clones and overlay their transcriptomic profile with cell fate outcomes after these cells had differentiated in ALI culture. Florescent clonal analysis suggested significant variability in the proliferation and differentiation ability of labeled BC clones. For example, the largest clones were 9 times larger, in terms of surface area, compared to average surface area of remaining clones. Through the barcoding/sequencing approach, we identified 70 unique clones, 49 of which consisted of more than 2 cells. The 3 largest clones contributed to 53% of all barcoded cells. Using canonical markers and published transcriptional signatures, we assessed the number of basal, ciliated, and secretory cells within each clone. In a subset of clones, differences in differentiation competence was suggested. In summary, our findings support functional heterogeneity within clonal populations of airway basal cells. The ability to track individual human basal cells using lentiviral barcoding, paired with single-cell RNA-Sequencing allows for the correlation of transcriptional profiling with functional outcomes.

Keywords: Airway Basal Cell, Lentiviral Barcoding, Stem Cell Program

985

WDR4 REGULATES INTESTINAL STEM CELL PROLIFERATION VIA THE REGULATION ON RIBOSOMAL BIOGENESIS

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WD40 repeat protein, Wdr4, is known to control the differentiation of embryonic and germline stem cells (GSCs) via protein-protein interactions. However, the physiological significance of Wdr4 in animal health and somatic stem cells remains unclear. Here, we report that Wdr4 loss in Drosophila causes shortened lifespan, locomotor defects, and increased susceptibility to stress. Wdr4 depletion also results in aberrant midgut morphology and dramatically increased proliferation of intestinal stem cells (ISCs), via JNK and Notch signaling activation, leading to increased progenitor cells accompanied by decreased absorptive enterocytes (ECs). To maintain gut integrity, the remaining ECs increase cell size by extra rounds of endoreplication. In addition, ECs display disrupted cell-cell contact, leading to leaky gut, increased immune response probably due to infection, and redox stress, which is known to non-cell autonomously promote ISC proliferation. Furthermore, the machine-learning and RNA-seq results suggest that Wdr4 controls ribosomal biogenesis, reminiscent of its role in controlling GSC homeostasis via the interaction with Mei-p26 (a TRIM-NHL tumor suppressor) that suppresses ribosomal biogenesis via degrading oncogene Myc, which is known to activate JNK signaling. Interestingly, guts bearing mettl1 knockdown in ISCs, which is a catalytic subunit of the tRNA methyltransferase in mammals' phenocopies, the wdr4 mutant gut with increased proliferation and ribosomal biogenesis. Most interestingly, overexpressing human WDR4 partially rescues wdr4 mutant gut phenotypes, indicating a conserved role of Wdr4 in ISCs from insects to mammals. These results document the complexity of ISC regulation by intrinsic and local signals and may shed light on the role of steady-state ribosomal biogenesis for stem cell homeostasis.

Funding Source: MOST and ICOB intramural funding support this research

Keywords: ROS, JNK, ribosomal biogenesis, Myc, Intestinal Stem Cell

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STEM CELL SECRETED PROTEINS IN INTESTINAL HOMEOSTASIS

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Epithelial stem cells maintain tissue homeostasis throughout adult life. Loss of the balance between cell loss and production is associated with declining tissue function in ageing and diseases such as cancer. Stem cells are therefore tightly regulated by their local microenvironment and have been studied extensively as signal-receiving cells. However, previous work from our lab and others has shown that stem cells make substantial contributions to their own microenvironment through autocrine signalling and regulation of the niche. The intestinal stem cells of the Drosophila midgut are an ideal model system to characterise stem cell contributions to the niche as their cell fate and major regulatory pathways are conserved to mammalian epithelia. We have taken advantage of the genetic tools available in Drosophila to perform a targeted RNAi screen of conserved secreted proteins that are expressed specifically in intestinal stem cells. Quantification of cell density, proportions of stem, progenitor, and differentiated cells has allowed us to identify stem cell-derived proteins that regulate adult tissue homeostasis. These include the septate junction protein Tsf2 and regulators of the extracellular matrix. Further characterisation has shown that Tsf2 regulates homeostatic turnover, shows changes in expression with age and impacts lifespan without altering barrier function. Ongoing and future work will test for conservation of expression patterns and function in mammalian epithelial stem cells.

Funding Source: Academy of Medical Sciences/Wellcome Trust/Department of BEIS/British Heart Foundation/Diabetes UK Springboard Award SBF005\1022 Newcastle Liverpool Durham BBSRC-DTP (BB/M011186/1) studentship

Keywords: Epithelia, Homeostasis, Drosophila

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DEFINING THE ROLE OF HMGA2 IN THE CUTANEOUS RESPONSE TO UVB AND MELANOCYTE STEM CELL MIGRATION

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High mobility group AT -hook 2 (Hmga2) is a non-histone binding chromatin remodeling protein which is highly expressed during early stages of development and in many tumor types but has negligible expression in healthy somatic tissue. However, there is emerging evidence that it plays a role in the activation and migration of melanocyte stem cells from their hair follicle niche into the interfollicular epidermis in response to ultraviolet-b (UVB) light exposure. Using engineered mouse models and whole-mount imaging, we show that Hmga2-/- animals have a reduction in UVB-induced melanocyte stem cell migration to the interfollicular epidermis. We further show that Hmga2 expression is found within the interfollicular epidermis shortly after UVB, where it is associated with hyperkeratosis and immune cell recruitment into the skin. Since Hmga2 expression is primarily regulated by the micro-RNA Let-7, we next interrogated whether Let-7 signaling was altered in the Hmga2-/- mouse model to understand how the observed cutaneous effects may be related to Let-7 signaling. A multifaceted approach using transcriptomic analysis and loss-of-function mouse models confirms that Let-7 signaling is disrupted following loss of Hmga2. This study highlights a novel role for Hmga2 in somatic tissue, providing further insights into

the processes underlying UVB-mediated melanocyte stem cell migration.

Funding Source: This work is funded by the NIH (5R01AR075755-03 to ACW), the Cornell University Stem Cell Program Training Fellowship (to LRD), and the Cornell University Center for Vertebrate Genomics Scholar Award (to LRD)

Keywords: Melanocyte Stem Cell, UVB, Inflammation

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UNIFYING IMMUNOLOGICAL AND MICROBIOTA-DEPENDENT MECHANISMS IN THE SKIN-GUT AXIS UNDERLYING THE ATOPIC MARCH

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Atopic dermatitis (AD) is the most diagnosed skin condition that affects approximately 20% of children and 5-10 % of adults. Alarmingly, AD patients often exhibit co-morbidities in other barrier tissues such as the gastrointestinal tract, including a higher incidence of inflammatory bowel disease, a clinical observation referred to as atopic march. Despite our growing appreciation that skin epithelial barrier dysfunction, microbial dysbiosis and uncontrolled immune responses can initiate atopic diseases, the underlying mechanisms that drive these atopic trajectories have not been fully elucidated. Human and mouse genetic studies have shown that transmembrane protein 79 (Tmem79) is an important skin-barrier related gene involved in the development of AD. We recently identified Tmem79 as a specific Wnt antagonist that promotes the degradation of Frizzled receptors. Here, we utilized multiple conditional deletion mouse models to specifically delete Tmem79 in skin epithelial cells or intestinal epithelial cells. Intestinal regeneration capacity was examined in an experimental model of colitis using dextran sulphate sodium (DSS). DSS sensitivity was primarily driven by skin-specific but not intestine-specific deletion of Tmem79. Skin-specific deficiency of Tmem79 compromised the integrity of the gut epithelial barrier and impaired intestinal regeneration, characterized by a reduction in proliferating Ki67+ cells, enteroendocrine cells and goblet cells. Co-housing experiments demonstrate microbial transfer confers protection against DSS challenge in mice lacking Tmem79 in the skin. RNA-sequencing revealed enriched inflammatory signatures in colonic epithelial cells of mice with skin-specific deletion of Tmem79. Moreover, an elevation in IL-17-producing γδ (γδ17) T cells and T helper 17 (Th17) cells was observed in the skin-draining lymph nodes and colon of skin-specific conditional knockout mice, indicating skin-derived inflammation is a key driver impacting intestinal stem cell regeneration. These novel findings signify a fundamental immunologic and microbiota-dependent mechanism underpinning the atopic march that may have broader implications for other atopic diseases, including asthma, food allergy and allergic rhinitis.

Funding Source: -BCH Internal Pilot Study Grant -American Cancer Society Research Professorship Grant -Fulbright Postdoctoral Fellowship

Keywords: Atopic march, Regeneration, Immune response

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DIFFERENTIAL METABOLIC ADAPTATIONS DEFINE RESPONSES OF WINNER AND LOSER ONCOGENIC MUTANT STEM CELLS IN SKIN EPIDERMIS IN VIVO

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Skin stem cells detect and correct aberrancies induced by oncogenic mutations. Different oncogenes invoke different modes of epithelial tolerance: while wild-type (WT) cells outcompete β-catenin-Gain-of-Function (βcatGOF) mutant cells, HrasG12V mutant cells outcompete WT cells, yet are integrated into functional tissue. We asked how metabolic states change as WT stem cells interface with mutant cells, and how this ultimately drives different cell competition outcomes. To do this, we combined two experimental modalities -1. optical redox imaging to visualize endogenous levels of the co-enzymes NAD(P)H & FAD, adapted within our in vivo imaging platform to enable long term tracking and visualization of metabolic states of stem cells within the live mouse, and 2. 13C6- glucose tracer- mass spectrometry to measure metabolic fluxes directly. By tracking the redox ratio (NAD(P)H/FAD) with single cell resolution in the same mouse over time, we discover that both βcatGOF and HrasG12V mutations lead to a rapid drop in redox ratios (NAD(P)H/FAD), before other epidermal aberrancy, making it a first line of response to the mutations. However, the winner cells in each model (WT cells in βcatGOF and mutant cells in HrasG12V model), rapidly recover their redox ratios, irrespective of the mutation induced. Glucose flux studies reveal that both βcatGOF and HrasG12V mutant epidermis upregulate flux and fractional contribution of glucose through TCA cycle, and thereby glucose oxidation, in line with the observed drop in NAD(P)H/FAD. Contrastingly, the "winner" mutation HrasG12V specifically upregulates pyruvate to lactate rates (glycolysis readout) while the "loser" βcatGOF mutation downregulates this step. Using pharmacological inhibition of mitochondrial activity, we find that upregulation of glucose oxidation is essential for the full penetrance of both mutant phenotypes. Hence, unique metabolic adaptations define the hallmarks of winners and losers during cell competition in vivo and drive tissue outcomes. These novel findings contrast with the expectations of Warburg effect, a fundamental concept in cancer metabolism, wherein proliferative cells upregulate glycolysis at the expense of downstream mitochondrial oxidation, and instead reveal metabolic requirements for oncogenic tolerance in skin stem cells.

Keywords: Metabolic rewiring, Stem cells in cancer, Warburg effect

Abstract Withdrawn

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OPTIMIZATION AND CHARACTERIZATION OF HUMAN ADULT ALVEOLAR ORGANOID CULTURES

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Stem cells of the lung have a complex hierarchy and show incredible plasticity and regenerative capacity. Nevertheless, respiratory diseases are one of the main causes of death with increasing incidences. Chronic obstructive pulmonary disorder, and other pulmonary diseases, are poorly understood. Before we can start to understand the mechanisms of these pathologies, we need to gain better understanding of how the human lung normally regenerates. Organoids are a valuable tool to study epithelial regeneration in vitro. While airway organoids are well-established, alveolar organoids, modeling the distal parts of the lungs, have proven challenging. Current protocols sort for alveolar type 2 (AT2) cells from fresh tissue and culture them in Wnt-activating media conditions. However, these conditions don't allow for long-term growth. Indeed, it is unclear if the general AT2 population contains a bona fide stem cell population or if they are facultative stem cells with limited growth capacity. Furthermore, differentiation conditions for alveolar type 1 (AT1) cells are poorly defined. Here, we strive to optimize human adult alveolar organoid cultures by testing different sorting and culturing strategies by manipulating common regeneration and developmental pathways in a temporal fashion. Our goal is to identify conditions that allow for long-term expansion of the alveolar stem population, and separate conditions for AT2 and AT1 differentiation.

Keywords: Organoids, Lung, Alveolar

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THROUGH COLLAGEN FIBROBLASTS INHIBIT EPITHELIAL REGENERATION IN GROWING MURINE SKIN

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Organs that undergo rapid cellular turnover depend on the orchestration of different cell types to regulate proper regeneration and maintain organismal health. In vitro, fibroblasts are essential for epithelial stem cell proliferation and expansion, but it is still unknown whether fibroblasts regulate epithelial cell behaviors in vivo. Here we show that fibroblasts downregulate epithelial proliferation through collagen during murine postnatal growth, which contrasts with their role to promote proliferation in adult homeostasis. We discover that reducing fibroblast number during postnatal growth decreases collagen density and ultimately results in higher epithelial stem cell proliferation and density. With a reduction of fibroblasts, the epidermis becomes thicker maintaining the water-barrier function. Furthermore, we rule out inflammation as a mechanism driving epidermal phenotypes. Instead, we find that a collagen deficiency in fibroblasts induced by COL1A1 knock-out is sufficient to increase epithelial proliferation. When investigating

the role of fibroblasts in adult mice, we find that fibroblast ablation decreases epithelial proliferation but not the integrity of dermal collagen. Our results demonstrate that fibroblasts inhibit epithelial proliferation via collagen in postnatal growth, converse to the canonical mitogenic role of fibroblasts in adult mice. To understand the molecular mechanism, we are actively investigating the role of signaling that uses collagen as a ligand to inhibit epithelial proliferation.

Funding Source: CSC-Yale Program, NIH DP1, and HHMI Scholar Awards.

Keywords: cell-cell interaction, proliferation, extracellular matrix

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CROSSTALK BETWEEN HAIR FOLLICLE STEM CELLS AND SENSORY NEURONS IN THE SKIN

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In mammals, the skin is not only the main barrier to the external environment, but also the largest sensory organ. Wholemount staining with tissue clearing unveils a strikingly intimate association between sensory nerves and stem cells residing in the upper bulge region of hair follicles. I've identified candidate ligand-receptor interactions between sensory neurons and hair follicle stem cells through single cell RNA-seq analysis, and established both an in vitro organoid co-culture system and in vivo genetic manipulation methods to interrogate their function. Our work has elucidated the mechanisms of how epithelial stem cells orchestrate the innervation of their niche as well as the physiological significance of stem cell-nerve interactions.

Funding Source: C. H. Li Memorial Fellowship; National Cancer Center Postdoctoral Fellowship; Charles Revson Postdoctoral Fellowship

Keywords: stem cell, sensory nerve, inter-tissue communication

1003

AN ORGANOMIMETIC STEM CELL PLATFORM TO ELUCIDATE GUT MICROBIOME-HOST INTERACTIONS

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The intestinal microbiome plays an important role in human health and disease. However, there is a considerable amount of the human intestinal microbiome that is uncharacterized due to difficulty conducting studies outside of the host while maintaining physiological relevance. Metagenomic studies across multiple publicly available human microbiome databases have discovered a bacterium with high prevalence in the human microbiome that has never been isolated or cultivated before. To our knowledge, we are the first to have successfully co-cultured this bacterium with intestinal tissue derived from human embryonic stem cells (hESCs). We speculate that tissues derived from hESCs will offer a more reproducible, standardized, and genetically homogeneous platform than primary tissue as hESCs don't have inconsistency among different samples including sample retrieval variability, genetic background, medical history, age, or gender. By using an organomimetic platform of the human intestine capable of recapitulating tissue composition, 3D structures, and oxygen gradient we aim to elucidate the role of this bacterium in gut homeostasis. Our bulk RNA sequencing data showed 250 genes differentially expressed in hESC-derived intestinal tissue following co-culture. Among other pathways, there was a significant up-regulation of the IL-17 pathway which has been previously shown to be essential for regulating mucosal host defense against many invading pathogens. Therefore, our preliminary data suggests a potential crucial role of this bacterium in immune regulation to maintain gut homeostasis.

Keywords: In vitro intestinal model, Microbiome-host interaction, Tissue engineering using stem cells

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CLUSTERS OF SENESCENT CELLS PROMOTE ACTIVITY OF NORMAL SKIN STEM CELLS FOR INCREASED REGENERATION

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In normal tissues, fate of resident stem cells is tightly controlled by specialized niche cells. In the skin, hair follicle stem cells are principally regulated by signals from mesenchymal cells of the dermal papilla – key hair follicle niche cells. Under normal conditions, niche signals maintain hair stem cells in the resting state and activate them only transiently at the onset of new hair growth. In hairy melanocytic nevus – a benign and common skin condition in humans, long hairs grow excessively, suggesting overabundance of stem cell activating signals. Compared to normal skin, nevus skin is enriched for oncogene-induced senescent melanocytes, that distribute in form of small clusters near hair follicles. Using genetic mouse models for melanocytic nevus, that harbor human-like oncogene activating mutations in either Nras or Bras, we show human-like hair overgrowth phenotype. Molecular profiling of nevus melanocytes identified a number of enriched signaling factors, that constitute the so-called Senescent Associated Secretory Phenotype (SASP). Focusing on individual SASP factors, we have identified key molecular drivers of the hairy nevus effect and new signaling pathways that prominently activate quiescent hair follicle stem cells. Overall, our findings from the hairy nevus model suggest that SASP factors from senescent cells can have beneficial, promoting effects on tissue resident stem cells, and

that profiling SASP factors can be a fruitful strategy for identifying new stem cell-regulating mechanisms.

Keywords: cellular senescent, adult stem cells, regeneration

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RETINOIC ACID PROMOTES HAIR REGENERATION BY RESOLVING LINEAGE PLASTICITY IN WOUNDED SKIN

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Stem cells upended from their niche upon injury display a wide-ranging lineage plasticity that enables their direct contribution to repair. However, this unsettled state persists in both chronic wounds and cancer. Here we identify retinoic acid (RA) as a critical metabolite at the intersection between homeostatic regeneration and wound repair in the skin. Using high-throughput approaches and 3D cultures, we defined conditions able to resolve lineage plasticity in hair follicle stem cells (HFSCs). Temporal control of RA activity in combination with established HFSC niche signals enabled the ex vivo recapitulation of stepwise differentiation programs that yield each hair lineage. During wound repair, RA availability in the HFSC niche is transiently reduced to favor skin re-epithelialization and can be functionally rescued to promote hair growth. Together, these findings underscore the potential for targeting lineage plasticity to restore tissue function in diseased states and report the minimal requirements to produce a suite of hair cell types outside the body.

Keywords: Lineage plasticity, Wound repair, Skin and hair follicle

1009

FRIZZLED5 MAINTAINS HOMEOSTASIS OF ADULT INTESTINAL EPITHELIUM THROUGH ORGANIZATION OF CHROMATIN ACCESSIBILITY

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The homeostasis of the intestinal epithelium is maintained through complex yet poorly understood intestinal stem cell (ISC) dynamics. Here, we demonstrate that Frizzled5 (Fzd5), a Wnt pathway receptor, acts as a cell fate determinator and regulates adult intestinal homeostasis. Fzd5 is broadly expressed alongside the crypt-villus, with enrichment in crypt-resident transition state cells. Knockout of Fzd5 in Lgr5+ ISCs led to diminished self-renewal. Krt19+ cells' ability to trace all epithelial cell lineages was abrogated in the absence of Fzd5. Instead, they formed sporadic labeling cells belonging to enterocytes. However, neither of these two models disrupted crypt integrity. We further performed Fzd5 knockout in all epithelial cells, utilizing the Villin-Cre-ERT2 model, and found severe crypt deterioration. The crypt loss was mainly due to stem cell diminish, proliferation inhibition, aberrant differentiation, and increased apoptosis. We proposed the model that Fzd5 governed crypt cell variable competence in which they become biased towards renewal or differentiation. ScRNA-seq revealed RNA profiling change primarily in ISCs and transition state cells. And CUT&Tag of transcription initiator Pol II and histone activator H3K4me3 demonstrated that the differences in transcription mounts could be ascribed to a similar change in the chromatin level. Integrated ScRNA-seq and ScATAC-seq analyses showed that the Fzd5 controlled chromatin accessibility and regulated stem and lineage-related gene expression in ISCs and transition state cells, mainly through activating Wnt and repressing Smad2 signalings. In addition, Wnt3A and Wnt5A acted as major ligands for Fzd5 in our organoid culture system, confirming both canonical Wnt and non-canonical Wnt were involved. Collectively, these data reveal that Fzd5, a Wnt receptor receiving signals derived from the niche, is a crucial intestinal homeostasis regulator, which provides novel insights into ISCs dynamics.

Keywords: Fzd5, Intestinal stem cell dynamics, Single-cell transcriptome and epigenome

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GENERATING FUNCTIONAL HUMAN THYMIC EPITHELIAL CELLS FROM INDUCED PLURIPOTENT STEM CELLS

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The thymus plays an essential role in the maturation of T cells and their ability to distinguish self from non-self antigens. Thymic atrophy associated with aging is a well-known factor conferring the decline in immunity, making this group more susceptible to infectious diseases and cancer. Several factors contribute to the deterioration of the immune system, including the failure to stimulate the maturation of lymphocyte progenitors, resulting in a reduced pool of naive T cells, limited T cell receptor diversity, impaired antigen recognition, and decreased T and B effector cell functions. These determinants combine to impart immune senescence in aging humans. This is one of the main reasons why common vaccines are less effective in the elderly than younger individuals. Despite significant advances in developmental biology and immunology, sufficient progress has yet to be made in understanding human thymus development, function, and age-related atrophy. Most of our understanding of human thymus development and functions is based on rodent models that differ in their lymphoid and stromal cell subpopulations, the critical components of lymphocyte differentiation. Advancements in induced pluripotent stem cell (iPSC) technology and humanized mouse model establishment provide unique platforms for understanding human thymocyte function. iPS cells enable the reprogramming of an adult cell to generate CD34+ hematopoietic stem cells (HSCs) and thymocyte precursors, which are essential for developing new therapies. Our goal has been to use iPSC technology to generate an autologous humanized mouse model by generating iPSC-derived

thymic and HSCs. Our group has established several iPSC cell lines derived from melanoma patients. Utilizing these, we developed iPSC-derived thymic epithelial cells expressing common thymic epithelial markers such as EpCAM, FOXN1, and STAB1. We have generated organoids with the iPSC-derived thymic epithelial cells, which can induce differentiation of HSCs into CD45+ hematopoietic progenitor stem cells and CD3+ thymocyte progenitor cells. Our next steps are to test the iPSC-derived thymic organoids in vivo to recreate the immune system in immune-deficient mice. This model will serve as the platform for understanding immunotherapy resistance in melanoma patients.

Funding Source: This work was supported by NIH grants P01 CA114046-13, R01 CA258113, and R01 CA255295

Keywords: Inducble Pluripotent Stem Cells, thymic epithelial cells, lymphocyte differentiation

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HUMAN PLATELET LYSATE PROMOTES THE ECTODERMAL LINEAGE DIFFERENTIATION OF HAIR FOLLICLE-DERIVED STEM CELLS

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Hair follicle stem cells (HFSCs) residing in the bulge of hair follicles are typically involved in the maintenance of skin homeostasis. Besides being highly proliferative, HFSCs can generate neurons as well as glial cells with a potential application in neurodegenerative diseases. Melanocytic differentiation is another propensity of HFSCs, that can be explored for treating pigmentary disorders. However, the clinical application of HFSCs requires xeno-free culture conditions. Recently, human platelet lysate (hPL) has been explored as an alternative to fetal bovine serum. Therefore, the present study investigated the influence of hPL in retaining stem cell phenotype and ectodermal differentiation of HFSCs. Full-thickness skin tissues were digested in 0.1% dispase and the hair follicles were separated out. The explants were cultured in DMEM supplemented with 5% hPL. Basic characteristics, such as morphology and growth kinetics were analyzed along with the expression of stem cell markers, such as CD29, Nanog, and OCT4 by flow cytometry. Neuronal induction medium was supplemented with β-mercaptoethanol, basic fibroblast growth factor, epidermal growth factor (EGF), 3-isobutyl-1-methyl xanthine, and dibutyryl cyclic adenosine monophosphate (dBcAMP). Melanocytic induction medium consisted of human melanocyte growth supplement, EGF, dBcAMP, α-melanocyte stimulating hormone, and endothelin-1. Following induction, the cells were characterized by morphology, immunofluorescence assay, real-time polymerase chain reaction (qRT-PCR), and cytochemical staining. HFSCs in hPL displayed positivity for CD29 as well as Nanog and Oct4. Further, cells induced into neurons exhibited neuronal-like morphological features with dendritic branching patterns and expressed neurogenic markers, such as nestin, β3-tubulin, Sox10, GLB1, GFAP, MAP2, and nerve growth factor. Cells grown in melanocyte induction media stained positive with L-DOPA dye, expressed melanocyte-specific markers, such as tyrosinase, tyrosinase-related protein 1, and dopachrome tautomerase. The findings validate that HFSCs could serve as a putative source of stem cells for ectodermal cell therapy. Further, hPL is an excellent serum-free culture supplement as it supports the expansion and differentiation of HFSCs.

Funding Source: This work was supported by Nitte (Deemed to be University), Mangaluru, India.

Keywords: Hair follicle stem cells, Ectodermal differentiation, Human platelet lysate

TOPIC: GERMLINE AND EARLY EMBRYO

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HUMAN PARTHENOTE STEM CELLS: THE CHALLENGES

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Pluripotent stem cells derived from unfertilized human eggs hold the promise of uniform gene editing at the haploid state. Haploid egg chromosomes genetically modified following extrusion of the second polar body (MII eggs) and before artificial activation would pass on the genetic modification to all daughter parthenote stem cells, whether haploid or undergoing diploidization. Derivation of human parthenote stem (hPS) cells has been hampered by federal funding restrictions, ethical concerns about women donating eggs for research, the paucity of human eggs for study, and the lack of response of human eggs to artificial activation protocols successful in animal models. Our previous studies of freshly collected, or cryopreserved, MII human oocytes revealed activation rates of approximately 90%, progression to blastocysts of approximately 22%, but no stable stem cell lines. Most of the activated MII human eggs arrested at the 6- to 8-cells. To improve the artificial activation of human eggs, we have utilized eggs discarded by egg banks for fertility treatment. Each cycle of egg collection yields oocytes at various stages of maturation: some are at MII and cryopreserved for fertility treatments, and others are dysmorphic or immature and discarded. We have studied such discarded eggs with two purposes: to ascertain their potential to undergo successful activation, and to attempt to re-set their maturation state to improve their potential. An ambient temperature transport system was developed for the discarded eggs to avoid cryopreservation. To evaluate their meiotic maturation competence on arrival, half (88 eggs) were stained with Hoechst 33258 and fluorescent phalloidin. Of those, 99% were alive, half were MII, 23% were MI, 19% were germinal vesicle (GV) stage, 6% had

activated enroute. Following overnight culture of the remaining 81 discarded eggs, 98% were alive, 29% were spontaneously activated, 50% were MII, 10% were MI and 7% were GVs. Pilot studies of activation potential of the discarded eggs reveal approximately 40% undergo cleavage following either Sr++, or Ca++ ionophore treatment. These results support the potential of human eggs routinely discarded during fertility procedures to assist in overcoming the barriers to successful derivation of hPS cells.

Funding Source: Bedford Research Foundation

Keywords: Human parthenote stem cells, Discarded human eggs, Artificial activation of human eggs

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TAURO-URSODEOXYCHOLIC ACID (TUDCA) A TOOL TO ENHANCE CHIMERIC EMBRYO DEVELOPMENT

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Organ demand outpaces the current supply of organs for transplantation. Regenerative medicine has sought alternative methods to generate an inexhaustible source of organs, with one approach being blastocyst complementation to produce exogenic organs in chimeric animals. However, developing highly efficacious and competent interspecies chimeras has been impeded by various interspecies chimeric barriers. One of these proposed barriers is interspecies cell competition and survival of the donor stem cells and host embryos. Tauro-ursodeoxycholic acid (TUDCA) is a bile acid that has been shown to have anti-apoptotic effects in embryos. Thus, TUDCA may be a tool to enhance chimeric competency. In this study, we assessed TUDCA's effect on cell growth and apoptosis in donor stem cells. We also investigated TUDCA's effect on mouse embryos by assessing early embryonic morphology and apoptotic cells. From our cell culture experiments, we found that lower concentrations of TUDCA trended towards no difference in cell growth and apoptosis, but high concentrations of TUDCA were cytotoxic. We also found that 100uM TUDCA concentration resulted in healthy mouse embryo morphology and reduced apoptosis. These findings suggest that TUDCA is a tool that lowers the level of apoptosis expressed by the host embryo but does not necessarily impact the donor cells cultured separately. TUDCA has the ability to alleviate some stress in the host embryo, making it possibly more amenable to donor cells used during blastocyst complementation.

Keywords: embryogenesis, chimeras, stem cells

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DIETARY INDUCTION OF ENDOMETRIAL EPITHELIAL REGENERATION

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Endometrial ailments such as endometriosis, infertility, and endometrial cancer impact 1 in 4 women in the US, accentuating the significant need to improve our understanding of endometrial biology. Endometrial stem cells regenerate the endometrium monthly, and their dysregulation is implicated in endometrial diseases. Recent studies have underscored the capacity for the metabolic reprogramming of stem cells, but little is known about the metabolic regulation of stem cell states in endometrial physiology and disease. Here, we explore how fatty acids affect endometrial stem cell regeneration. We performed a diverse fatty acid (FA) screen on human and mouse endometrial organoids and discovered a subset of ω-6 fatty acids converging on arachidonic acid (AA) with stem cell-enhancing effects. Using bulk and single-cell RNA sequencing analysis, we found AA to induce de novo stem cell states, dedifferentiation programs, and repair-associated stem cell reprogramming signatures in response to AA. Metabolic interactome modeling revealed AA to beget Prostaglandin E2 (PGE2) and activate the Ptger4 → cAMP → PKA signaling axis to promote stemness in mice and humans. This data demonstrates that dietary AA is a conserved promoter of endometrial regeneration that mimics the injury repair response through PGE2-Ptger4 signaling. These findings highlight the significance of dietary fatty acids in regulating endometrial tissue regeneration.

Keywords: Reprogramming, Endometrium, Metabolism

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FATTY ACID METABOLISM CONTROLS CELLULAR TRAFFICKING FOR FUNCTIONAL REPRODUCTION VIA NUTRITION SIGNALING

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Cell metabolism is recently known to control stem cell behavior for tissue homeostasis. However, the involved mechanisms are largely unclear. To have a systemic study of stem cell metabolism at an organismic level, we have conducted an RNAi-based screen for metabolic enzymes that control Drosophila ovarian germline stem cell (GSC) fate. One of the candidates, Acetyl-CoA Carboxylase (ACC) catalyzes acetyl-CoA to malonyl-CoA which promotes fatty acid synthesis (FAS) but suppresses fatty acid oxidation (FAO). Here, we report that ACC regulates cellular trafficking to

maintain GSCs and ensure GSC progeny proper differentiation, contributing to functional reproduction. Interestingly, nutritional deprivation feeding restored homeostatic balances in ACC-depleted germline. Our findings argues a role of ACC in maintaining cellular energy balance for germline homeostasis and may offer therapeutic chances for metabolic disorders by nutrient interventions.

Keywords: stem cell metabolism, nutrient signaling, cellular trafficking

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PIG EXTRAEMBRYONIC ENDODERM (XEN) CELLS CAN BE ESTABLISHED IN A CHEMICALLY DEFINED CULTURE SYSTEM

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Extraembryonic endoderm (XEN) cells can be derived from blastocyst primitive endoderm (PrE), becoming a useful tool for studying mammalian development, including early lineage segregation and embryo patterning in vitro. The establishment of stem cells representing the respective lineages in blastocysts has been robustly attempted in domestic animals, especially pigs, to reconstitute embryogenesis in vitro for comparative studies. Several studies on pig XEN cells have been conducted under serum-free conditions supplemented with signaling molecules but these culture conditions compromised the self-renewal ability of XEN cells when compared to FBS-containing media, possibly because of inappropriate extrinsic cues. Therefore, we developed a chemically defined culture system for pig XEN cells by dissecting the signals governing the core gene network of the PrE lineage and found that several signaling pathways are essential for maintaining their rapid proliferation rate. These cells recapitulated the molecular features and differentiation capacity of porcine PrE lineage, with PrE derivative-specific markers detected during proliferation and XEN-derived spheroid formation, respectively. In addition, species-specific characteristics of pigs were observed, including the involvement of lipid metabolism and NANOG/GATA co-expression in XEN cells. Taken together, we derived stable pig XEN cell lines that efficiently proliferate in a serum-free culture system, producing reproducible and homogenous pig XEN cells. They can be employed as a cell culture model representing extraembryonic endodermal lineages to recapitulate mammalian embryogenesis. Further, our culture systems can shed light on more precise key molecules involved in PrE lineage development by excluding the effect of serum-derived factors. Therefore, our findings can contribute to the expansion of the understanding of developmental biology and its biomedical applications.

Funding Source: This work was supported by the BK21 Four program, the Korea Evaluation Institute of Industrial Technology (KEIT) [20012411]; and the National Research Foundation of Korea (NRF) grant [2021R1A2C4001837 & 2023R1A2C1005026].

Keywords: XEN, Pig, Signaling pathway

1025

IDENTIFYING THE ROLE OF EPIGENETIC GENE REGULATORY MECHANISMS DRIVEN THROUGH MATERNAL OBESITY ON EMBRYONIC NEUROGENESIS.

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Neurodevelopmental disorders have a complex etiology that starts very early on during embryonic development through the involvement of specific gene regulatory mechanisms. A perturbed metabolic environment due to maternal obesity could cause dyslipidemia and adversely affect the developing central nervous system of the embryo. Research shows that maternal obesity alters developmental neurogenesis and predisposes the developing fetus to neurodevelopmental disorders. Among several gene regulatory mechanisms, altered histone modifications due to changes in the expression of histone-modifying enzymes or the levels of their substrates is proposed as a key mechanism of neurodevelopmental defects in offspring born to metabolically compromised pregnancies. This study uses the Wistar rat model of maternal obesity for the identification and functional validation of novel molecular mechanism/s of maternal obesity-mediated alterations on embryonic neurogenesis. Cortex tissue samples harvested from embryos of female Wistar rats fed with normal and high-fat diets are subjected to an array of molecular and biochemical techniques. Genome-wide high-throughput RNA-sequencing revealed that maternal obesity dysregulated many genes of key developmental/signaling pathways in the developing embryo brain cortex. Alterations in global histone acetylation and methylation during cortical neurogenesis associated with these differential gene expressions have been identified and analyzed through High-throughput ChIP-Seq analysis. Possible molecular mechanisms driving these specific histone modifications have also been tested for and proposed thus providing a complete molecular picture of the potential effect of a high fat-fed maternal environment on embryonic neurodevelopment. The knowledge thus gained is critical in the development of preventative strategies and verifying therapeutic or diet interventions for abnormal neurogenesis and faulty embryo brain development of metabolically compromised pregnancies.

Keywords: Maternal-obesity, cortical-neurogenesis, Neurodevelopmental-disorders

TOPIC: HEMATOPOIETIC, IMMUNE, AND ENDOTHELIAL

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RBFOX2-MEDIATED ALTERNATIVE SPLICING SELECTIVELY GOVERNS HEMATOPOIETIC STEM CELL SELF-RENEWAL

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Hematopoietic stem cells (HSCs) persist throughout life via self-renewal. How HSCs are differentially regulated from downstream progenitors remains poorly understood. We found that HSCs displayed a distinct mRNA alternative splicing pattern and preferentially expressed Rbfox2, an alternative splicing factor, compared with multipotent progenitors (MPPs). Deletion of Rbfox2 from the hematopoietic compartment specifically depleted HSCs, but not progenitors, in the adult bone marrow. In contrast, deletion of Rbfox1 did not affect the number or function of HSCs. Rbfox2-deficient HSCs, but not progenitors, exhibited compromised proteostasis, including an increased protein synthesis rate and accumulated misfolded/unfolded protein contents. Rbfox2 loss altered the alternative splicing of Mpl in HSCs by increasing the fraction of functional full-length Mpl isoform. Small molecules that restore proteostasis rescued HSC depletion in Rbfox2-deficient mice. Our work reveals that HSCs selectively rely on Rbfox2-mediated alternative splicing for their maintenance.

Keywords: hematopoietic stem cell, alternative splicing, proteostasis

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SOLUBLE SIGNALS TO IMPROVE VASCULAR INTEGRITY IN THE LUNG

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Disorders in vascular integrity are a prominent feature in many pulmonary vascular diseases. Paracrine signals are enriched throughout the whole lung tissue and are critical in regulating the homeostasis and regeneration of the functional pulmonary microvasculature. Here, we studied paracrine signal interactions in the native human lung microvascular niche and identified soluble factors that are critical in endothelial integrity. Leveraging the previously published single-cell dataset, we applied single-cell RNA-seq-based computational model Connectome to analyze ligand and receptor interactions in the native human lung microvascular niche. The spatial localization and secretion levels of soluble factors were confirmed using immunostaining and ELISA assays. The impact of soluble factors on cellular functions was assessed through electrical resistance impendence sensing and transwell assays. We also applied lipopolysaccharide (LPS) -induced lung injury model to confirm the effect of soluble factors on vascular integrity in animal settings. We identified that SLIT2, ANGPT1, ADM, VEGFD, and BMP5 strongly interact with microvascular endothelium in the human lung microvascular niche. Immunostaining and ELISA assays confirmed their spatial localization and secretion level in native human distal lung samples. Upon treatment with these ligands, we found that VEGFD could markedly increase the electrical resistance and decrease the permeability of human lung microvascular, arterial, and venous endothelial cells in vitro. Furthermore, treatment with VEGFD reduced pro-inflammatory response including vascular permeability and immune cell infiltration in LPS-induced lung injury model. This data demonstrates strong cell-cell crosstalk interactions in the pulmonary microvascular niche and suggests a potentially novel role of VEGFD in regulating vascular integrity and regeneration.

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Keywords: Lung microasculature, Multi-omics, Paracrine signal

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CATHEPSIN LA IS NECESSARY FOR THE DEVELOPMENT OF HEMATOPOIETIC NICHE AND STEM CELL COLONIZATION

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Hematopoietic stem cells (HSCs) reside in a transient sinusoidal hematopoietic niche during development. The embryonic hematopoietic niche develops as a highly branched sinusoidal network that supports HSC expansion. Later in development, the niche regresses to a less-branched structure where HSCs egress and migrate to an adult hematopoietic niche. To investigate mechanisms of the embryonic hematopoietic niche regression, we performed scRNA-seq on the niche endothelial cells (ECs) at two developmental time points, which revealed a dramatic transcriptional change of niche ECs. We found that the expression of cathepsins, the lysosomal proteases, in the niche ECs diminishes during development. Morpholino knockdown of cathepsin la (ctsla) led to a deformed and less-branched vascular niche as evidenced by a 25.5% decrease in intervascular cavities (n = 14). To test if macrophages, another major source of cathepsins, are responsible for niche deformity, we ablated macrophages with irf8 morpholino in ctsla morphant embryos. Regardless of the macrophage ablation, ctsla knockdown resulted in a niche with fewer branches and fewer intervascular cavities. (n = 8) Together, these data suggest that ctsla expression from embryonic niche ECs is likely responsible for the deformed vascular structure in an EC autonomous manner. To test if impaired niche leads to a hematopoietic defect, we live imaged Tg(runx1+23:mCherry) reporter line of HSPCs. Strikingly, the impaired niche of ctsla morphant contained significantly fewer HSPCs (51.2%, n = 17). Timelapse live imaging of Tg(CD41:GFP; flk:mCherry) reporter line of HSCs budding from the hemogenic endothelium demonstrated that a knockdown of ctsla does not affect the emergence of HSCs. EdU staining of HSPCs in the embryonic niche showed proliferation of HSPCs is not affected by ctsla knockdown (n = 12), suggesting that the decreased HSPCs in the ctsla morphant is attributable to impaired HSPC colonization of the niche. These results demonstrate that ctsla is necessary for neovascularization and HSC colonization of an embryonic hematopoietic niche.

Keywords: Hematopoietic niche, Cathepsin, Neovascularization

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SINGLE CELL INSIGHT INTO HAEMATOPOIETIC DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

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One of the challenges in regenerative medicine remains generating functional haematopoietic stem cells (HSCs) of clinical quantity. Utilizing data from single cell RNA sequencing (scRNA-seq) of human embryonic stem cells (hESC) derived cells, we explored the differences that exist between in vitro and in vivo haematopoiesis, with the aim that understanding these differences could help improve future chances of generating HSCs in vitro. Functional analysis into in vitro differentiation using an established haematopoietic differentiation protocol, confirmed that the current differentiation strategies may be myeloid and NK lineage biased compared to haematopoietic lineages from umbilical cord blood. The population lineage dynamics was also observed to change over time. Using scRNA-seq, we investigated dynamic gene expression of cell populations emerging during hESC differentiation. We observed that the haematogenic endothelium capable of generating the haematopoietic progenitors emerges as early as day 6 of differentiation. We also observed that priming of the arterial endothelial cells to the haematogenic endothelium, and subsequent emergence of haematopoietic progenitors is associated with an increase in ribosomal and mitochondrial gene activity. Finally, a comparative analysis of the hESCs generated dataset with publicly available dataset of embryonic haematopoietic tissues revealed that hESC derived haematopoiesis, associated with increased mitochondrial gene activity, is likely mimicking events of the yolk sac haematopoiesis, rather than the haematopoiesis occurring in the AGM region. Based on our data, we hypothesize that increased mitochondrial gene activity, likely promoted by the normoxic culture conditions, may be contributing to the challenge in generating HSCs from hESCs in the dish.

Keywords: Single Cell RNA Sequencing, Transcriptional landscape, Mitochondrial gene activity

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DETERMINING HOW BLOOD VESSELS ARE REGULATED DURING LIMB REGENERATION IN AXOLOTLS: DEVELOPING NOVEL GENETIC MANIPULATION METHODS

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Mammalian regenerative capacity is severely restricted and limbs cannot. Therefore, salamanders, such as axolotls, can provide important information on how tetrapods can regenerate limbs, which could lead to therapeutic intervention. Limb regeneration results in the growth of a fully functional, including requisite tissues, generated via the formation of a blastema which dedifferentiates into different cell types. However, how vasculature regrows and interacts with other cells during regeneration is not well understood. We are currently characterizing vasculature using immunohistochemistry and HCR in situ and generating transgenics to visualize and modulate vasculature during development and regeneration. Axolotl CRISPR knock-in Kdr (VEGFR2) founders express GFP and the avian TVA receptor, which permits viral entry; we will deliver cargo plasmids containing Cas9 and validated gRNAs identified through our transcriptional analyses. We have also used Tol2-mediated transgenesis to express endothelially-restricted Cas9. Finally, we have established axolotl HCR in situ hybridization (HCR) for endothelial-specific genes. We will use HCR to validate transgenic lines and characterize vascular identities. We will determine how vascular disruptions affect transcriptional profiles of all cell types via single-cell RNA sequencing

at time points to understand how blood vessels and other cell types interact during regeneration.

Funding Source: Human Frontier Science Program NIH

Keywords: Limb regeneration, Angiogenesis, Transgenesis

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INDUCTION OF MULTIPLE ENDOGENOUS CYTOKINES AS A NEW STRATEGY FOR MOBILIZATION OF HEMATOPOIETIC STEM CELLS FROM THE BONE MARROW TO THE BLOOD

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Pharmacological mobilization of cells from the bone marrow (BM) to blood is used for collection of hematopoietic stem cells (HSCs) for transplantation. Successful transplantation is determined by the number and regenerative potential of HSCs present in the mobilized blood. In case of allogenic transplantation, graft versus host disease (GVHD) driven by T-cells present in the graft remains one of the biggest challenges of mobilized blood transplantation. Thus, there is still a need for new mobilizing therapies, which will allow for collection of sufficient number of functional HSCs, while minimizing the risk of GVHD in the recipient. Currently, the most commonly used mobilizing drug is recombinant G-CSF (rhG-CSF). Our aim is to develop an alternative strategy, which induces endogenous G-CSF and other mobilizing factors to overcome the limitations of current clinical strategies. We discovered that cobalt protoporphyrin IX (CoPP) induces the mobilization of cells from BM. CoPP induces not only G-CSF, but also other cytokines with mobilizing properties, such as interleukin-6 (IL-6), CXCL1 (GROα) and CXCL2 (GROβ). To compare the kinetics of the CoPP- and rhG-CSF-induced mobilization, we analyzed the phenotype and numbers of blood cells in C57BL/6 mice injected daily with CoPP or rhG-CSF for 1 to 5 days. We observed the increase of hematopoietic stem and progenitor cells (HSPCs) in blood starting from day 2 in both groups. However, at day 4, in mice injected with CoPP the number of HSPCs increased sharply and was 4 times higher than in the mice treated with rhG-CSF. Additionally, we saw differences in granulocyte mobilization. The increased numbers of mature granulocytes appeared already 6h after CoPP injection, and further started to rise at day 3. In comparison, in the mice treated with G-CSF the numbers of mature granulocytes started to increase only at day 3. In summary, CoPP induces more efficient and rapid mobilization of HSPCs than rhG-CSF. Different kinetics of CoPP- and recombinant rhG-CSF-induced mobilization suggests that other cytokines may modulate the effect of G-CSF and possibly affect the functional properties of mobilized cells. CoPP could be considered as a new potential drug for mobilization, as it simultaneously induces multiple endogenous mobilizing factors.

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Keywords: Hematopoietic stem cells, Cell mobilization, Granulocyte colony-stimulating factor

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CONSERVED REGULATORS OF MITOCHONDRIAL ACTIVITY AND DYNAMICS MEDIATE DEVELOPMENTAL HEMATOPOIESIS

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Mitochondrial morphology and dynamics are known to affect blood stem cell differentiation, proliferation and aging. However, further roles of mitochondrial homeostasis in developmental hematopoiesis are unclear. We previously showed that the mitochondrial morphology and dynamics regulator and stem cell protein Asrij/OCIAD1 is essential for maintenance of human pluripotent stem cells in vitro as well as Drosophila blood progenitors in vivo. Asrij/OCIAD1 depleted cells possess elongated mitochondria, leading to precocious differentiation and lineage bias. Hence, we hypothesized that additional mitochondrial regulators may control hematopoiesis. High throughput studies and our immunoprecipitation experiments revealed that Asrij interacts with the mitochondrial AAA proteases YME1L and AFG3L2 in human immortalized cells. Knockdown of proteases in Drosophila lymph gland hematopoietic progenitors caused lethality and developmental delays. Interestingly, YME1L or AFG3L2 depletion from differentiated blood cells in circulation showed dramatic hyperproliferation and differentiation in the lymph gland. These effects could be rescued by pharmacologically quenching ROS by feeding larvae with N-acetyl cysteine (NAC) or genetically by overexpressing catalase. Thus, AAA protease-mediated mitochondrial homeostasis is essential to regulate inter-organ communication that maintains hematopoiesis in response to systemic cues. We also investigated the role of mitochondrial homeostasis in the endothelial to hematopoietic transition (EHT) in mouse embryonic hematopoiesis. Emerging HSCs have increased mitochondrial mass compared to the aortic endothelium. Further mitochondrial activity and potential are also increased, accompanied by changes in morphology and expression of fission-fusion regulators. Asrij null mice will further be used to study implications of perturbed mitochondrial homeostasis on EHT progression. This suggests that conserved mechanisms regulating mitochondrial homeostasis actively control developmental hematopoiesis.

Keywords: Hematopoiesis, Mitochondria, Development

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SELECTIVE SENSING AND REVERSAL OF ADAR1-MEDIATED BASE EDITING IN PRE-CANCER AND LEUKEMIA STEM CELLS

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Innate immunity-induced adenosine deaminase acting on RNA1 (ADAR1) base editing (BE) of adenosine to inosine (A-to-I) inhibits RNA viral replication and prevents retroviral integration into the human genome. However, inflammation-associated ADAR1p110 to p150 splice isoform switching drives cancer stem cell (CSC) generation and therapeutic resistance in 20 malignancies. Previously, predicting and preventing ADAR1p150-mediated malignant RNA editing represented a significant challenge. Herein we describe: 1) a selective lentiviral ADAR1 activity reporter that enables non-invasive sensing of A-to-I BE; 2) a quantitative ADAR1p150 intracellular flow cytometry assay; 3) inhibition of leukemia stem cell (LSC)-enriched ADAR1 expression and activity by a potent small molecule splicing modulator, Rebecsinib (17S-FD-895); and 4) successfully completed pre-IND studies with Rebecsinib. Whole transcriptome sequencing of primary hematopoietic stem and progenitor cells (HSPCs) and LSCs purified from myeloproliferative neoplasm and acute myeloid leukemia (AML) patient samples revealed a splice isoform switch in pre-leukemia progenitors that favored ADAR1p150 production. In contrast to catalytically inactive ADAR1 (E912A) mutant or ADAR2 proteins, the lentiviral ADAR1 nanoluciferase-GFP reporter showed a dose dependent increase in A-to-I BE that responded to overexpression of wild-type ADAR1p150. In functional stromal co-culture assays, Rebecsinib brought about decreased myelofibrosis pre-LSC viability, survival, and self-renewal, commensurate with reduced ADAR1p150 protein expression and reporter activity. Because Rebecsinib exhibits a favorable therapeutic index in AML LSC-engrafted in vivo models, we performed IND-enabling multi-species studies to assess the toxicokinetic and pharmacodynamic performance of this compound. With a clinically tractable formulation, Rebecsinib showed predictable pharmacological (PK/PD) properties combined with favorable bioavailability and stability, thereby enabling twice-weekly intravenous dosing regimens with no evidence of systemic toxicity. Together, these results lay the foundation for developing Rebecsinib as a clinical ADAR1p150 antagonist aimed at obviating malignant microenvironment-driven LSC generation.

Keywords: RNA editing, AML, leukemia stem cells

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INHIBITION OF HYPERACTIVATED LYSOSOMES RESOLVES INTRINSIC INFLAMMATION AND RESTORES YOUTHFUL PROPERTIES AND FUNCTIONS IN OLD HEMATOPOIETIC STEM CELLS

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Aging is the primary risk factor for many late-onset disorders. Aging of hematopoietic stem cells (HSCs) is specifically implicated in the rise of myeloid malignancies and immune deficiencies in the elderly. Despite major progress, mechanisms that promote aging of HSCs remain to be elucidated. As quiescence maintains HSCs' health and function throughout life, loss of quiescence with age is linked to old HSCs' defects including their myeloid-biased lineage commitment. We have identified dynamic lysosomal modulations as a switch in the precise control of HSC quiescence vs. priming/activation in young HSCs. Here we show that lysosomes are relatively depleted and lysosomal properties and functions are compromised, independently of the known defective autophagy, in old HSCs. Lysosomes were hyper-acidic and their activity enhanced in old relative to young HSCs. This was coupled with old HSCs' alteration of expression of lysosomal enzymes Cathepsin C and D and the vacuolar H+ -adenosine triphosphatase ATPase (v-ATPase). Specifically, we show that lysosomal defects led to a build-up of cytosolic damaged mitochondrial DNA that activated the cGAS-STING/IRF3/TBK1 pathway resulting in increased intrin-

sic inflammatory gene expression in old HSCs. The inhibition of lysosomal acidification by v-ATPase repression, ex vivo, restored lysosomal properties in old HSCs, and resulted in the normal expression of histone marks and intrinsic inflammatory genes similar to what was observed in young quiescent and healthy HSCs. This treatment also increased substantially the fraction of non-dividing old HSCs in culture, and the frequency of old HSCs-derived LTC-ICs. Importantly, ex vivo inhibition of lysosomal activity increased old HSCs' in vivo competitive repopulation ability by more than 8-fold over 21 weeks and balanced the production of lineages downstream of old HSCs. Overall, our work indicates that inhibiting lysosomal hyper-activation restores old HSCs' lysosomal properties, reduces intrinsic inflammation and restitutes stem cell function in old HSCs. These findings may be exploited for improving HSCs' potential in the elderly.

Keywords: Hematopoietic Stem Cells, aging, lysosomes

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DISSECTING THE ROLE OF FOS EARLY ACTIVATION GENE IN HEMATOPOIETIC STEM CELL QUIESCENCE AND ACTIVATION

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Most hematopoietic stem cells (HSCs) exist in a dormant state in the bone marrow of adult mice; however, HSCs can exit the marrow, travel through the circulation, expand, and differentiate when activated. Stimulation by mobilizing agents (such as G-CSF) drives quiescent HSCs to migrate into the bloodstream in greater numbers and also drives a quiescence to activation switch. Previous work from our lab identified Early growth response 1 (Egr1), a member of the EGR family of zinc finger proteins that interacts with members of the Activator Protein 1 (AP-1) class of transcription factors, as a key regulator of HSC migration and proliferation in adult mice. Recently, we further demonstrated involvement of the EGR1-interacting AP-1 protein FOS in the activation from quiescence of muscle stem cells in response to tissue injury. Based on these results, we hypothesize that FOS may similarly orchestrate the activation-associated migration and differentiation of HSCs, stimulated by G-CSF. To test this hypothesis, we administered G-CSF to FOS-GFP reporter mice and monitored subsequent activation of FOS expression. Two hours after G-CSF administration, 5.6+/-2.0% of bone marrow HSCs of young adult mice and 6.9+/-3.3% of HSCs from aged mice demonstrated induction of FOS expression. To determine the functional consequences of Fos induction in this subset of HSCs, we transplanted FOS+ and FOS- HSCs, competitively with congenic helper marrow cells, to monitor their engraftment, expansion, and differentiation capacities. In parallel, we evaluated the in vitro differentiation and proliferation capacity of the sorted FOS+ and FOS- HSCs by colony formation assay. Results to date indicate that Fos expression in HSCs is correlated with increased differentiation and loss of the stem cell pool. Finally, to examine the necessity of Fos induction for changing HSC functions, we analyzed constitutive or conditional deletion of Fos in HSCs and other blood cells, using Fosfl/fl mice crossed to either Vav1-iCre or HSC-SCL-CreERT mice. While studies of HSC-SCL-CreERT; Fosfl/fl mice are still ongoing, analysis of Vav1-iCre; Fosfl/fl mice demonstrate that deletion of Fos early in hematopoietic ontogeny phenocopies germline knockout of Fos, with significant disruption of bone formation and induced extra-medullary hematopoiesis.

Keywords: Hematopoietic Stem Cell, Fos (c-Fos), Quiescence to Activation Switch

1049

FINE-TUNING ENDOGENOUS ENDOTHELIAL VASCULATURE IN HUMAN PLURIPOTENT SPHEROIDS

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Vascularized spheroids made from human pluripotent stem cells have garnered interest as a novel platform for the study of angiogenesis, tissue engineering and drug discovery. However, current methods require lengthy culture time and complex media changes with expensive growth factors. Here, we describe the creation of vascularized spheroids within a week using human pluripotent stem cells (hPSCs) transduced with doxycycline-inducible ETV2. Involved in vascular development, ETV2 serves as a master regulator of endothelial cell specification. By modulating the expression of ETV2, we were able to tune the formation of vascular endothelial networks within hPSC spheroids. Interestingly, control samples with no ETV2 upregulation also produced open-lumen vascular networks cultured in basal media alone. Furthermore, our results show that addition of growth factors (VEGF, FGF2) may actually hinder development of vascular networks, compared to endogenous ETV2 and control basal conditions. Preliminary quantitative PCR results show increased Ephrin-B2 expression in spheroids cultured with growth factors in contrast to those without, implying the ability to adjust vascular fate. Overall, our data suggest that the optimal conditions for promoting vascularization in these spheroids may be simpler than previously thought. Our results demonstrate that ETV2-transduced hPSCs efficiently form functional, self-assembled vascularized spheroids in a short amount of time, providing a valuable tool for further fine-tuning of vessels as well as the in vitro study of angiogenesis and the discovery of anti-angiogenic drugs.

Keywords: Stem cells, Endothelial cells, Spheroid

1051

SELECTIVE TARGETING OF LEUKEMIC STEM CELLS THROUGH MODULATION OF SELENOPROTEIN BIOSYNTHESIS BY PSTK

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The exquisite differences in the susceptibility to oxidative stress of normal hematopoietic stem cells (HSCs) and leukemic stem cells (LSCs) is a result of their differing metabolic processes. In contrast to HSCs, LSCs have a higher dependence on antioxidant and detoxification pathways to defend against the damaging effects of ROS generated through mitochondrial oxidative respiration. Our study discovered that modulating the expression of the critical enzyme, phosphoserine tRNA kinase (PSTK) in the selenoprotein biosynthesis pathway can selectively kill cancer cells. Abolishing PSTK leads to an expansion of HSCs, but impairs the differentiation of B and T cells; partial inhibition of PSTK has minimal impact on HSC functionality. Nevertheless, even moderate PSTK reduction in LSCs results in a disruption of selenoprotein synthesis, elevated intracellular ROS and induction of ferroptosis. The inhibition of PSTK through a degrader shows remarkable therapeutic efficacy against acute myeloid leukemia (AML) in vivo, with limited side effects on normal HSCs. These findings highlight the heightened vulnerability of leukemia cells to PSTK depletion, and underscore the potential for precise regulation of PSTK levels as a therapeutic strategy for AML treatment without affecting HSC functionality.

Keywords: Oxidative stress, selenoprotein biosynthesis, acute myeloid leukemia

TOPIC: KIDNEY

1053

CHARACTERIZATION OF PROGENITOR CELLS IN THE ADULT MAMMALIAN KIDNEY

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Homeostatic renal filtration relies on the integrity of podocytes, which function in glomerular filtration. These highly specialized cells are damaged in 90% of chronic kidney disease, representing the leading cause of end-stage renal failure. While podocytes are thought to have a severely limited capacity for renewal in homeostatic conditions, recent studies highlighted modest podocyte renewal in adult mice following injury. Nonetheless, the mechanisms regulating podocyte renewal following injury in the adult organism remain largely unknown and controversial. Using a mouse model of Adriamycin-induced nephropathy, we report that proper recovery of filtration function following podocyte injury in wild-type mice requires up-regulation of the endogenous gene encoding the protein component of telomerase TERT. To assess the cascade of events involved in podocyte renewal, we used a mouse model of TERT conditional overexpression that allows forceful renewal of kidney podocyte following a pulse of TERT. Specific and stochastic lineage-tracing approaches carried out in this model provide evidence that telomerase drives the activation and clonal expansion of podocyte progenitor cells to give rise to monoclonal glomeruli. Using high throughput sequencing approaches further allowed us to unveil the core pathways involved in TERT pro-regenerative functions in the adult kidney. To characterize podocyte progenitors, we carried out a kinetic single nuclei RNA-seq analysis upon TERT-induced regeneration. Such approach provides insight into the regenerative trajectory of the progenitor cell population during podocyte renewal and further highlight the factors triggering state transitions of these cells. Our findings demonstrate that the adult kidney bears intrinsic regenerative capabilities involving the protein component of telomerase, paving the way for innovative research toward the development of chronic kidney disease therapeutics.

Keywords: Kidney, Telomerase, Regeneration

1055

IN VITRO AND IN VIVO MOLECULAR SIGNATURES OF HUMAN PLURIPOTENT STEM CELL DERIVED NEPHRON PROGENITOR ORGANOIDS TO SUPPORT A POTENTIAL REGENERATIVE KIDNEY CELL THERAPY

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Novel therapies are needed to address the unmet clinical need of patients suffering from chronic kidney disease (CKD) and cell therapy is an innovative strategy that has the potential to deliver effective, curative treatment options to renal patients. There are several recent reports that have developed protocols to differentiate human pluripotent stem cells (hPSCs) into kidney-lineage cells using organoid models and we have further developed a robust and scalable method to produce kidney organoids for cell therapy applications. hPSCs were used to generate nephron progenitor cells (NPCs) through a previously described stem cell differentiation protocol for the formation of kidney organoids. On day 10-11 of differentiation, NPCs were transplanted under the renal capsule of a NOD background mice model, where we studied the in vivo maturation of NPCs up to five weeks after implantation. In parallel, we studied the in vitro differentiation progression and maturation of NPC kidney organoids generated in the same way. RNA sequencing of NPC kidney organoids during in vitro differentiation shows broad representation of renal cell types by expression of markers representing several cell populations present in the developing nephron. There is increased expression over time in vitro of key podocyte- and tubular cell markers, and in vitro maturation leads to strong enrichment of glomerular related makers and also upregulation of markers connected to a functional phenotype such as tubular transporters. We can currently show that NPC kidney organoids implanted under the kidney capsule continue differentiation towards mature renal cell types. Histological evaluation and huRNA sequencing indicate progression of NPC-differentiation in vivo, predominantly towards tubular like cells, and upregulation of renal functional pathways in grafts 5 weeks after implantation.

Keywords: Cell Therapy, Kidney Orgaoids, Kidney Disease

TOPIC: LIVER

1057

CELL-TRANSPLANTATION-INDUCED REPROGRAMMING STATE IS ESSENTIAL FOR HEPATOCYTE ENGRAFTMENT

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For cell-based therapy, successful initial engraftment is critical. However, the mechanism of cell engraftment during transplantation remains elusive. Recent studies have focused on under-

standing the effects of cell transplantation on the host microenvironment, while donor cell behavior after transplantation is largely unknown. Here, we elucidated that the transplanted hepatocytes (Tx-Heps) undergo a rapid reprogramming state transition, which is essential for cell engraftment. Through transcriptome sequencing and in situ staining analysis based on Tx-Heps, we demonstrated that Tx-Heps displayed a reprograming signature during engraftment. Moreover, in vivo and in vitro functional experiments have confirmed that Tx-Heps acquired bi-potential differentiation ability. Notably, this reprogramming state is conserved in different transplantation models and species. Remarkably, lineage tracing experiments clearly showed that all successfully engrafted Tx-Heps undergo this reprogramming state. Importantly, hepatocyte-specific Arid1a ablation inhibited the formation of this reprogramming state and impaired cell engraftment. In conclusion, these findings depict that cell reprogramming in a given cell transplantation system, endowing Tx-Heps with the features of resistance to stress, survival and cell differentiation, facilitating cell engraftment as a new paradigm for understanding mechanisms concerning regulation of systems for addressing basic issues in cell transplantation, tumor metastasis/resistance and cell plasticity.

Keywords: Hepatocyte reprogramming, Cell engraftment, Cell transplantation

TOPIC: MESENCHYMAL STROMAL CELLS, ADIPOSE, AND CONNECTIVE TISSUE

1059

THE LANDSCAPE OF LONG NON-CODING RNAS IN HUMAN MESENCHYMAL STEM CELL-DERIVED SMALL EXTRACELLULAR VESICLES/EXOSOMES

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The regenerative properties of mesenchymal stromal cells, also known as medicinal signaling cells (MSCs), are facilitated by the secretion of small extracellular vesicles (sEVs), also known as exosomes. The therapeutic potential of these sEVs can be increased through various licensing approaches, including cytokines, hypoxia, chemicals, and genetic modifications. The non-coding RNAs present within MSC-derived sEVs have been shown to play a crucial role in tissue regeneration. Despite extensive research on miRNA fingerprints, the landscape of long non-coding RNAs (lncRNAs) in MSC-sEVs has yet to be fully explored. In this study, the authors thoroughly characterized lncRNA signatures in human adipose-derived MSC sEVs, both with and without cytokine licensing, revealing unique lncRNA profiles distinct from their intracellular counterparts. The authors also identified 194 “medicinal signaling lncRNAs” specific to MSC-sEVs and 27 “licensing-responsive lncRNAs” upregulated by cytokines. An analysis of the lncRNA-protein interactome revealed a tightly connected network involved in chromatin remodeling, SWI/SNF superfamily-type complex, and histone binding. Overall, this study sheds light on the landscape of lncRNAs in MSC-sEVs and advances our understanding of their therapeutic potential.

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Keywords: MSCs, Exosomes, lncRNAs

1061

DIFFERENCES IN ANGIOGENIC AND IMMUNOMODULATORY CAPACITIES OF EXTRACELLULAR VESICLES DERIVED FROM BONE MARROW- AND ADIPOSE-DERIVED MESENCHYMAL STROMAL/STEM CELLS AND IMPACTS OF IN VITRO EXPANSION

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Recently, therapies utilizing extracellular vesicles (EVs) derived from mesenchymal stromal/stem cells (MSCs) have begun to show promise in clinical trials. However, EV therapeutic potential varies with MSC tissue source and in vitro expansion through passaging. To find the optimal MSC source for clinically translatable EV-derived therapies, this study aims to compare the angiogenic, osteogenic, and immunomodulatory potentials and the protein and miRNA cargo compositions of EVs isolated from the two most common clinical sources of adult MSCs, bone marrow and adipose tissue, across different passage numbers. Primary adipose- and bone marrow- derived MSCs (ASCs and BMSCs) were isolated from adult Lewis rats. Although both MSC sources secreted EVs with similar mean sizes and average amount of protein per EV at passage 2 (P2), passage 4 (P4), and passage 8 (P8), BMSCs showed a significantly higher EV yield per cell than ASCs at P2 ($p < 0.01$, $n > 6$) and P4 ($p < 0.05$, $n > 6$), but not at P8 ($n > 3$). Transmission electron microscopy confirmed the morphology of EVs at P2, P4, and P8 from ASCs and BMSCs. According to preliminary proteomics analysis, BMSC-EVs showed a higher number of unique protein types than ASC-EVs at the same passage, while BMSC-EVs and ASC-EVs each showed distinct protein profiles at different passage numbers. In vitro tube formation assays employing human umbilical vein endothelial cells (HUVECs) suggested that P2 ASC-EVs showed a significantly higher angiogenic capacity than BMSC-EVs ($p \leq 0.5$, $n = 5$). These effects got stronger at P4, as indicated by increased numbers of nodes, branches, meshes and total tube length ($p \leq 0.5$, $n = 5$). However,

differences between seemed to disappear at P8 (n = 2). Interestingly, proliferation assays showed a higher HUVEC proliferation rate when co-cultured with BMSC-EVs than with ASC-EVs at P2 (n = 3), P4 (n = 3), and P8 (n = 2). Meanwhile, BMSC-EVs demonstrated significantly stronger M2-macrophage immunomodulatory capacity than ASC-EVs under LPS stimulation at P2, P4, and P8 as suggested by increased expression of Arg1 and IL-10 in qRT-PCR data (n ≥ 3), although no clear trends were observed without LPS stimulation. Further in vitro comparison studies of EV are currently underway to identify the optimal MSC-derived EV population for clinical bone regeneration therapies.

Keywords: MSC, EV, tissue engineering

1063

MESENCHYMAL STEM CELLS TRANSFER MITOCHONDRIA IN A 3D BIOPRINTED CHIMERIC MODEL SYSTEM

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Mitochondria are one of the most complex and vital organelles in eukaryotic cells. In recent years, it has been shown that through intercellular mitochondrial transfer, this important organelle provides a critical role in tissue homeostasis, damaged tissue repair, and tumor progression under physiological conditions. However, the mechanism of mitochondrial transfer and its effect on various cellular microenvironments has not yet been defined. Understanding the metabolic effects of mitochondrial transfer and exploring the signaling leading to the intercellular mechanisms could provide advancements in both translational medicine and cell therapy for cancer progression and age-related diseases. Mesenchymal stem cells have been proposed as a therapeutic cell type for the rejuvenation of damaged and aged tissues. While there is good evidence in the literature for mitochondrial transfer from MSCs, there does not exist a sufficient high-throughput, biomimetic system for studying the molecular impacts of this process. Therefore, we adapted a novel 3D bioprinting system to study mitochondrial transfer in biomimetic 3D cultures. Using a fluorescent GFP-MITO lentivirus, we were able to label mitochondrial protein in adipose derived stem cells (ASCs) to track mitochondrial transfer activity. The cells were co-cultured in 2D tissue flasks and printed into hydrogels using our 3D bioprinter. Through fluorescent microscopy, mitochondrial protein was observed traveling from stem cells to epithelial cell lines. We hypothesize this organelle trafficking aids in cell rescuing to stabilize mitochondria and improve metabolic function leading to successful rejuvenation of damaged and aged cell types. Further research to establish mitochondrial transfer, its mechanism(s), and molecular effects could lead insight into how this cellular communication rescues and normalizes metabolic factors of the stem cell microenvironment.

Keywords: Mitochondrial Transfer, 3D Bioprinting, Mesenchymal Stem Cell

1065

EXOSOMES TO PROMOTE A PRO-REGENERATIVE ENVIRONMENT IN UTERO AND REDUCE THE SEVERITY OF SPINA BIFIDA

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Transamniotic therapy mediated by placental mesenchymal stem cells (MSCs) has recently showed promise for the treatment of congenital disorders, including spina bifida (SB). MSCs accomplish the important task of communication with surrounding cells through a complex repertoire of soluble and vesicular emissions which act in tissue trophic, oxidative stress scavenging, anti-fibrotic, anti-inflammatory, anti-apoptotic and systemically responsive manner. We tested the therapeutic potential of the exosomes (EXOs) released by amniotic fluid (AF)-derived MSCs to modulate the in-utero environment and mitigate the severity of SB in Fkbp8 knockout mice. Fkbp8 -/- mice exhibit 100% penetrance and represent a highly relevant model, with strikingly similar features of human SB. Biodistribution data show that fluorescently labelled EXOs injected intraperitoneally in pregnant mice (E5) preferentially accumulate within the uterus where they exert a therapeutic role. Data demonstrate that the injection of MSC-EXOs (109) i.p. into pregnant Fkbp8+/- dams resulting from heterozygous crosses at E8-12 is associated to a reduction in length of the SB lesion at E18.5 (3.06mm) compared to the PBS-treated homozygotes (5-8mm). The regenerative/protective effect of EXOs is also observed in the skeleton structure following injection, with an ongoing vertebrae closure visible at the level of the last thoracic and all lumbar vertebrae compared to untreated mice. The insights obtained from these preliminary studies support the potential role of AF-MSCs to be used for the development of cell-free strategies to improve the prognosis of patients with SB.

Keywords: Spina bifida, Exosomes, amniotic fluid-mesenchymal stem cells

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ENGINEERING MULTILAYERED CELL SHEETS USING HUMAN DENTAL PULP STEM CELLS UNDER XENO-FREE CULTURE CONDITIONS FOR HARD TISSUE REGENERATIVE MEDICINE

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Regenerative therapies using human dental pulp-derived mesenchymal stem cells (DPSCs) need to be safe and highly reproducible. We previously reported an effective culture method for DPSCs using type 1 collagen (COL) coating of cultureware under xenogeneic serum-free culture (XFM) conditions. COL-XFM cultures of DPSCs produced abundant extracellular matrix proteins and formed a cell sheet structure (CS). However, CS formation required a long period (^{3}2 weeks). The aim of this study was to establish a new culture method capable of rapidly and reproducibly forming CS for cell therapy.

DPSCs and iliac bone-derived bone marrow stem cells (BMSCs) were cultured under COL-XFM. All experiments were performed using cells at passages 3-6. DPSCs cultured at a specific cell density adhered to the culture dish at an early stage due to high expression of integrin α2β1 (a specific interactor with type I collagen) and showed high proliferation. In addition, DPSCs formed a multi-layered CS featuring the production of multiple types of collagen for 10 days, and apoptotic cells were not observed. This multilayered CS (multi-CS) formed calcified nodules by induced osteogenic differentiation. Moreover, CS encapsulated with hydroxyapatite/β-tricalcium phosphate and implanted subcutaneously in immunodeficient mice resulted in significantly enhanced formation of bone-like hard tissue compared to BMSCs. Conversely, BMSCs cultured under the same conditions as DPSCs exhibited low integrin α2β1 expression and low proliferation. Although they reached confluence, BMSCs did not form CS. We have established a new culture method for rapid formation of a mechanically strong multi-CS structure. Moreover, multi-CS can be easily adapted to making cell/scaffold constructs, providing highly reproducible outcomes. This new culture method is expected to permit safe and effective hard tissue engineering.

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Keywords: Human dental pulp stem cell, Cell sheet, xenogenic serum-free culture

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AUTOLOGOUS IN UTERO MESENCHYMAL STEM/STROMAL CELL THERAPY FOR MYELOMENINGOCELE - A PILOT STUDY

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We have established that early gestation placenta-derived mesenchymal stem/stromal cells (PMSCs) can potentially be used for treatment of myelomeningocele (MMC, spina bifida), a neural tube defect. The current standard of care for spina bifida is in utero skin closure over the defect during the second trimester of pregnancy. Our ongoing CuRe Trial (NCT04652908) for spina bifida utilizes banked allogeneic PMSCs seeded on sub-intestinal submucosa (SIS) extracellular matrix (PMSC-ECM) placed on the site of defect at the time of repair. Allogeneic cell banking, testing and maintenance involves continued labor and costs, and the effect of long-term storage on the quality of banked cells is undetermined. If feasible, offering autologous therapy as an option to MMC patients is a worthwhile alternative. MMC is diagnosed by ultrasound at 18-22 weeks of pregnancy, but it can potentially be detected during the first trimester (11-14 weeks) using nuchal translucency ultrasound imaging. Chorionic villus sampling (CVS) is a surgical procedure to obtain first trimester placental villus tissue for genetic testing. In this pilot study, we tested the feasibility of an autologous therapy using the gold standard ovine MMC model. A cotyledon from the placenta was surgically obtained at the time of defect creation surgery gestational age (GA) 75 days (first trimester). Chorionic villi then underwent enzymatic digestion and isolated cells were seeded for expansion. At passage 2, the cells were transduced with GFP expressing lentiviral vector and underwent further expansion for a total of four passages. The cells isolated were tested for MSC phenotype by PCR and neuroprotective capability by an established in vitro anti-apoptotic assay. 24 hours prior to the time of defect repair surgery (GA 105 days, 2nd trimester) the cells were seeded onto the SIS-ECM at a density of 300,000 cells/cm2 and incubated at 37ºC, 5% CO2. 6 fetuses were treated with PMSC-ECM patch. The three lambs that survived had a sheep locomotor rating (SLR) score of 14, 15 and 15 at birth (median score 15). An SLR score of 0 indicates complete paralysis and a SLR score of 15 indicates normal ambulation. Historical control lambs that were treated with ECM only had a median SLR score of 6.5. This pilot study demonstrated the feasibility of an autologous therapy for MMC patients.

Keywords: Mesenchymal stem/stromal cells, spina bifida, autologous therapy

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TREATMENT OF MOUSE MODELS OF EXPERIMENTAL AUTOIMMUNE DISEASES BY ADMINISTRATION OF ACTIVATED ADIPOSE-DERIVED MESENCHYMAL STEM/STROMAL CELLS

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Adipose-derived mesenchymal stem/stromal cells (ASCs) have gained attention as a treatment for fibrotic disease. They have anti-inflammatory and fibrotic effects and modify the microenvironment at the site of engraftment. ASCs are typically isolated via enzymatic digestion using collagenases. Low-molecular-weight heparin (LMWH) exhibits multiple functions including anti-inflammatory, antifibrotic, and cell function-promoting effects. LMWH stimulation is expected to increase the therapeutic effect of ASCs by promoting cellular functions. This study aimed to confirm the therapeutic effect of LMWH-activated ASCs (hepASCs) in mouse models of several autoimmune diseases. ASCs were cultured in an LMWH-supplemented medium. LMWH significantly increased the number of ASCs and enhanced their anti-inflammatory, and antifibrotic effects. Systemic lupus erythematosus (SLE) is an autoimmune disease with multi-organ manifestations such as skin, lung, heart, and kidney. Lupus nephritis is one of the life-threatening complications in SLE, which correlates with high mortality rate. Lupus nephritis mouse model were prepared. Systemic sclerosis is a refractory autoimmune disease that causes inflammation, fibrosis, and vascular endothelial damage in systemic organs. Interstitial lung disease is a condition that may cause respiratory failure. Interstitial inflammation of the lungs induces fibrosis in diffused alveolar damage. Mice with bleomycin-induced pulmonary fibrosis and systemic sclerosis were prepared. They were intravenously administered normal ASCs or hepASCs. The collagen content decreased significantly in the normal ASCs and hepASCs administered groups compared with that in the disease model mouse group. The relative mRNA expression of inflammation-related genes was significantly lower in the treatment group than in the pathological model group. hepASCs showed higher anti-inflammatory and antifibrotic effects than normal ASCs and may be a promising candidate for autoimmune diseases treatment.

Funding Source: This work was supported by JSPS KAKENHI (Grant Number 21K16312).

Keywords: stem cell-based therapy, Adipose-derived mesenchymal stem cells, autoimmune diseases

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EPIGENETIC REGULATIONS OF ADIPOSE-DERIVED STEM CELLS DURING SPHEROID FORMATION AND PERIPHERAL NERVE REGENERATION

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The neuropathogenesis was triggered after nerve injury. We are interested to discover the potential therapeutic approach for the regeneration of the peripheral nervous system (PNS). In adipose-derived stem cells (ASCs), we discovered the sphere formation which is important for morphological changes in adult stem cells and material modification using biomaterials. We found the fibroblast growth factor receptor was significantly increased during neural lineage cells (NLC) induction. Further analysis of ASC-derived spheres discovered the involvement of histone deacetylase (HDAC) 5 nucleus translocation during NLC induction. The HAT activities were decreased and the trimethylation of H3K4 and H3K9 were increased during spheroid formation. The supplement of FGF9 during NLC induction facilitated the Schwann cells (SCs) fate commitment via the FGF9-FGFR2-Akt phosphorylation pathway. The fate committed SC can participate in the myelin sheath formation during nerve regeneration. We also investigated the epigenetic changes of different HDACs after PNS injury. The HDAC inhibitor (HDACi) sodium phenylbutyrate (PBA) was discovered to reduce SCs inflammation and improve sciatic nerve regeneration after injury. The PBA inhibited nuclear factor kappaB (NFkappaB)-p65 phosphorylation and translocation by regulating the HDAC3 expression and activity. Taken together, the epigenetic regulation on neural spheroid formation in ASCs and the cellular responses of Schwann cells in the microenvironment after PNS injury play important roles for nerve repair and regeneration.

Funding Source: NSTC Taiwan

Keywords: adipose stem cell, neuronal induction, peripheral nerve regeneration

1075

TRANSPLANTATION OF HUMAN CRANIAL BONE-DERIVED MESENCHYMAL STEM CELLS AND REHABILITATION ENHANCE MOTOR FUNCTIONAL RECOVERY IN MICE AFTER TRAUMATIC BRAIN INJURY

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Traumatic brain injury (TBI) is a major global health problem. The recovery and healing from TBI are often difficult due to the limited self-repair capabilities of brain tissues after trauma. Therefore, stem cell transplantation and rehabilitation are suggested to treat TBI. In this study, we transplanted human cranial bone-derived mesenchymal stem cells (hcMSCs) into a mouse model of TBI, and investigated the effects of different post-transplantation rehabilitation approaches for the recovery of motor function. In the present study, human frontotemporal cranial bone waste following neurosurgical procedures were used for the isolation of hcMSCs. Twenty four hours after TBI induction in mice, hcMSCs or PBS were transplanted intravenously. After transplantation, they were classified into a no-exercise group, a low-frequency exercise group with treadmill exercise 3 days a week (LF Ex), and a high-frequency exercise group with treadmill exercise 7 days a week (HF Ex). Treadmill exercise (TM) was performed for 20 minutes at a speed of 6 m/min. Beam walking test and rotarod test were used for motor function evaluation. Injured brain tissues were analyzed at days 8 and 35 days after TBI induction for mRNA and protein expression analysis by real-time polymerase chain reaction and western blotting, respectively. At 35 days after TBI induction, hcMSCs transplantation + HF Ex group significantly improved motor function compared to the no exercise group, the hcMSCs transplantation + LF Ex group, the LF Ex group, and the HF Ex group. Furthermore, we found that the mRNA and protein expression levels of hepatocyte growth factor (HGF), nerve growth factor (NGF), and Growth-associated protein 43 (GAP-43) were significantly higher in the hcMSCs transplantation + HF Ex group compared to other groups. Previous studies have demonstrated that NGF accelerates neural differentiation and increased expression of GAP-43 enhances synaptic regeneration and high expression of HGF ensures the efficacy of hcMSCs in promoting functional recovery following TBI. Therefore, the combined use of hcMSCs transplantation and rehabilitation may provide a promising therapeutic option for treating TBI.

Funding Source: This study was financially supported by the Program for Developing and Supporting the Next-Generation of Innovative Researchers at Hiroshima University, Japan.

Keywords: Traumatic brain injury, Mesenchymal stem cells, Rehabilitation

1077

ALTERED MICRORNA EXPRESSION PROFILE IN EXTRACELLULAR VESICLE OF THE BONE MARROW MESENCHYMAL STROMAL CELLS IN ACQUIRED APLASTIC ANEMIA PATIENTS

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Bone marrow mesenchymal stromal cell (BM-MSC) derived extracellular vesicles (EVs) play a crucial role in determining the fate of hematopoietic stem and progenitor cells (HSPC) in the BM niche. Acquired aplastic anemia (AA) is a state of bone marrow failure characterized by peripheral pancytopenia and hypoplastic marrow with profound alteration in the HSPC numbers and functions. Recently, we have reported that EVs from BM-MSC of AA patients inhibit the proliferative and colony forming ability of HSPCs and promote apoptosis. It is now well established that one of the important components of EVs mediating their effector functions are microRNAs (miRs). Thus, we hypothesized that the miRs in BM-MSC EVs of AA patients may contribute to the impaired hematopoietic functions of HSPCs. Therefore, the aim of the study was to identify differentially expressed (DE) miRs, and their target pathways in AA BM-MSC EVs in comparison to normal controls (NC). To conduct the study, EVs were isolated using Total Exosome Isolation Kit and characterized following the MISEV 2018 guidelines. RNA was isolated from BM-MSC EVs of AA (n=6) and NC (n=6). RNA quality was evaluated by RIN number followed by library preparation and NGS based Illumina miR profiling. Bioinformatic pipeline included DESeq and miRWalk2 to identify DE miRs and target genes, respectively. KEGG database embedded in miRNet tool was used for Pathway analysis and miR-gene network (p=< 0.05). This study was approved by Institutional Ethics Committee and Stem Cell Research Committee. Our results show that morphology and size of EVs from AA and NC BM-MSC were similar. Both groups exhibited the expression of CD63, CD81 and TSG101 (EV markers) with negligible expression of calnexin (non-EV marker). miR profiling revealed that EVs from AA BM-MSC had 681 DE miRs out of which 11 were significantly upregulated and 9 downregulated (>log2fold). Pathway and network analysis revealed interaction between significantly altered miRs with genes involved in cell cycle, proliferation, apoptosis, T-cell activation, and hematopoiesis regulation. Our data highlights that EVs from AA BM-MSC have significantly altered miRs which may be of functional importance in AA pathobiology. Future studies are warranted to elucidate the diagnostic and therapeutic potential of these EV miRs in AA.

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DBT Wellcome Trust India Alliance Grant (IA/I/16/1/502374) and DST INSPIRE Fellowship (IF170881).

Keywords: Extracellular Vesicles of Bone Marrow-MSC, microRNA, Aplastic Anemia

TOPIC: MUSCULOSKELETAL

1079

A MULTIPLEX PROTEIN ASSAY TO SCREEN OSTEOGENIC PROPERTIES OF CALCIUM PHOSPHATE BIOMATERIALS - IN VITRO SELECTION AND IN VIVO VALIDATION IN A SUBCUTANEOUS IMPLANTATION MODEL

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The intrinsic healing capacity of bone falls short in critical-sized defects. Autologous bone grafts, which are considered a gold standard treatment for these defects, are associated with considerable drawbacks and the clinical performance of existing synthetic bone graft substitutes remains inferior to their biological counterpart. This led to development of a growing number of new biomaterials for bone regeneration and with it, the demand for extensive in vitro and in vivo testing. Furthermore, a poor correlation between in vitro and in vivo results in bone regeneration research indicates a need to improve methodologies used to assess biomaterials in vitro. Therefore, we developed a targeted protein multiplex assay containing a combination of markers related to osteogenesis, angiogenesis and inflammation, all relevant processes in bone regeneration, as a screening tool to identify increased osteogenic differentiation in human mesenchymal stem cells exposed to different biomaterials. A library of calcium phosphate (CaP)-based biomaterials, widely used synthetic bone graft substitutes, with inorganic additives was established and cells were cultured on these CaP particles. All materials were biocompatible and no cytotoxic effect was detected. The protein multiplex expression profile of cells exposed to these materials allowed us to identify materials predicted to have an increased osteogenic, angiogenic or inflammatory potential. Twelve materials were chosen which showed the most differences in protein profiles for subsequent in vivo evaluation in a mouse subcutaneous implantation model over 8 weeks. Osteogenic properties were shown through ectopic bone formation. Macrophage polarization was assessed as an indicator for the inflammatory response 7 days after implantation. Angiogenic potential was determined by blood vessel quantification. Based on this ongoing work, we aim to describe the correlation between in vitro and in vivo results, justifying this screening tool for the in vitro assessment of biomaterial performance for bone regeneration.

Funding Source: This research is funded by the Netherlands Organisation for Scientific Research Vidi grant (15604), the Dutch Province of Limburg (LINK project) and the Interreg Vlaanderen/Nederland project 'BIOMAT-on-microfluidic-chip'.

Keywords: Protein multiplex, human mesenchymal stem cells, calcium phosphate-based biomaterials

1081

GPCRS-G12/13-RHOA SIGNALING AXIS LOCKS MUSCLE STEM CELL IN QUIESCENCE

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GPCRs have emerged as important regulators that keep muscle stem cells (MuSCs) from exiting the quiescent and undifferentiated condition. However, the topography of the quiescent MuSC-specific GPCR signaling network has not to be elucidated yet. Here, we depicted a landscape of GPCR expression during MuSC quiescence, by comparative analysis the transcriptomic variations between quiescent and activated MuSCs. Meanwhile, using pharmacological and inducible-genetic approaches, we identified two niche-derived GPCR ligands, endothelin-3 (ET-3) and neurotensin (NT), which bind to EDNRB and NTSR2 respectively, and subsequently trigger the G12/13 signaling pathway that reinforces MuSC quiescence through tuning of RhoA activity. In summary, our findings open a window on the GPCR-mediated regulatory architecture in quiescent MuSC, facilitating the identification of novel targets and repositioning of therapeutic agents for prevention and mitigation of myopathies associated with the disruption of MuSC quiescence.

Keywords: Muscle stem cell, quiescence, GPCR

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INTERSPECIES GENERATION OF FUNCTIONAL MUSCLE STEM CELLS

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Satellite cells, the stem cells of skeletal muscle tissue, hold a prodigious regeneration capacity. However, low satellite cell yield from autologous or donor-derived muscles preclude adoption of satellite cell transplantation for the treatment of muscle diseases including Duchenne muscular dystrophy (DMD). To address this limitation, we investigated whether sufficient quantity of satellite cells can be produced in allogeneic or xenogeneic animal hosts. First, we generated intraspecies mouse chimeras by injection of CRISPR/Cas9-corrected DMD-induced pluripotent stem cells (iPSCs) into mouse blastocysts carrying an ablation system of host satellite cells. Analysis of adult chimeras revealed exclusive generation of iPSC-derived satellite cells and derivative myoblasts. Similarly, injection of genetically-corrected DMD-iPSCs into rat blastocysts produced interspecies rat-mouse chimeras harboring mouse muscle stem cells. Notably, iPSC-derived muscle stem cells produced in either allogenic or xenogeneic animal hosts efficiently restored dystrophin expression in limb muscles of DMD mice following intramuscular transplantation. Our study thus provides a proof-of-principle for generation of therapeutically-competent stem cells between divergent animal species, raising the possibility of procuring human stem cells in large animals for regenerative medicine purposes.

Keywords: Myogenic stem cell niche regeneration, Blastocyst complementation, Duchenne muscular dystrophy

1085

MODULATION OF SIGNAL PATHWAYS IN HUMAN SKELETAL STEM CELLS TO UPREGULATE CARTILAGE REGENERATION

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The vertebral column is a unique skeletal structure composed of intercalating vertebrae bone and intervertebral discs (IVD). This structure is not only essential for supporting the body's structure and nervous system, but responsible for providing movement as well. Though crucial for normal body function, the vertebral column is susceptible to a wide range of genetic and injury-related disorders such as IVD degeneration and osteoarthritis. In an attempt to alleviate these conditions, research is being conducted on using highly regenerative resident stem cell populations as potential therapies. In the past, we have isolated skeletal stem cell (SSC) populations and demonstrated their committed downstream progenitors give rise to bone, cartilage, and marrow stroma in mice and humans. Different from mesenchymal stromal cells, bona fide SSCs represent a pure cell population that is intrinsically pluripotent. Using these isolation techniques, we discovered resident SSCs in both mice and human IVDs. Within species, these populations are distinct from each other depending on the source tissue location (cervical, thoracic, and lumbar) and display diversity in their capacity to differentiate into bone or cartilage. Using single cell-RNA sequencing, we found that human SSCs from each vertebral compartment upregulate specific and distinct factors. Some of these factors are conserved in mice and have been found to increase regeneration of damaged IVDs and vertebrae. We are now testing these factors in human vertebral specimens to evaluate if these signaling pathways are conserved and to provide translational information for vertebral regeneration. The data gathered in this study sets the foundation for the clinical translation of stem cell-based therapies for preventing and reversing IVD-related musculoskeletal diseases.

Keywords: Skeletal stem cells, IVD, Cartilage

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ACTIVATION OF SKELETAL STEM CELLS FOR INTERVERTEBRAL DISC REGENERATION

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The intervertebral disc (IVD) is a highly specialized, fibrocartilaginous structure that deteriorates at a rate faster than any other connective tissue in the body. This condition is commonly referred to as IVD degeneration and a critical challenge for IVD repair is the development of effective treatments that reverses the fibrocartilage damage. Due to their cell intrinsic properties of self-renewal and differentiation, the utilization of tissue resident stem cells holds promise as a stem cell-based approach to combat IVD degeneration. Our lab was the first to identify, isolate and functionally characterize bona fide skeletal stem cells (SSCs) and their committed downstream progenitors that give rise strictly to bone, cartilage, and marrow stroma in mice and humans. SSCs are distinct from mesenchymal stromal cells which represent highly heterogenous mixtures of cell types. More recently, we used our isolation methods to discover tissue resident SSCs within mouse and human IVD and vertebra. Additionally, we found that the fate decisions of IVD SSCs can be guided by specific intrinsic and extrinsic cues. Furthermore, these factors can be transplanted into damaged spinal tissue to increase cartilage and bone in IVD and vertebrae tissues, respectively, using a novel mouse tail injury model. Collectively, our data shows that these factors can be used as potential stem cell-targeting therapy for combating degeneration of IVD as well as vertebra tissue. We aim to further this study by testing these factors using human spinal samples and set the foundation for the clinical translation of stem cell-based therapies for preventing and reversing IVD-related musculoskeletal diseases and hence, improve vertebral regeneration.

Funding Source: F31AR079265

Keywords: stem, regeneration, disc

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IDENTIFICATION AND CHARACTERIZATION OF NOVEL SKELETAL STEM CELL POPULATIONS IN MICE AND HUMANS

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Skeletal tissues possess an amazing capacity to regenerate. However, this regenerative capacity decreases with age and comorbidities. In older individuals, skeletal tissues heal slowly and imperfectly despite advances in orthopedic surgery and rehabilitation. Current experimental approaches involve tissue engineering and stem cell-based regenerative therapies. Indeed, stem cells are responsible for growth, maintenance, and repair of skeletal tissues. However, they remain poorly characterized at the cellular and molecular levels which is a clear limitation for their clinical use. Our aim was to identify and characterize novel skeletal stem cell populations in murine and human tissues. To achieve this, we used genetic lineage tracing, spectral 3D confocal imaging, computational image analysis, single cell transcriptomics and in vitro assays on mouse and human tissues. We show that the adult mouse skeleton contains self-renewing, multipotent skeletal stem cells (SSCs) with osteogenic, chondrogenic and adipogenic potential. These bona fide SSCs express Sox9 and are located in the resting zone of the growth plates and in periosteum. We further show that they persist after epiphyseal fusion in mature and old animals. Transcriptome analysis revealed that these cells express other putative SSCs markers, as well as genes involved in skeletal development, stem cell self-renewal, and fate decision. This data provides testable drug targets to pharmacologically manipulate SSCs fate decisions in situ. We showed that human tissues contain SSCs akin to murine tissues. This is the first experimental proof of self-renewal in postnatal SSCs in vivo. These findings provide actionable insights for the use of culture-expanded stem cell product for regenerative medicine product or pharmacological targeting of these stem cells in situ.

Funding Source: Stem Cell Network; the Arthritis Society; the Canadian Orthopedic Foundation; The Ottawa Hospital

Keywords: skeletal stem cells, musculoskeletal tissues, bone, cartilage

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LIGAMENTOCYTE DEDIFFERENTIATION TO A TRANSIENT PROGENITOR STATE FOR SCAR-FREE LIGAMENT REGENERATION IN ZEBRAFISH

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Acute ligament injuries are common and associated with long-term risks of recurrent ligament tears and osteoarthritis resulting from instability of the repaired joint. In mammals, poor healing results from a failure in differentiation during repair; cells rebuilding the healed ligament form a fibrotic scar rather than differentiating into ligamentocytes, as they do not express ligamentocyte marker Scleraxis (Scx). In contrast, we recently described how adult zebrafish ligaments heal with scxa+ cells following an acute transection injury. Here we define the early cellular and epigenetic drivers of ligament regeneration in adult zebrafish. Our studies reveal ligamentocyte dedifferentiation and later fate specification as key drivers of scar-free healing. Mature mammalian ligamentocytes are quiescent both in homeostasis and after acute injury. In contrast, we find injury-driven cell cycle re-entry in normally quiescent adult zebrafish ligamentocytes. Repeated live imaging of two independent ligamentocyte transgenic reporter zebrafish, scxa:mCherry and thbs4a_p1:eGFP, shows initial downregulation and later re-expression of hallmark ligament genes during the regenerative healing process. Additionally, lineage tracing of mature ligamentocytes driven by a newly generated thbs4a_p1:CreER allele, with tamoxifen-mediated BFP to DsRed reporter conversion prior to injury, shows that pre-existing ligamentocytes contribute to the final regenerated ligament. scRNAseq of joints across the healing time course highlight a transient population of injury-specific cells that reactivate developmental ligamentocyte genes. Furthermore, snATACseq analysis of joints during early regeneration points to dynamic chromatin accessibility changes associated with mature ligamentocyte genes. Together, these results indicate that adult zebrafish ligamentocytes have the unique potential to de-differentiate and proliferate to act as a progenitor pool in adult ligament healing. Through these studies, we developed the first adult vertebrate injury model for scar-free ligament regeneration. In future, by further characterizing the regulators of dedifferentiation and ligament fate restoration in a regenerative model, we aim to identify targets for therapies to improve mammalian ligament healing.

Funding Source: NIH NIDCR R00 DE027218 and F31 DE031970

Keywords: Ligament, Zebrafish, Regeneration

TOPIC: NEURAL

1093

A MULTIMODAL NEUROPROTECTIVE STEM CELL-TISSUE ENGINEERING SOLUTION FOR TREATING RETINITIS PIGMENTOSA

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Stem cell therapies have shown great promise to restore vision in patients affected by retinal degenerative diseases. However, there are still challenges that remains due to the efficacy gap between in vitro studies and in vivo cell transplantation. This is partly due to the hostile microenvironments in which these cells are transplanted in. In a degenerative tissue, the host immune system and hostile conditions cause major challenges that need to be addressed. In retinitis pigmentosa (RP), the loss of vision is due to the death of both rod and cone photoreceptors. The absence of rods causes the loss of critical metabolic factors directly affecting cone survival and function. Therefore, cell therapies for RP are focusing on protecting, repairing, and replacing cone photoreceptors. To address this key issue, we have created a 3D hydrogel-based stem cell product that secretes neuroprotective factors in the vitreous to preserve cone photoreceptors. This biocompatible hydrogel mimics the in vivo extracellular matrix to offer a thriving environment for cellular proliferation and viability. Our biomaterial encapsulates a novel enriched target cell population that produces specific neurotrophic factors which are lost in RP. We have engineered this technology by combining material science and cell biology and have examined its effects in vitro on cone cells and in vivo with the rd-1 mice model (retinal degenerative mice). Our study shows that our technology is a multimodal therapy that targets specific neurodegenerative pathways such as neuro-glycolysis and neuro-inflammation. The neurotropic factors present can enhance glucose uptake by cone photoreceptors and reduce the expression of W6/32 markers indicating a reduction in neuro-inflammation. Following the injection of our stem cell-matrix product in the vitreous of rd-1 mice (P26) we observed a 10-fold increase in cone survival (central retina) which translated into the mice ability to perceive light. This technology is the first of its type to be gene agnostic and which can target multiple pathways that are affected in RP patients.

Keywords: Retina, Tissue Engineering, Retinitis Pigmentosa

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INVESTIGATING THE RESTORATIVE FUNCTIONS OF SLEEP IN NEURAL STEM CELLS AND REGENERATION

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Adequate sleep is considered restorative as circadian rhythm is reported to control adult stem cells from different sources to ensure tissue homeostasis. However, very little is known about the mechanistic control of stem cell functions by sleep. Despite sleep being an essential non-negotiable and conserved bodily function, human beings are the only mammals that actively avoid sleep in favor of other activities, e.g., work productivity or consumption of electronic media before bedtime, which delay sleep onset, reduce sleep duration, and compromise sleep quality. Women may need longer sleep duration than men and are more likely to suffer from anxiety and depression, which may also be related to their 40% higher prevalence of insomnia, although causal evidence is lacking and underlying mechanisms are unclear. Utilizing plasma samples from a clinical trial involving healthy women with a randomized crossover design of extended, objectively monitored sleep restriction (delaying bedtime by 1.5 hours over their regular schedule) that mimics “real-life” derangement of sleep duration (ClinicalTrials.gov NCT02835261), we performed metabolomic and proteomic profiling of the samples. We identified metabolites and proteins differentially enriched in adequate and restricted sleep conditions. Joint network pathway analysis showed enrichment of terms like long-term potentiation and depression, neurotrophin signaling, circadian entrainment, glutamatergic synapse and glutamine and glutamate metabolism under restricted sleep condition, highlighting a strong link between sleep restriction and neuronal development phenotypes. Using neural stem cells (NSCs) differentiated from human embryonic stem cells, we found treatment with metabolic candidates enriched in sleep restriction led to aberrant G1 phase of cell cycle, and defective differentiation of NSCs into neurons, astrocytes, and oligodendrocytes. We speculate that alterations in sleep can influence the daily dynamics of NSC divisions, and our study may shed light on how sleep shapes adult stem cell functions in neural development.

Keywords: Sleep restriction, Neural Stem Cells, Multiomics

Clinical Trial ID number: NCT02835261

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SNIP1 AND PRC2 COORDINATE INTRINSIC APOPTOSIS, CELL DIVISION, AND NEUROGENESIS IN THE DEVELOPING BRAIN

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Neural progenitor cells (NPCs) are stem cells in the central nervous system that give rise to neurons and glia. During development, NPCs extensively undergo proliferation, apoptosis, and differentiation in order to provide the structural organization of the brain. Aberrant NPC functions have been implicated in a number of neurological disorders and cancers including autism spectrum disorders, schizophrenia, and medulloblastoma. However, little is known about how NPCs properties are orchestrated at a molecular level. Here, we report the discovery of Snip1 as a key regulator of these NPC properties. The conditional deletion of Snip1 in the mouse embryonic brain causes dysplasia with robust activation of intrinsic apoptosis. Our transcriptomic analysis shows that loss of Snip1 reduces genes critical for cell cycle, neurogenesis, and cortical development, while inducing apoptotic genes and genes involved in developmental pathways. Mechanistically, Snip1 binds at the promoters of its target genes and facilitates the co-occupancy of the polycomb repressive complex PRC2 and H3K27me3 deposition. Our motif analysis further shows the preferential occupancy of Snip1 and PRC2 at loci with motifs including E2F proteins, RELA and SP2. Deletion of PRC2 partially restores the gene expression profile of the Snip1-depleted brain and significantly improved brain dysplasia. Our findings reveal that Snip1 exerts loci-dependent regulation of PRC2 and H3K27me3 to transcriptionally regulate the NPC functions in the developing brain.

Keywords: brain development, chromatin modifications, CUT&RUN

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KYNURENINE PATHWAY EFFECTS ON NEURAL STEM CELL FUNCTIONS

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In the adult brain, neurogenesis depends on neural stem cells (NSCs) residing in distinct niches such as the subventricular zone

(SVZ) which lines the lateral ventricles. Hallmarks of neurodegenerative disorders include decreased neurogenesis and impaired NSC functions. While the effects of adult neurogenesis and preserved NSC functions are well documented, the regulation of such processes remains unclear; however, there is reason to believe the kynurenine pathway may play a role. Here we examine how adult mouse neural stem cells respond to various concentrations of kynurenine and its downstream metabolites, kynurenic acid (KA) and quinolinic acid (QA), in vitro. Transgenic mice lacking the kynurenine pathway enzyme kynurenine-3-monooxygenase (KMO-/-) exhibit elevated levels of KA and decreased levels of QA, thus we expect exogenous treatment with KA to bolster proliferation while treatment with QA should negatively impact proliferation. These experiments demonstrate how altered kynurenine metabolite concentrations seen in neurodegenerative disorders affect cell proliferation and the prevalence of the quiescent state. We also explore potential mechanisms of action through the N-methyl-D-aspartate receptor, which is agonized by QA and antagonized by KA, and the aryl hydrocarbon receptor, which can utilize both kynurenine and KA as ligands. In vivo experiments show increased proliferation within the SVZ of KMO-/- mice, as well as hyperproliferative growths into the lateral ventricle. Furthermore, there are increases in doublecortin-positive SVZ neuroblasts, suggesting increased olfactory bulb interneuron formation. In habituation-dishabituation behavioral experiments there are indeed significant differences in olfaction between KMO-/- and wild-type mice. We hope to further elucidate mechanisms of action beyond the in vitro model to better understand how kynurenine metabolism can play varied roles in damage recovery and neurodegeneration.

Funding Source: NIH-NIDSR01NA102448 William and Ella Owens Medical Research Foundation

Keywords: neural stem cells, kynurenine pathway, neurogenesis

1101

A SERIES OF DEVELOPMENTAL MILESTONES DEFINES THE EMERGENCE OF THE ADULT NEURAL STEM CELL STATE IN THE HIPPOCAMPUS

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The dentate gyrus region of the hippocampus is one of only two regions in the adult mammalian brain where a population of neural stem cells is maintained throughout adulthood. Previously, we showed that Hopx-positive embryonic neural stem cells which contribute to developmental cytogenesis in the mouse dentate gyrus transition into quiescent adult neural stem cells, suggesting that a common lineage of neural stem cells contributes to developmental and adult neurogenesis. Quiescence is a hallmark feature of many adult stem cells, and presumably the transition to quiescence marks the shift from developmental to adult neural stem cell. However, the mechanisms underlying this critical transition remain completely unknown. Here we investigated the dynamic properties of dentate gyrus neural stem cells during the early postnatal transition to a quiescence in male and female mice. We identified a timeline of molecular, cellular, and metabolic changes that define the transition to an adult neural stem cell state, including changes in cell cycle dynamics, the transcriptome, autophagy, and cellular ROS levels. While some neural stem cell changes coincided with the transition to quiescence, others occurred before or after cell cycle exit, indicating that a series of milestones underlies the transition to an adult neural stem cells state. Together, our work supports a model wherein the transition to an adult neural stem cell state is not a singular switch from proliferation to quiescence, but is instead a more protracted, multi-step developmental process. Our study presents a framework from which future studies can build upon to investigate mechanisms driving the establishment and maintenance of the quiescent adult neural stem cell pool. Though this study focused on neural stem cells in the brain, many organ systems in the body harbor a population of quiescent adult stem cells, and the principles identified in the brain may apply broadly to the developmental establishment of other somatic stem cell populations.

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Keywords: hippocampus, neural stem cell, quiescence

1103

IN VIVO AND EX VIVO CHARACTERIZATION OF TRANSPLANTED HUMAN IPSC-DERIVED DOPAMINE NEURONS FOR THE TREATMENT OF PARKINSON'S DISEASE

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Parkinson's Disease (PD) is marked by loss of dopaminergic cells in the ventral midbrain that results in pathophysiological motor and cognitive deficiencies. Over the last decade, cell therapies have aimed to replace the lost dopamine (DA)-producing cells to reverse the pathophysiological symptoms of PD. Induced pluripotent stem cell (iPSC)-derived progenitor DA neurons transplant-

ed into the striatum of PD model animals show great promise as an effective treatment for PD. To become an effective therapy, the transplanted cells must contain a sufficient number of DA-producing neurons that can properly integrate into the host brain. An important part of this requires transplanted cells to make synaptic connections with the host brain for optimally timed DA release during motor initiation. This functional integration is critical for long-term functional efficacy. Here, we characterize a research-grade human iPSC-derived DA neuron cell therapy product, DA02, applying behavioral, molecular, and functional approaches during cell engraftment and maturation. We found that DA02-transplanted rats demonstrate complete rescue of amphetamine-induced rotations in 6-OHDA lesioned rats at four-month timepoint post-engraftment. Using gene expression approaches, we show that DA02 cells contain mature markers of dopaminergic neurons. Furthermore, we observe no evidence of off-target neurons such as serotonergic or GABAergic cells. Using patch clamp electrophysiology, we observe spontaneous synaptic events that indicate proper graft-host interactions. Features of intrinsic excitability such as action potential waveform are also similar to those of canonical DA neurons. In summary, these data provide important evidence of proof-of-concept for feasibility of an iPSC-derived DA cell therapy product for the treatment of PD.

Funding Source: BlueRock Therapeutics

Keywords: Dopamine, Parkinson's Disease, Electrophysiology

1105

AN INVERSE AGONIST OF ROR-GAMMA, SR2211, PROMOTES OLIGODENDROCYTE MATURATION AND REMYELINATION IN VIVO

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Multiple Sclerosis (MS), the most common demyelinating disorder in young adults, is characterized by an immune-mediated loss of myelin, which could lead to severe neurological disabilities. Available treatment options for MS are limited to modulation of immune responses to slow down the progression of the disease, but they do not directly promote remyelination. A complementary treatment that would effectively promote protection and/or restoration of damaged myelin would provide a significant benefit for MS patients. Therefore, our lab has been working to identify biologically active small molecules (SMs) that can stimulate endogenous oligodendrocyte precursor cells (OPCs) to differentiate into mature and functionally active myelin producing oligodendrocytes (OLs). To that end, we developed a human embryonic stem cell (hESC) reporter system and performed high-throughput drug screening using hESC-derived OPCs, which identified SR2211, an inverse agonist for retinoic acid receptor-related orphan receptor gamma (ROR-gamma), as a small molecule that can enhance the maturation of hOLs from hOPCs. Interestingly, inhibition of ROR-gamma has been shown to block the differentiation of Th17 cells and the production of IL-17, which is associated with MS progression. However, the role of ROR-gamma in myelination is not clear. To examine the translatability of the OL-maturation and myelination-promoting role of SR2211 in vivo, we established a cuprizone-induced demyelination mouse model, which is frequently used to evaluate remyelination independent of immunomodulation. Five weeks after the initiation of cuprizone feeding, a timepoint at which there is significant demyelination in the corpus callosum, mice on cuprizone diet received either vehicle, SR2211, or Miconazole (positive control) daily through intraperitoneal injection for two weeks. Our initial analysis of the brain sections with Black-Gold staining and immunofluorescent staining for OPC/OL markers (PDGFRA, OLIG2, CC1, and MBP) showed promising improvement in myelination of the corpus callosum of SR2211-treated mice. Our study suggests that ROR-gamma could be a promising target for developing remyelination-based therapy for multiple sclerosis (MS) and other demyelinating diseases.

Funding Source: National Eye Institute

Keywords: Multiple Sclerosis, Human Embryonic Stem Cells Differentiation into Oligodendrocyte Progenitor Cells and Matured Oligodendrocyte, Remyelination Promoting Small Molecule

1107

UNEXPECTED MHC CLASS II EXPRESSION IN PUTATIVE PROGENITOR SUBPOPULATIONS OF ADULT HUMAN NEUROSPHERES

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The stem/progenitor hierarchy governing adult-born neurons and glia is well-characterized in rodents and other mammals, but poorly understood in humans. In order to probe for functional evidence of multipotent progenitors, and to search for new markers to facilitate their prospective isolation, we harnessed the outgrowth of primary neurospheres derived from fresh postnatal human brain tissue in order to enrich for cells retaining cell division and differentiation activity. Using high-coverage single-cell RNA sequencing (scRNAseq), we identified a rare population within postnatal human brain neurospheres expressing several genes associated with a neural stem cell identity. We further identified several cell surface receptors uniquely expressed by this cluster, including CD74, whose protein-level expression was confirmed using flow cytometry. CD74 performs many functions, most notably as a chaperone for MHC Class II complex assembly, as well as a receptor for macrophage migration inhibitory factor (MIF). Since treatment with exogenous MIF did not affect expansion of postnatal neurosphere cultures, nor fate choice upon differentiation, we turned our attention to MHC Class II. By scRNAseq, we determined that components and positive regulators of the MHC Class II complex were exclusively expressed by the CD74+ immature progenitor cluster. As MHC II is classically associated with anti-

gen presentation, its expression on a subset of non-immune cells such as a putative neural progenitor is unexpected and intriguing. Ongoing work is aimed at uncovering the significance of MHC II heterogeneity in postnatal human neurospheres. Additionally, we are investigating whether MHC II expression in non-immune cells of the adult human cortex plays a role in neural-immune interactions, possibly through regulation of phagocytosis or synaptic pruning, in the context of severe epilepsy.

Funding Source: CIRM Bridges 3.0 - Scholars Program

Keywords: Adult Human Neural Progenitor, MHC Class II, Single Cell Transcriptomics

1109

TIME POINT SPECIFIC ABLATION OF GDNF-EXPRESSING NPCS IN SPINAL CORD INJURY REVEALS LONG TERM ROLE IN REGENERATION

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Neural Precursor Cell (NPC) transplantations are a promising field of research for treating spinal cord injury (SCI). However, it is currently unknown to what extent the transplanted cells contribute to regeneration through the release of trophic factors that stimulate and protect endogenous tissue, and the generation of glial cells and neurons to replace lost neuronal tissue. Recent research from our lab has shown that transplantation of NPCs which overexpress glial-derived neurotrophic factor (GDNF), a protein endogenously expressed by NPCs, rescues neuronal differentiation in the transplanted NPCs, improves white matter sparing after injury, and improves the degree of functional recovery when compared to conventional NPCs. By expressing the GDNF protein under a progenitor cell state promoter, and inducing ablation of progenitor cells with the HSV-TK system, we can elucidate the mechanism and critical time period by which the overexpression of GDNF facilitates the previously observed recovery after transplantation. Previous studies conducted in our laboratory have recorded increased functional recovery as early as 2 weeks after transplantation, at which point grafted cells have not yet fully differentiated and integrated with host circuits. We observed that the rats that sustained SCI and were given transplants of the NPC lines expressing GDNF showed improved functional recovery when compared to conventional NPCs. When compared to the constitutively active promoter expressing GDNF, the progenitor state promoter line of GDNF-expressing NPCS facilitated similar levels of recovery, as seen in the catwalk, grip strength, and inclined plane behavioural tests. This could indicate that the progenitor promoter expresses GDNF for the necessary period of time to facilitate the benefits that we observed in the constitutively active EF1a::GDNF group. Ongoing tissue analysis will elucidate how the grafted undifferentiated GDNF-expressing NPCs contribute to functional recovery after the transplantation.

Keywords: Neural Stem Cells, Spinal Cord Injury, Cell transplantation

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ISOLATION AND THERAPEUTIC APPLICATION OF NEURAL STEM CELLS FROM THE NERVES OF ADIPOSE TISSUE

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Diseases affecting the nervous system are notoriously difficult to treat, necessitating the development of new therapies for this urgent unmet clinical need. Neural stem cell (NSC) regenerative therapy is a promising approach to alleviating diseases of the nervous system by replacing damaged neurons/glia, or by producing neurotrophic factors that protect and stimulate their repair. This approach requires an autologous source of NSC for adequate engraftment. The concept that cells from fat tissue can be isolated and induced to acquire the traits of neurons and glia has been known for 20 years, but the identity of this neurogenic population has remained elusive. Using elegant cell reporter models, we report that these neurogenic cells originate from the neural crest-derived Schwann cell population in both subcutaneous and visceral adipose tissue. Further experiments indicate that the neurogenic potential of adipose-derived cells was confined specifically to the Schwann cell-derived population, rather than mesenchymal stem cells. These cells represent only a minority of the heterogenous stem cell population from adipose tissue. Based off our findings we report novel methodologies to isolate NSCs from the nerves within adipose tissue from mice and human clinical samples. To understand the biological mechanisms underlying this process, RNA-sequencing was performed and showed that Schwann cells can dedifferentiate in vitro and subsequently acquire NSC-like properties. To test their functional potential, NSCs from the adipose were transplanted into mouse models of neurointestinal disease (gastroparesis and Hirschsprung disease). The cells engrafted, migrated, differentiated into neurons and glia, and exhibited calcium signaling properties consistent with neurons. Importantly, these cells rescued the functional deficits in both models. Furthermore, we demonstrate that adipose-derived NSCs can fully reform functional nerves in a sciatic nerve gap model. NSCs could be successfully isolated from clinical samples of subcutaneous and visceral adipose tissue obtained by laparoscopy from lean and obese subjects with ages ranging 10 to 80 years old. The biological insight provided by these studies offers an opportunity to develop better cell therapies from autologous fat for nervous system diseases.

Keywords: neural stem cell, adipose, nerve

1113

UNDERSTANDING THE SPATIOTEMPORAL EFFECTS OF GDF11 IN REJUVENATION OF THE AGED MOUSE BRAIN.

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Brain endothelial cells (ECs) form the blood-brain barrier (BBB) which functions, at least in part, to maintain brain homeostasis. ECs deteriorate with aging and neurodegenerative disease, leading to BBB dysfunction and a concomitant reduction in adult neurogenesis. However, the infusion of young blood into old mice via heterochronic parabiosis can reverse some of these changes. GDF11 is a circulating factor in the blood and has beneficial effects on brain vasculature and neurogenesis in aged mice. MRI scanning of old mice after 28 days of GDF11-treatment revealed a spatiotemporal increase in cerebral blood volume (CBV) in the brain. Interestingly, we observed increased CBV in different regions of the cortex and dentate gyrus of the hippocampus (a niche of adult neurogenesis). This is consistent with our previous findings of increased brain vessel density following GDF11 administration. Because systemic GDF11 appears not to cross the BBB, we hypothesize that it acts directly on the vascular cells. To this end, we performed a time course analysis of the aging mouse brain after GDF11 treatment to understand its sequential effects. We found that GDF11 treatment for 14 days increased the CD31+ blood vessel area in the cortex and hippocampus. Moreover, EC tight junction proteins, including Claudin5 and ZO1, and other young brain EC components, such as VEGF signaling, also increased, indicating improved functionality of the existing vessels. Furthermore, we observed increased doublecortin and c-fos-positive cells in the dentate gyrus, suggesting stimulation of neurogenesis and enhanced neuronal activity. Together, these data confirm that GDF11 acts in multiple brain regions to improve vascular and neuronal outcomes. Next, we are investigating the mechanism by which GDF11 exerts its effects in the aging brain to improve its therapeutic efficacy.

Funding Source: 1. R01AG072086 NIH/NIA 2. Simons Foundation (Collaboration on Plasticity in the Aging Brain) 3. R01NS117407 NIH/NINDS

Keywords: GDF11, Aging brain rejuvenation, adult neurogenesis, Blood brain barrier, endothelial cells

1115

EFFECTS OF MITOCHONDRIAL BIOGENESIS MODULATION ON NEURAL STEM CELLS IN VITRO AND AFTER TRANSPLANTATION IN A SPINAL CORD INJURY MODEL

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Central nervous system (CNS) cells have a high bioenergetic demand making them vulnerable to mitochondria dysfunction. In spinal cord injury (SCI), the secondary injury phase is characterized by changes in the microenvironment that affect mitochondria such as oxidative stress, loss of access to nutrients, and the presence of pro-inflammatory signals such as C1q. Human neural stem cells (hNSC) have shown great promise in clinical therapies for traumatic injuries due to their ability to generate new cells of the CNS and secrete neuroprotective cargo. We have derived and characterized several human neural stem cells (UCI-hNSC) lines and their potential for restoring locomotor function (efficacy) after SCI in mice. NSC lines exhibit variation in “mitochondria fitness traits” (MFTs) that is linked to their capacity for in vivo repair. In this study, we hypothesize that resilience to stressors as well as enhancing NSC mitochondrial function, could enhance NSC repair capacity. Transcriptomic comparison of an efficacious line (UCI161) vs. a non-efficacious line (UCI152) revealed key differences in MFT categories: bioenergetics, biogenesis, permeability transition, redox potential, and mitophagy and/or autophagy. UCI161 were shown to have higher Nrf2 levels, a key transcription factor involved in stem cell self-renewal and antioxidant response. Mitochondrial functional assays of mitochondria membrane potential (MMP) and ATP production revealed that UCI161 were better responders to bioenergetic drugs, whereas UCI152 responded optimally to biogenesis enhancement. Conversely, treatment of hNSC with pro-inflammatory C1q resulted in loss of TFAM, a key biogenesis transcription factor, which led to loss of

mitochondria area, mtDNA instability, and activation of the inflammasome pathway. MFT screening of UCI161 and UCI152 with FDA-approved mitochondrial compounds identified a biogenesis drug candidate that increased TFAM and mitochondrial levels. Rag1 mice underwent a unilateral C5 cervical SCI followed by administration of UCI hNSC lines, hNSC pre-treated for 2 days, or hNSC plus drug supplementation via diet. Functional recovery was assessed using locomotor tasks: Catwalk gait analysis (Noldus), horizontal adder beam, and Dynamic Weight Bearing (Bioseb).

Keywords: Mitochondria Biogenesis, Neural Stem Cells, Spinal Cord Injury

1117

ENRICHED DIFFERENTIATION OF HUMAN OTIC SENSORY NEURONS DERIVED FROM DENTAL PULP STEM CELLS

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Sensorineural hearing loss (SNHL) is one of the most common human sensory disorders. Most cases of SNHL are due to the degeneration of key structures of the sensory pathway in the cochlea such as the hair cells and the spiral ganglion neurons (SGNs). Because of the lack of regenerative capacity of the sensory epithelium and SGNs, SNHL is permanent. Different stem cell-based approaches aiming at cell replacement are currently the subject of intensive research as putative therapeutic strategies for SNHL. Nevertheless, the availability of renewable source of stem cells and efficient protocols for in vitro generation of otic neurosensory structures remain among crucial requirements to develop cell replacement for SNHL. Human dental pulp stem cells (hDPSCs) represent a readily accessible source of cells from an ectodermal origin to treat SNHL, but are new in the field of inner ear regenerative therapy. To this this end, we have developed a stepwise in vitro guidance procedure, which mimics the most important steps of in vivo otic development, to differentiate hDPSCs into otic neuronal progenitors (ONPs) and then to otic sensory neurons (OSNs). Initially, the hDPSCs were propagated as monolayer cultures expressed known mesenchymal and neural crest markers. Transition to culturing hDPSCs as 3D neurospheres grown under BMP pathway inhibition followed by BMP4 treatment resulted in a significant upregulation of otic/placodal and ONP gene markers after 7 days in vitro. Furthermore, either ONP or OSN-like cells were obtained when the neurospheres were plated on a 2D Matrigel-coated substrate and exposed to Shh and Wnt pathway modulators for 1 week followed by treatment with NT3 and BDNF neurotrophins for 1 week. The differentiated cells displayed characteristic otic neuron bipolar morphology and upregulated a comprehensive panel of ONP and OSN gene markers. This study demonstrates that hDPSCs can be driven to differentiate inner ear neuronal lineages and thereby promotes their potential to be employed in cell-based therapies for autologous auditory nerve repair.

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Keywords: human dental pulp stem cells, otic development, spiral ganglion neurons

TOPIC: PANCREAS

1119

EXPANSION OF INSULIN-PRODUCING ISLET CELLS THROUGH MYCL-MEDIATED REPROGRAMMING FOR TISSUE REGENERATION

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β cells have a limited capacity for regeneration, which predisposes towards diabetes. Here, we show that, of the MYC family members, Mycl plays a key role in proliferation of pancreatic endocrine cells. Genetic ablation of Mycl causes a reduction in the proliferation of pancreatic endocrine cells in neonatal mice. By contrast, the expression of Mycl in adult mice stimulates the proliferation of β and α cells, and the cells persist after withdrawal of Mycl expression. A subset of the expanded α cells give rise to insulin-producing cells after this withdrawal. Transient Mycl expression in vivo is sufficient to normalize the hyperglycaemia of diabetic mice. In vitro expression of Mycl similarly provokes active replication in islet cells, even in those from aged mice. Finally, we show that MYCL stimulates the division of human adult cadaveric islet cells. Our results demonstrate that the induction of Mycl alone expands the functional β-cell population, which may provide a regenerative strategy for β cells.

Keywords: β cell, reprogramming, Mycl

1121

MECHANISMS OF ID3 UPREGULATION

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Pancreatic ductal adenocarcinoma (PDAC) carries one of the highest rates of mortality among cancers. Examination of RNA-seq data from patient tumors revealed that high levels of the transcriptional repressor Inhibitor of DNA Binding 3 (ID3) are associated with poor survival in PDAC. ID3 inhibits the activity of basic helix-loop-helix transcription factors through protein: protein interactions that are notoriously difficult to target with small molecules. Therefore, it is important to identify potentially targetable signaling pathways that regulate ID3 expression. Here we investigated the role of bone morphogenic protein (BMP) signaling on ID3 expression. When the PDAC lines PANC1 and 779e were treated with the BMP inhibitor Noggin for four hours, they exhibited reduced ID3 expression at both the protein level, shown by Western blot and the RNA level, shown by qRT-PCR. To determine which BMP receptor was responsible for ID3 expression, PDAC cells were treated with dorsomorphin homolog 1 (DMH1),

a BMP receptor that specifically targets the ALK2 BMP receptor. Twenty-four-hour treatment with DMH1 profoundly reduced ID3 expression. Further, treatment of PANC-1 and 779 with recombinant BMP2 significantly upregulated ID3 protein and RNA, an effect that was reversed by addition of Noggin or DMH-1 to BMP2 treated cells. To determine whether BMP control of ID3 occurred through canonical signaling we examine the phosphorylation status of the BMP effector pSMAD1,5,8 by Western blot. pSMAD1,5,8 phosphorylation correlated closely with ID3 expression as it was induced by BMP2 and repressed by both Noggin and DMH1. Using SMAD1,5,8 phosphorylation as a readout of BMP signaling, we examined pancreata from the KPC mouse model of PDAC that expresses mutant forms of Kras and p53 in the pancreas. Pancreatic cancer lesions expressed high levels of pSMAD 1, 5, 8 and moreover the pSMAD1,5,8 co-localized with ID3. Together the data suggest that canonical BMP signaling regulates ID3 expression in PDAC and small molecule inhibitors of BMP signaling could be used in the treatment of pancreatic ductal adenocarcinoma. The BMP signaling pathway could be more easily targeted with drug than the transcriptional repressor ID3 in future treatment.

Keywords: pancreatic ductal carcinoma, Inhibitor of DNA Binding 3, cell signaling

1123

PERFUSED FULL-SCALE BETA CELL GRAFTS TO SUPPORT MATURATION IN VITRO AND IMMEDIATELY DELIVER INSULIN IN VIVO

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Several studies have shown that beta cells require an in vivo maturation period after months long injection or implantation. This may be related to lack of a mature niche and lack of sufficient blood supply. For comparison, native pancreatic islets require blood flow of 10-30ml/min to enable physiological response to glucose fluctuations. To improve graft maturity pre-implantation and provide immediate blood supply and function post implantation, we therefore designed a perfused biomimetic scaffold that simulates the native beta cell niche better than current encapsulation techniques. We developed a scalable technology to enable interposition of 15um thick semi-permeable biomimetic membranes between parallel hierarchical channels built to support 600k IEQs (n=15 full-scale scaffolds). We first seeded small-scale grafts with primary rat islets and found that after 24 hours culture, insulin was produced at a stimulation index >5 comparable to static controls. To verify that perfusion would enable bi-directional diffusion across our membrane and convection of a product similar in size to insulin, we seeded HEK cells that secrete luciferase in response to IFN (93,552 at Day2; 4,137,350 at Day7 (RLUs)). Once scaffold function was confirmed, we differentiated hypo-immune induced pluripotent stem cells (Hypo1, Pancella) into endothelial cells and beta cells (adherent Millman Lab Protocol), producing insulin in the scaffold perfusate as long as 18 days in vitro. A graft was loaded with ~15,000 stem-cell beta-cell clusters, while the vascular channel system was lined with endothelial cells, and attached extra-corporeally to the circulation of a healthy porcine recipient. The graft with a sub-therapeutic dose secreted c-peptide into the systemic circulation at a rate of ~24pmol/hr (810 pmol/hr if scaled to 500k clusters). The perfused biomimetic graft design enables pre-implant maturation, glucose sensing and insulin secretion across a mechanical and endothelial based immune barrier, provides immediate blood supply at physiologic levels after implantation, supporting beta-cell engraftment and long-term function. Having recently shown hypo-immune human iPSC derived endothelial coverage in the graft vasculature, validation at scale with human islets and further large animal survival studies are next.

Funding Source: Juvenile Diabetes Research Foundation

Keywords: perfused, beta-cell, implant

1125

ROBUST ESTABLISHMENT AND EXPANSION OF HUMAN PANCREATIC DUCT ORGANOIDS IN PANCREACULT ORGANOID MEDIUM KITS

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Organoids are a novel in vitro culture system that promotes the growth of primary and PSC-derived cells in three-dimensional culture to generate structures that recapitulate tissue morphology. We have developed PancreaCult™ Organoid Medium Kits (Human), which combine PancreaCult™ Organoid Initiation Medium (OIM) and serum-free PancreaCult™ Organoid Growth Medium (OGM) into a robust and standardized workflow for the establishment and expansion of pancreatic duct organoids. To establish organoids, fresh or cryopreserved islet-depleted exocrine tissue was seeded in Corning® Matrigel® domes and cultured in PancreaCult™ OIM. After 3 days, the medium was changed to PancreaCult™ OGM, and the organoids were passaged and maintained in this medium thereafter. Organoids were passaged as fragments every 4 - 7 days. Organoids were successfully established from 5 out of 5 donors with a 2- to 6-fold expansion in cell numbers every 7 - 14 days. Real-time PCR and immunofluorescence micros-

copy demonstrated that organoids expressed pancreatic duct markers (PDX1, SOX9, KRT19, CFTR, CA2, and MUC1), as well as the proliferation marker Ki-67 and the stem cell gene LGR5. Pancreatic duct organoids could be maintained for at least 10 passages (n = 3) and cryopreserved as fragments that could be used for rapid re-establishment of cultures at later time-points. In addition to normal pancreatic duct cultures, PancreaCult™ OGM supported the efficient long-term expansion of three pre-established pancreatic ductal adenocarcinoma (PDAC) organoid lines and could be modified to suppress normal cell growth to select for tumor cells with activating KRAS mutations. Our results demonstrate that PancreaCult™ Organoid Medium Kits provide a robust and flexible in vitro culture system for the establishment and expansion of normal pancreatic duct cells as well as the long-term expansion of PDAC organoids.

Keywords: organoid, pancreatic, PDAC

TOPIC: NO TISSUE SPECIFICITY

1127

AUTOMATED CELL AND COLONY MANAGEMENT FOR GENERATION OF HUMAN INDUCED PLURIPOTENT STEM CELL CLONES

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Human Induced Pluripotent Stem Cells (iPSC) are a transformative technology and could revolutionize how patients are treated for some of the most disabling of chronic illnesses. They can be used to regenerate tissues or serve as important human models for disease mechanism and novel drug testing. However this progress has been hindered as iPSC expansion are labor intensive and subject to large variation in outcomes depending on the skill and subjective judgment between technicians and laboratories. The goal of this work is to develop automated repeatable workflows using the Cell X platform for: 1) Cell Source Selection, 2) Automation of “preferred” clone picking, 3) Automated identification and removal (“weed”) of unwanted differentiating cells, 4) Automated cell confluency monitoring of selected clones overtime, 5) Automated feeding and 6) Documentation of clone attributes to measure quantitatively morphological attributes. Human skin fibroblasts were reprogrammed using the CytoTune Sendai kit. Automated clonal selection and expansion were performed using Cell X robot, which is contained within an enclosed BioSpherix Xvivo systen. Large field of view of the wells were imaged every 2 days using Cell X, which allowed for tracking of cells from fibroblast isolation to colony formation. Automated weeding of residual fibroblasts and spontaneous differentiation to allow iPS clone growth was also performed. Once colonies reached sufficient size, automated quantitative cell/colony morphology analysis was used to segment colonies and quantify critical quality attributeS. Colonies with desired characteristics were then selected and automatically picked. The entire colonies were removed with minimal fluidic shear force using multiple overlapping pick points, then transferred into 12-well plate. Automated media changes were performed at set time intervals during the expansion process. We demonstrated here that reproducible and standardizable protocols and tasks can be defined using Cell X platform to perform critical steps in iPSC fabrication. This platform will contribute to the development of fully automated cell processing and fabrication methods to be developed in a manner consistent with the demands of a GMP environment that is entirely free of manual manipulation and subjective decision making.

Keywords: IPS, manufacturing, morphology

1129

EXPLORING THE MODALITIES OF X CHROMOSOME INACTIVATION MAINTENANCE ACROSS PRIMATES

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X chromosome inactivation (XCI) is a well-established process to equalize the dosage of gene products from the X chromosome between females and males. The master regulator of this phenomenon is the long noncoding RNA XIST which accumulates in cis on the future inactive X chromosome and acts as a platform for the recruitment of various protein complexes including chromatin remodelers. This induces a series of events such as the global loss of euchromatin active histone marks and the gain of heterochromatin repressive ones leading to the wide transcriptional silencing of the X chromosome. Once established, the X inactive state was thought to be stable and transmitted during subsequent cell divisions. However, recent emerging studies reported and linked unusual maintenance of XCI with numerous pathologies as autoimmunity and cancer susceptibility. Decades of extensive studies in mice revealed the dynamics, and molecular mechanisms of XCI establishment and maintenance, some of which appears to be diverse from that of primates. Notably, in a recent study, the establishment of XCI during cynomolgus macaque development was unveiled, highlighting the diversity of XCI strategies across mammals and driving us to revisit major questions about the importance of dosage compensation. In this study, we used human and non-human primate primed embryonic stem cells in a post-XCI state to comprehend the underlying mechanisms of XCI maintenance and its degree of evolutionary conservation among closely related species. Using RNA Antisense Purification (RAP-seq) and CUT&RUN, we both mapped at a high resolution XIST binding sites and characterized the chromatin landscape of the inactive X. This revealed marked differences between species. The biological significance of such differences was approached by inducible XIST loss of function experiments. Intriguingly, loss of XIST expression has distinct molecular and cellular consequences depending on the context. While the underlying mechanisms are currently being investigated, these data point to major differences in the early steps of XCI among primates.

Keywords: X chromosome inactivation, Epigenetics, Evolution

1131

EXPLORING THE ROLE OF LET-7 IN REVERSING STEMNESS OF HIGH-GRADE SEROUS OVARIAN CANCER

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High-Grade Serous Ovarian Cancer (HGSOC) is the most malignant form of ovarian cancer accounting for up to 70% of all ovarian cancer cases with a recurrence rate of more than 80% after treatment for advanced cases. Cancer studies have demonstrated that cancer stem cells (CSCs) and the epithelial-mesenchymal transition (EMT) cause an increase in cancer aggressiveness, including invasion, metastasis, and drug resistance. The Let-7 family of miRNAs function as tumor suppressors that inhibit growth and metastasis by suppressing pluripotency factors and oncogenes. Our previous study revealed Let-7i is substantially downregulated in HGSOC and its levels indirectly correlated with cancer aggressiveness. To better understand how to reverse aggressiveness in HGSOC, the objective is to understand the role of Let-7 in CSCs and its connection to EMT. We hypothesize that overexpressing Let-7 will reverse stemness in CSCs, partially through effects on EMT factors. To begin the investigation, RT-PCR was conducted to establish a baseline of Let-7 expression levels in multiple patient-derived cells where their expression levels were low while EMT factors (SNAIL, TWIST, ZEB1, and ZEB2) were highly expressed. We explored two patient-derived HGSOC samples, one of which was from a primary tumor, and the other from ascites, representing a metastasized tumor sample. To analyze the stemness, SOX2/OCT4 expression was measured through RT-PCR. Let-7 was overexpressed in the PDX4 and PDX6 cells which was confirmed through RT-PCR. Let-7 overexpression led to a decrease in the expression of the EMT factors SNAIL, ZEB1, and TWIST and a decrease in the expression of SOX2/OCT4. We conclude that an increase in Let-7 is correlated to a decrease in stemness in cancer stem cells. Further studies will determine the influence of let-7 on specific EMT factors and a spheroid assay will be conducted to determine the influence on stemness.

Funding Source: CIRM Bridges

Keywords: High-grade serous ovarian cancer, Let-7, Reverse stemness

1133

IDENTIFYING GENES REQUIRED FOR BLASTEMA SPECIFICATION AND REGENERATION IN THE AXOLOTL

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Almost 2 million Americans live with limb loss, and there is no treatment that restores the full functionality of the original limb. Mammals cannot regenerate jointed appendages, yet salamanders robustly regenerate complete limbs through the formation of a blastema. The blastema, a mass of dedifferentiated mesenchymal cells, forms and proliferates shortly after injury, contributing to all mesenchymal structures of the regenerating limb. We must identify the genes required for blastema specification if we hope to recapitulate blastema formation and limb regeneration in humans. Several studies have profiled the blastema transcriptome, but, without a molecular blastema marker, they have been unable to isolate nascent blastema cells before they coalesce into a visible blastema. Kazald2 expression initiates within scattered mesenchymal cells shortly after injury and persists as they coalesce into a discrete blastema, disappearing after the blastema has differentiated. I am generating KAZALD2-GFP reporter axolotls to isolate and profile the transcriptome of nascent blastema cells shortly after injury. Our understanding of blastema formation is also limited by the lack of blastema-less axolotl mutants. Reverse genetic studies targeting blastema-enriched or developmental genes have produced mutants which suffer from delayed regeneration, but still ultimately regrow limbs. I am developing a forward genetic CRISPR screen to identify blastema-required genes in the Axolotl. I will transfect axolotl AL-1 cells with a gRNA library targeting ~1500 transcription factors, and transplant these cells into the axolotl limb, where I will test their ability to integrate into the blastema. I expect both efforts to reveal novel blastema specification genes.

Keywords: Blastema, Axolotl, Regeneration

1135

EXAMINING THE THERAPEUTIC POTENTIAL OF HESC-DERIVED CORNEAL ENDOTHELIAL CELL INJECTION AS AN ALTERNATIVE TO DESCEMETS MEMBRANE ENDOTHELIAL KERATOPLASTY

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Cornea is the outermost, transparent tissue of the eye comprising five layers with corneal endothelium (CE) as the innermost layer. CE maintains corneal transparency by mediating hydration through barrier and pump functions. The genetic and environmental insults lead to corneal endothelial cell (CEC) loss and if left untreated results in blindness. Corneal endothelial dystrophies are the leading cause of corneal transplantations performed in the United States each year. Currently, keratoplasty is the only treatment option for corneal endothelial dystrophies. Although keratoplasty has been effective in treating corneal edema, the global shortage of transplantable-grade donor CE and graft rejection remains an overwhelming impediment and compels for alternative therapies. We previously reported the efficacy of cryopreserved human embryonic stem cell (hESC)-derived CECs to form a functional CE on denuded Descemet's membrane (DM) in rabbits and monkeys. Here, we extended our approach to developing an alternative treatment modality for Descemet's membrane endothelial keratoplasty (DMEK) by successfully demonstrating that injection of cryopreserved hESC-derived CECs into the anterior chamber of rabbit and monkey eyes after removal of central 4mm DM and CE by Descemetorhexis forms a functional CE and the DM on the posterior stroma. All animals developed transparent corneas and CEC injected eye demonstrated corneal

thickness comparable to the untreated eye. CEM-530 specular microscopy confirmed a uniform layer of CE exhibiting hexagonal/ polygonal cells. Moreover, optical coherence tomography showed anatomically normal cornea in the CEC-injected eye similar to the untreated eye. The immunohistochemical analysis illustrated the human-specific expression of collagen type VIII alpha 1 in the regenerated DM and zona occludens-1, and ATPase sodium/potassium subunit alpha1 expression in the regenerated CE. To the best of our knowledge, this is the first report confirming the efficacy of cryopreserved hESC-derived CECs to form a functional CE and the DM on the posterior stroma. These data will lead to the development of a minimally invasive, and donor tissue-independent, treatment modality and will make a paradigm by offering an unlimited source for the treatment of CE and DM dysfunction.

Funding Source: Maryland Stem Cell Research Fund

Keywords: cornea, corneal endothelial cell, human embryonic stem cell

1137

SOX2 MEDIATES OCT4 SUBDOMAIN DYNAMICS

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The complexity of the mammalian genome is owed in part to multiple layers of transcriptional regulation, including the use of multiple transcription factors for the activation/inhibition of a single gene. In pluripotency, Oct4/Sox2 coregulation is one of the most well-researched examples of this and yet a high-resolution structural data isn't available. Data from Oct1/Sox2 structures combined with Oct4/Sox2 molecular simulations suggest that the addition of Sox2 affects the number and type of contacts as well as the affinity of each of Oct4's subdomains. We successfully crystalized the first two Oct4/Sox2/DNA ternary complexes on native targets Utf1 and HoxB1. Oct4/Sox2/Utf1 complex revealed a unique extended conformation in which the DNA binding domain (DBD) linker segment is entirely helical and the homeodomain (POUHD) was not bound to DNA, while the Sox2 HMG and Oct4 POU specific (POUS) domains resemble prior Oct1 structures. A loosely bound POUHD in the Utf1 complex was confirmed by SAXS. Comparison of DNA geometry among Oct/Sox/DNA structures showed an absence of straightening in the Utf1 POUHD half-site, likely due to its suboptimal binding sequence. DBD mutants confirmed the importance of each subdomain in DNA binding, transactivation, and maintenance of pluripotency. Furthermore, adding Sox2 partially rescued mutant activity more for POUHD compared to POUS mutants. However, reprogramming ability was lost for the majority of mutants regardless of subdomain. Molecular dynamics simulations (MDS) with the POUHD modeled into the Utf1 complex showed while this extended conformation does not allow POUHD binding to proximal DNA, it promotes distal DNA sampling. Together these data describe previously unknown details about Oct4 subdomain dynamics in response to Sox2 co-binding, potentially shedding light on POU family binding-site search mechanisms.

Keywords: transcription factors, pluirpotency network, cooperative gene regulation

1139

FGF2, PART TWO: EVALUATION OF HOMOLOGS FOR USE IN HUMAN AND ANIMAL CELL CULTURE FOR THE LIFE SCIENCES AND CULTIVATED MEAT

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Fibroblast growth factor 2 (FGF2, or basic fibroblast growth factor) plays a critical signaling role across cell regeneration, growth, differentiation, migration, and survival, and has been instrumental in the maintenance and differentiation of stem cells, particularly in serum-free medium formulations. Recent improvements in FGF2 stability and performance have centered on the human isoform for use in conspecific cell culture. Notably, earlier work showed differential high performance of cross-species homologs in human pluripotent stem (PS) cell culture. Considering the rapidly expanding interest in food-grade cultivation of agriculturally relevant animal tissue stem cells, these data suggest that cross-species usage of high-performing homologs could provide benefit for both the life science and cultivated meat industries. We previously showed that a wide range of nearly a dozen different species homologs of human FGF2 (72-99% identity) could be readily produced and purified with low-cost methods. To explore their functional value for cell culture, we evaluated these same candidates for proliferation of animal-derived cell lines, and found candidates that showed improved growth compared to others despite species similarity. We further explored the value of top-performing homologs in the culture of human PS cells, utilizing our HiDef-B8 stem cell maintenance medium with the stabilized human FGF2 component removed. These results highlight the value of growth factor homolog screening when optimizing the growth of human- and animal-derived cell lines. Subsequent efforts will explore other growth factors present in expansion and differentiation media used in life sciences and cultivated meat to identify top-performing candidates for potency, cell morphology, growth rate, and cost, enabling the use of affordable and high-functioning defined ingredients for cell culture.

Funding Source: Good Food Institute RFP Award No. REES042021

Keywords: FGF2 homologues, tissue stem cells, cultivated meat

TRACK: TISSUE STEM CELLS AND REGENERATION (TSC)

Session 2: Even

10:45 AM – 11:30 AM

TOPIC: CARDIAC

970

BIOMATERIAL HEART-IN-A-DISH MODELS USING HUMAN PLURIPOTENT STEM CELL CARDIOMYOCYTES FOR TRANSPLANTATION AND DRUG DEVELOPMENT

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Chronic heart failure currently does not have a cure or effective treatment and is the leading cause of mortality and morbidity, accounting for 64.3 million patients worldwide. Human pluripotent stem cell derived cardiomyocytes (hPSC-CMs) offer a reproducible resource of cells. However, a significant challenge lies around identifying physiologically relevant models for cardiovascular disease modelling. Here we explore different models for understanding the complexities of the heart. Proprietary micropatterned polymer coated chemo-topography model identified from screening the TopoChip (~2000 micro-topographies) and polymer (~300 polymer chemistries) micro-arrays have been fabricated using hot-embossing techniques to produce platforms for drug development. Heart-in-a-dish platforms have been used to investigate differences between healthy and diseased hPSC-CMs assessed by their contraction using CellOptiqTM and structural interactions quantified using artificial intelligence guided image analysis and custom sarcomere banding analytical tools. Micro-topographies improved structural integrity of hPSC-CMs by promoting cell elongation (aspect ratio 6-7) and alignment (sarcomere arrangement and bilityrize) whilst our proprietary polymer coating improved hPSC-CM function by increasing contraction amplitude and beat rate (~60-70 beats per minute). The combinatorial technologies can improve multiple maturation factors for better hPSC-CM modelling. Small-scale drug screening assays indicate improved toxicity assessments. The fully-synthetic "chemo-topography" platform completely eliminates the need for common animal-derived coatings currently used to support hPSC-CM attachment and reduces cost by ~300 fold. We expect this to benefit both supply as well as application of hPSC-CMs which include drug cardiotoxicity screening and scale-up production to tissue-like implantable models of chronic heart failure.

Funding Source: EPSRC: Next Generation Biomaterials Discovery & Impact Acceleration Account, UoN: Interdisciplinary Centre for Analytical Science and Strategic Innovation funds, Animal Free Research UK (AFR-UK) and BHF.

Keywords: Human pluripotent stem cell cardiomyocytes, Biomaterial micro-topographies, Fully-synthetic, Xeno-free polymers

972

EFFICIENT DIRECT LINEAGE REPROGRAMMING OF FIBROBLASTS INTO INDUCED CARDIOMYOCYTES USING NANOTOPOGRAPHICAL CUES

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Induced cardiomyocytes (iCMs) generated via direct lineage reprogramming offer a novel therapeutic target for the study and treatment of cardiac diseases. However, the efficiency of iCM generation is significantly low for therapeutic applications. Here, we show the efficient direct conversion of somatic fibroblasts into iCMs using nanotopographic cues. Direct conversion into iCMs on nanopatterned substrates resulted in a dramatic increase in the reprogramming efficiency and maturation of iCM phenotypes compared with that on flat substrates. Additionally, the enhanced reprogramming by the substrate nanotopography was derived from changes in the activation of focal adhesion kinase and specific histone modifications. Taken together, these results suggest that nanotopographic cues can serve as an efficient stimulant for direct lineage reprogramming into iCMs.

Keywords: Direct reprogramming, induced Cardiomyocytes(iCM), Nanotopographical cues

976

GRAPHENE NANOSHEETS MEDIATE EFFICIENT DIRECT REPROGRAMMING INTO INDUCED CARDIOMYOCYTES

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In vivo cardiac reprogramming is a potential therapeutic strategy to replace cardiomyocytes in patients with myocardial infarction. However, low conversion efficiency is a limitation of in vivo cardiac reprogramming for hear t failure. In this study, we showed that graphene nanosheets mediated efficient direct reprogramming into induced cardiomyocytes in vivo. We observed that the administration of graphene nanosheets led to the accumulation of H3K4me3, which resulted in direct cardiac reprogramming. Importantly, the administration of graphene nanosheets combined with cardiac reprogramming factors in a mouse model of myocardial infarction enhanced the effectiveness of directly reprogrammed cell-based cardiac repair. Collectively, our findings suggest that graphene nanosheets can be used as an excellent biomaterial to promote cardiac cell fate conversion and provide a

robust reprogramming platform for cardiac regeneration in ischemic heart disease.

Keywords: Direct reprogramming, Induced Cardiomyocytes, Graphene Nanosheets

Abstract Withdrawn

TOPIC: EPITHELIAL INCLUDES SKIN GUT LUNG

980

SEX DIFFERENCES IN MELANOCYTE STEM CELL BEHAVIOR REVEALS THERAPEUTIC STRATEGIES FOR EPIDERMAL RE-PIGMENTATION

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Conditions of epidermal de-pigmentation, such as vitiligo, can be caused by cutaneous melanocyte loss. Although phototherapy and T cell suppression therapy have been widely used to induce epidermal re-pigmentation in vitiligo patients, full pigmentation recovery is rarely achieved due to our poor understanding of the cellular and molecular mechanisms governing this process. Here, we identify unique melanocyte stem cell (McSC) epidermal migration rates between male and female mice, which is due to sexually dimorphic cutaneous inflammatory responses generated by ultra-violet B exposure. Using genetically engineered mouse models, and unbiased bulk and single-cell mRNA sequencing approaches, we determine that manipulating the inflammatory response through cyclooxygenase expression and activity regulates McSC proliferation and epidermal migration in response to UVB exposure. Furthermore, we demonstrate that macrophages significantly influence epidermal melanocyte re-population via cyclooxygenase activity. These findings suggest a new therapeutic strategy for re-pigmentation.

Funding Source: NIH 5R01AR075755-03

Keywords: hair, melanocyte, epidermis

982

LONG TERM CULTURE OF PORCINE LGR6+ FOLIATE TASTE ORGANOIDS

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The prevalence of acute and chronic taste symptoms in the current SARS-CoV-2 epidemic has highlighted the importance of taste perception to health and has made evident the paucity of clinical treatments for taste disorders. This is because our understanding of taste stem cell function and differentiation lags behind that of other organ-resident stem cells. To date there are limited reports of studies on rodent taste organoids but none on higher-order animals. Recent clinical trials where pig organs have been transplanted into humans have highlighted the similarity of pig organs to human ones. Hence, we set out to propagate porcine taste organoids focusing on the least understood

papillae, the foliate papillae located on the side of the tongue. The pig foliate tissue was digested and plated using basement membrane extract as static 3D cultures, and dynamic cultures using the CLINOSTAR® system. Static cultures gave rise to small reticulate taste organoids within a few days in culture in media that promotes the maintenance of taste stem cells. These organoids in passage 0 (P.0), originated both from single cells and small cell/tissue clumps. Over time the P.0 organoids grew into multiple dense structures comprising a proliferating outer cell layer that enclosed taste receptor cells (TRCs) oriented towards the interior of the organoid. These organoids once established, were expanded beyond passage 15 and were passaged every 10-14 days in culture. In general, the organoids underwent an initial period of expansion and a subsequent spontaneous differentiation period giving rise to mature TRCs. Gene expression analysis of these organoids revealed Lgr6-expressing stem cells with no Lgr5 expression evident. Pig foliate organoids harvested at Day 14 and beyond exhibited the expression of Type I, II, III, and mature TRC markers such as ENTPD2, GNAT3, CA4, and KCNQ1, respectively. Previously, we showed that TRCs synthesize insulin and likewise the pig organoids also contained insulin, and insulin transcription factors MAFA and PAX4. The application of neurotrophins alleviated the adverse effect of the cancer drug Vismodegib on pig taste organoid proliferation and organoid formation. Our work indicates that pig taste organoid cultures are a relevant model for studying taste stem cell dynamics and their differentiation into TRCs.

Funding Source: NIH/NIA Intramural Funds 1ZIAAG000291-14

Keywords: taste organoids, regeneration, insulin

984

IDENTIFICATION OF FACTORS CONVERTING ALVEOLAR TYPE 2 TO TYPE 1 CELLS USING HUMAN IPS CELLS

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During alveolar homeostasis and injury repair in adult lungs, cuboidal type 2 alveolar epithelial stem cells (AT2) proliferate and differentiate into thin-and-flat shaped type 1 alveolar epithelial cells (AT1) which cover the majority of the lung alveolar surface for gas-exchange. Previous studies using human AT2 cells reported fetal bovine serum promoted AT2-to-AT1 differentiation and it has been difficult to identify the global signals that regulate their differentiation, because of the difficulty in stable culture of functional human AT2 cells. Induced pluripotent stem (iPS) cells are suitable for in vitro studies on human AT2 cells. We previously reported that suppression of canonical WNT signaling promoted differentiation from iPS cell-derived AT2 (iAT2) to AT1 (iAT1). However, accumulating literature suggested that additional signals would be involved in AT2-to-AT1 differentiation, motivating us to screen compounds in the non-biased setting. Here, we aimed to search signaling pathways for inducing AT1 and we conducted compound screening using human iPS cells. First, we established an “on-gel culture method” of iAT2 optimized for the screening. Next, in order to precisely detect AT1, we generated AGER-mCherry-HiBiT knocked-in reporter iPS cells. After inducing alveolar progenitor cells from AGER reporter iPS cells, we generated alveolar epithelial cells in the “on-gel culture” and screened chemical compounds which induced AGER-positive cells. As a result of screening, several compounds were found to induce AGER-positive cells. We then asked whether those candidate compounds could promote iAT2-to-iAT1 differentiation, resulting that LATS-IN-1, a YAP signaling activator, was identified. When YAP-TAZ signaling was constitutively activated, AT2-to-AT1 differentiation were promoted. These findings could be beneficial for elucidating the mechanism of human alveolar regeneration.

Funding Source: Funded by Kyorin Pharmaceutical Co. Ltd and Japan Agency for Medical Research and Development (JP19bm0704037 to S.G.)

Keywords: human iPSC-derived alveolar epithelial cells, chemical compound screening, AT2-AT1 differentiation

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SIZE-DEPENDENT G1/S TRANSITION CONTROLS SKIN STEM CELL SIZE

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In adult tissues, heterogeneity in stem cell cycle progression underlies the spatiotemporal dynamics of how tissues turnover. We show that cell size homeostasis which couples cell cycle progression to cell growth is a major contributor to the heterogeneity of stem cell cycle timing. Using week-long longitudinal intravital imaging, we resolved 3D skin stem cell growth in vivo at single cell resolution within a living mouse. We find that the coupling of G1/S transition to cell size accounts for the majority of the heterogeneity of cell cycle timing, above other geometric or topological cell-level and tissue-level features. Furthermore, we find that disrupting the dilution of retinoblastoma protein (RB), which couples G1/S transition to cell size in cultured cells, also leads to the disruption of cell size homeostasis in vivo. This work identifies that cell size homeostasis is largely autonomous in adult skin stem cells, and is a major determinant of their cell cycle dynamics.

Funding Source: NIGMS 1K99GM138712

Keywords: intravital imaging, epidermal stem cells, cell cycle

988

H2AZ1 AND H2AZ1 REGULATE DIVERGENT GENE NETWORKS DURING EPIDERMAL HOMEOSTASIS

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The histone variant H2A.Z experienced duplication early in the vertebrate lineage, generating the H2AZ1 and H2AZ2 isoforms, which differ by only three amino acids. Studies have implicated H2AZ1 in survival, cell fate, and differentiation choices in embryonic and some adult tissue stem cells. The functional differences between H2AZ1 and H2AZ2 remain understudied. Therefore, we leveraged primary human keratinocytes as our model system and applied genomic approaches with 2D and organotypic culture models to define the roles of H2AZ1 and H2AZ2 in epidermal homeostasis. In human epidermis, the outermost terminally

differentiated layer is continually shed and replaced by an underlying pool of progenitor keratinocytes. Western blot and qPCR revealed that progenitor keratinocytes express high levels of H2AZ1 and H2AZ2. shRNA-mediated depletion of either H2AZ1 or H2AZ2 significantly impaired keratinocyte proliferation and migration in 2D culture and inhibited stratification in organotypic culture. To understand these defects, we utilized RNA-sequencing, revealing that H2AZ1 and H2AZ2 knockdown increase the expression of non-overlapping gene sets related to extracellular matrix. During differentiation, H2A.Z protein decreases but a residual remains. We performed ChIP-sequencing with an H2A.Z antibody and confirmed a genome-wide decrease in H2A.Z binding with remaining peaks enriched in promoter regions. In the differentiated condition, RNA-sequencing revealed that both H2AZ1 and H2AZ2 knockdown leads to down-regulation of cell cycle and DNA replication programs that exceeds what is observed in normal differentiation. H2AZ1 knockdown continued to dysregulate the extracellular matrix program while H2AZ2 uniquely impaired terminal differentiation. We establish that both H2A.Z isoforms are essential and cannot compensate for one another in epidermal homeostasis despite their structural similarity, suggesting that the H2A.Z isoforms may distinctly regulate stem cell function in other tissues.

Funding Source: Research supported by NIH NIAMS R01-AR075015 and NCI F31-CA261114

Keywords: epidermis, H2A.Z, keratinocyte

990

DYSREGULATION OF STEM CELLS COMPROMISES SKIN BARRIER FORMATION IN ATOPIC DERMATITIS PATHOGENESIS

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Atopic dermatitis (AD) is the most common chronic inflammatory skin disease in children. The pathophysiology of AD remains poorly defined but involves skin barrier dysfunction, immune dysregulation and microbiome dysbiosis. Accumulating evidence supports skin barrier dysfunction as an initiating factor in AD. Tmem79 is a predisposing gene for AD in mice and humans. Here, using Tmem79 global knockout (KO) and skin-specific conditional knockout (cKO) mice, we demonstrated that Tmem79 in the skin plays a major role in epidermal hyperplasia and skin barrier defects. Furthermore, we showed that epidermal hyperplasia is due to increased basal stem cell proliferation and altered differentiation patterns, subsequently leading to barrier abnormalities. Histological and ultrastructural images further revealed that the terminally differentiated layers of Tmem79 cKO epidermis display morphological differences, which are strongly associated with abnormal skin barrier formation. Additionally, impaired skin barrier function is accompanied by infiltration of γδ T cells, neutrophils and mast cells. Mechanistically, using a genome-wide CRISPR-Cas9 screen, we identified TMEM79 as a specific inhibitor of the WNT pathway via promoting degradation of the Frizzled receptors. TMEM79-KO human keratinocytes exhibited Wnt signaling activation in both proliferative and differentiated states. Importantly, elevated Wnt signaling induced by TMEM79 deficiency can be fully suppressed by Wnt signaling inhibitors in vitro. Collectively, we uncovered a novel mechanism involving hyperactivation of Wnt signaling to regulate skin barrier formation, which may be a viable therapeutic target for AD.

Funding Source: 1. Kirby Innovation Award Program 2. BCH Internal Pilot Study Grant 3. American Cancer Society Research Professorship

Keywords: stem cell proliferation and differentiation, skin barrier dysfunction, Atopic dermatitis

992

ZNF711 COOPERATES WITH THE WNT/β-CATENIN SIGNALING PATHWAY TO REGULATE DIFFERENTIATION AND GROWTH OF INTESTINE STEM CELLS AND COLORECTAL CANCER

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The Wnt/β-catenin signaling governs development and homeostasis by regulating proliferation and cell fate. In the gastrointestinal tract (GI), a Wnt/β-catenin target gene program controls stem cell self-renewal and differentiation into the epithelia, which is responsible for digestion, nutrient absorption, and barrier formation. However, somatic mutations, such as in the adenomatosis polyposis coli (APC) gene, lead to Wnt signaling hyperactivation and improper expansion of stem cells which fuels tumorigenesis and culminates in colorectal cancer (CRC). CRC is the third most common cancer and the second most common cause of cancer-related death worldwide. 90% of CRCs involve Wnt signaling dysregulation, while APC mutations account for more than half of all CRC cases. Therefore, studying Wnt signal transduction and defining novel Wnt signaling regulators has critical significance in understanding stem cell biology and developing novel therapeutic strategies. By performing a cDNA expression screening, we identified ZNF711, an X-linked zinc finger transcription factor of unknown function, as a potent stimulator of TCF/β-catenin-dependent transcription. Using a Wnt-specific reporter gene assay, we uncovered that Znf711 synergizes with Rspo1. Furthermore, we identified that Znf711 N-terminal acidic transactivation domain (TAD) binds to TCF4 and that both TAD and a C-terminal DNA binding domain (DBD) of Znf711 are essential for Wnt signaling activation, suggesting that both TCF4 and DNA binding are required for its activity. The knockdown (KD) of ZNF711 inhibits the Wnt signaling in human CRC cell lines (DLD-1, SW480, HT29, and LS174T) while also being accompanied by impaired growth and increased differentiation. Additionally, overexpression of Znf711 in mouse intestinal organoids allows their growth without Wnt3a

supplementation, evidencing a Wnt-related function. Finally, the KD of Znf711 in mouse intestine organoids and patient-derived CRC organoids hinders proliferation and triggers differentiation. In sum, our data define Znf711 as a new transcription factor that cooperates with TCF/LEF to orchestrate stem cell maintenance and tumorigenesis. These findings delineate a novel mechanism in which Wnt signaling dictates stemness with therapeutic potential for CRC treatment.

Funding Source: R01DK121945, and American Cancer Society research professorship Grant #RP-20-116-06-COUN to X.H

Keywords: Wnt signaling, colorectal cancer, stem cell proliferation and differentiation

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SOX17 INITIATES AN IMMUNE EVASION PROGRAM IN EARLY COLORECTAL ADENOMAS AND CANCERS

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A hallmark of cancer is to avoid immune destruction, yet this process has been primarily investigated in the setting of locally advanced or metastatic cancer. Much less is known about how premalignant or early invasive tumor cells evade immune detection and elimination. To understand this process in early colorectal cancers (CRCs), we investigated how naïve mouse and human colon cancer organoids that were engineered in vitro to harbor APC-null, KrasG12D, and p53-null (AKP) mutations adapted to the in vivo native colonic environment. Comprehensive transcriptomic and chromatin analyses revealed that the endoderm specifying transcription factor SOX17 became strongly upregulated in primary in vivo AKP-derived organoids compared to their naïve counterparts. Surprisingly, whereas SOX17 loss did not affect AKP organoid propagation in vitro, its loss dramatically reduced the ability of AKP tumors to persist in vivo. The small fraction of SOX17-null tumors that grew displayed notable IFNg-producing effector-like CD8+ T cell infiltrates in contraposition to the highly immune suppressive microenvironment in wild-type counterparts. This inability of SOX17-null AKP tumors to engraft and grow was rescued when they were transplanted into CD8+ T cell-depleted or immunocompromised colons. Mechanistically, in both endogenous APC-null pre-malignant adenomas and organoid-derived AKP CRCs, SOX17 suppresses the ability of tumor cells to sense and respond to IFNg which prevents an anti-tumor T cell response. SOX17 engages a fetal intestinal program that drives differentiation away from Lgr5+ tumor cells to produce immune evasive Lgr5- tumor cells with lower MHC-I expression. We propose that SOX17 is a master transcription factor turned on during the early steps of colon cancer to orchestrate an immune-evasive differentiation program that permits CRC progression.

Keywords: colorectal cancer, stem cell, immune evasion

996

ENVIRONMENTAL OXYGEN REGULATES DENTAL EPITHELIAL STEM CELL PROLIFERATION VIA ENERGY METABOLIC-EPIGENETICS INTERPLAY

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The microenvironment, including oxygen (O2), plays a role in regulating cell behavior. However, the mechanism of regulation in epithelial stem cells is not well understood. In this study, we examined the effect of environmental oxygen on the proliferation of dental epithelial stem cells in continuously growing mouse incisors. Our results showed that slow-cycling dental epithelial stem cells were localized away from blood vessels and had higher expression of Hif1α and lactate dehydrogenase, but lower expression of markers for oxidative phosphorylation. This indicates that these stem cells are maintained in a glycolysis-dominant energy metabolic state under low oxygen conditions. Culturing dental epithelial cells under hypoxia increased the expression of glycolysis marker genes and lactate production, which is known to promote histone acetylation. Furthermore, when mouse incisors were cultured under hypoxia, the number of Ki67 positive cells in dental epithelial cells decreased, while acetylated histone H3 and H4 positive cells increased. Finally, treating cells with tricostatin, which induces histone acetylation, reduced the number of Ki67 positive cells. These findings suggest that hypoxia maintains slow-cycling dental epithelial stem cells through lactate-induced histone acetylation, highlighting the interplay between energy metabolism and epigenetics in regulating epithelial stem cells.

Keywords: epithelial stem cells, metabolism, epigenetics

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AIRWAY EPITHELIAL STEM CELLS: REGENERATIVE PROPERTIES, DIFFERENTIATIVE POTENTIAL AND SAFE EXPANSION FOR TISSUE-ENGINEERING APPROACHES

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Respiratory diseases affect millions of people globally, emerging as one of the major causes of disability and death overall. Many pathological conditions can alter the structure and function of the different tracts of the respiratory system, substantially affecting the patients' life. In the case of wide structural alterations, the standard surgical strategies are ineffective or totally inapplicable and among the numerous reconstructive techniques and tissue-engineering (TE) approaches tested so far, no one has become a well-established and routinely-applied clinical procedure.

One of the major difficulties encountered in these clinical approaches is the regeneration of a self-renewing epithelium covering the transplanted graft, imperative to preserve respiratory functions. In TE strategies, the adult airway epithelial cells would be the most appropriate cellular source for restoring the airway epithelium. However, many difficulties have been encountered in the in-vitro expansion of these cells. Indeed, they were described as able to divide efficiently for a very limited number of passages, beyond which they lose their differentiative potential and the ability to form a functional barrier. In the present study, we proved the ability of a clinical-grade culture system, to sustain the long-term proliferative and differentiation potential of airway epithelial cells safely and effectively. Crucial, we identified the stem cells of the airway epithelium and proved their self-renew capacity. Furthermore, we hypothesized the use of some transcription factors as putative molecular markers to be adopted to evaluate the percentage of stem cells within an airway culture. Finally, the multiple analyses conducted at the single-cell level allowed us to understand the mechanisms that underlie the cellular specialization process. Therefore, here we propose a model showing how airway epithelial renewal and differentiation processes could occur. We believe that the information about regeneration, maintenance, and specialization of the respiratory epithelium gained from this study coupled with the established quality controls to be adopted from the biopsy collection up to the development of a TE construct represents important steps towards a safe and successful TE approach for airway reconstruction.

Keywords: Respiratory epithelial stem cells, self-renewal and differentiation, airway tissue-engineering

1000

THE MIR-200 FAMILY INHIBITS EMBRYONIC SPECIFICATIONS OF SEBACEOUS PROGENITORS BY TARGETING BMP PATHWAY

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Mammalian skin plays a critical role as the barrier. Role of stem cells (SCs) including epidermal and hair follicle (HF) SCs in the skin development and maintaining the homeostasis are well studied. So far, it is believed that HF-SCs differentiate and lead to the development of another epithelial skin appendage, sebaceous glands (SGs). Here, I am studying how the SG-fate is determined by finetuning one or a combination of signaling pathways by the miR-200 family of miRNAs (miR-200a, 200b, 200c, 141 and 429), one of the highly expressed miRNA family in epithelial cells. These miRNAs coordinately regulate important physical properties of the cells including that control actin cytoskeleton, cell migration, proliferation, lipid biosynthesis. Interestingly, epithelial specific induction of miR-200a, 200b and 429 at the onset of epidermal fate specification (embryonic day 11) but not the later time point largely abolishes the specification and morphogenesis of sebaceous glands. This suggests that miR-200 miRNAs negatively control sebaceous gland formation in a stage-specific manner. Single-cell RNA-seq and cell trajectory analysis confirm the specific blockage of sebaceous gland fate specification by targeting a subset of upper HF cells. Using an unbiased genomics approach, we have also detected miR-200 targets Nog, a BMP inhibitor, in keratinocytes. This suggests miR-200 miRNAs can change the expression pattern of BMP targets by downregulating Nog and results in perturbing the balance between signaling pathways. These studies together will define the molecular network required for sebaceous gland fate specification and morphogenesis and reveal the mechanism of miR-200 miRNAs in their inhibitory function of gland morphogenesis.

Keywords: skin stem cells, sebaceous gland development, microRNA

1002

INTERROGATING THE CELL-OF-ORIGIN OF BRCA2 MUTANT BREAST CANCER TO IDENTIFY NOVEL TARGETS FOR CHEMOPREVENTION

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It is currently estimated that approximately one woman dies every minute of breast cancer across the globe. While the greatest risk factor for breast cancer development is having female reproductive organs, the cumulative life-time breast cancer risk for women who carry pathogenic mutations in their BRCA2 gene is significantly higher than non-carriers. To date, there have been no prospective studies or chemoprevention trials for BRCA2mut/+ breast cancer, highlighting a need for the development of targeted breast cancer prevention strategies for BRCA2 mutation carriers. Transcriptional and proteomic profiling coupled with downstream ex vivo functional assays were performed to interrogate the mammary stem cell hierarchies of premenopausal BRCA2 mutation carriers and non-carriers, revealing dysregulation across the luminal compartment and an expansion of aberrant luminal progenitors displaying disrupted proteostasis. These data describe developments in our understanding of the cellular alterations present in putative BRCA2mut/+ breast cancer cells-of-origin, and uncover potential therapeutic targets for breast cancer prevention in these patients.

Keywords: mammary stem cell hierarchy, BRCA2, patient

1004

RNA METHYLTRANSFERASE METTL3 IS ESSENTIAL FOR GENOMIC INTEGRITY AND SURVIVAL OF SMALL INTESTINAL STEM PROGENITORS

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Background: N6-methyladenosine (m6A), the most common covalent modification of mRNA, profoundly impacts mRNA translation, stability, splicing, and more. Recent studies demonstrated essential roles for m6A in stem cell survival, proliferation, pluripotency, and differentiation. Almost all m6A on mRNA is added by the METTL3-METTL14 methyltransferase complex. METTL3 and METTL14 are both considered essential members of the complex and deletion of either protein is used interchangeably to study the role of m6A in a target tissue. Gap in knowledge: Two recent studies demonstrate that METTL14 deletion in the intestinal epithelium leads to a severe stem cell death phenotype in the colon with no detected phenotype in the small intestine, but the consequence of METTL3 deletion has not been evaluated in homeostasis of the intestinal epithelium We hypothesized that METTL3 deletion would phenocopy published studies of METTL14 deletion in the intestine. Results: We induced deletion of METTL3 in the intestinal epithelium using Vil1-CreERT2;Mettl3-fl/fl mice (METTL3 KO). METTL3 KO mice lost weight in the days following tamoxifen injection and demonstrated near 100% mortality within two weeks. Histopathological analysis revealed unexpected severe defects in the small intestine including crypt enlargement and villus shortening. We identified abundant apoptosis, gamma-H2AX, and P21 staining in the highly proliferative stem progenitor cells (cells in the transit amplification zone of the crypt), indicative of DNA breaks, cellular senescence, and cell death. We deleted METTL3 in small intestinal organoids, which rapidly resulted in organoid death, mirroring in vivo results. Re-expression of a catalytic inactive METTL3 could not rescue METTL3 KO organoids, suggesting m6A-mediated roles for METTL3 in the small intestine that are independent of METTL14. Ribosome profiling of METTL3 KO organoids demonstrated downregulation of translation of ~1000 unique transcripts including many with critical roles in double stranded DNA break repair. Conclusion: METTL3, but not METTL14, is essential for small intestinal homeostasis at the level of intestinal epithelial stem progenitors. Our data support disparate roles for METTL3 and METTL14 in the small intestine and raise the possibility of novel divergent roles for METTL3.

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Keywords: m6A, METTL3, Intestinal stem cell

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REJUVENATION OF TYPE II ALVEOLAR EPITHELIAL CELLS USING INTERRUPTED REPROGRAMMING TO REVERSE THE HALLMARKS OF AGING

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Autologous lung cell therapy applications for the elderly are limited by suitable cell sources given cellular health declines with age. Alveolar type 2 (AT2) cells, noted to be the distal lung epithelial cell progenitor cell, have shown to be useful as a therapeutic cell source for a number of lung pathologies including Pulmonary Fibrosis. We look to identify aging hallmarks and use a novel transient reprogramming strategy to reverse damage resulting from age and disease. Our reprogramming approach is based on doxycycline-mediated induction of pluripotency stem cell genes, Oct4, Klf4, c-Myc, and Sox2 (OKMS), resulting in transient expression for a defined period, thus generating rejuvenated aged AT2-induced progenitor-like (AT2-iPL) cells. Our findings show that aged (≥65 years old) human AT2 cells have limited clonal potential and are functionally deficient compared to young (≤35 years old) AT2 cells; aged alveolospheres are on average smaller and have less colony forming capacity in our 3D organoid model. Their also appears to be a different distribution of morphologies of these alveolospheres between aged and young donors which is being explored further. Investigating some of the aging hallmarks associated with physiological aging, we saw aged AT2 cells have increased DNA damage and increased gene expression for senescence biomarkers including p21 and SA-β galactosidase. After we have applied our interrupted reprogramming process to aged AT2 cells, we will characterize AT2-iPL cells and assess if they have restored functionality like young AT2 cells. Thus, providing a rejuvenated population for therapeutic use with the elimination of cell-intrinsic deficits after the reprogramming process.

Funding Source: CIHR

Keywords: Primary Human Lung Organoids, Ageing, Cellular Rejuvenation

1008

RECOMBINANT LAMININ PROTEINS PROVIDE A BIOLOGICALLY RELEVANT NICHE FOR PRIMARY CELLS IN VITRO, MIMICKING THE NATURAL NICHE FOR BOTH ADULT STEM CELLS AND PRIMARY CANCER CELLS

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Major efforts have been made on cell culture protocols for cellular therapies, however, studies on cell substrates for pathologically relevant cell cultures are less frequent. Cancer cell models in particular are often based on cell-cell contacts leaving out the essential cell-extracellular matrix (ECM) responses and despite that 85% of all cancers are epithelial cancer in direct contact with the Ecm of the basement membrane. Laminins are a large ECM protein family enriched within the basement membranes, underling epithelial tissues, and an essential part of the stem cell niches. The laminins have multiple, often cell type–specific functions, such as adhesion, differentiation, migration, phenotype maintenance, and resistance to apoptosis. We have analysed the expression patterns of the laminin proteins, in healthy and cancer tissues. We can demonstrate that there is a shift in laminin gene expressions within multiple cancer forms compared to healthy tissues. Both on protein and on relative expression level, within multiple major cancer types such as, lung adenocarcinoma, renal cancers, pancreatic cancer, glioma, and melanoma. The different laminin proteins have different activities and their presence in a tumour can have positive or negative impact on 5-year survival probability, for example, in lung adenocarcinoma where high LAMA3 expression is associated with a negative 5-year survival vs high LAMA2 expression is associated with a positive 5-year survival (P=0,00051, n=494 resp. P=0,0027, n=500). We believe that these in vivo insights can be translated to improve in vitro conditions for primary cells. Cells from lung, skin, and gut, can be maintained in vitro without the need for serum or other undefined components when a biologically relevant laminin isoform is used as substrate, also with direct effects on the cell survival, proliferation and migration, parameters of major focus within drug development assays.

Keywords: stem cell niche, extracellular matrix, cancer

1010

LOSS OF NF-KAPPAB INDUCING KINASE INCREASES SUSCEPTIBILITY TO COLORECTAL CANCER VIA TOP-DOWN TUMORIGENESIS FOLLOWING DIMINISHED REGENERATIVE CAPACITY OF STEM CELL NICHE

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Colitis-associated colorectal cancer (CAC) is an inflammatory subtype of colorectal cancer, in which greater than 20% of Inflammatory Bowel Disease (IBD) patients develop CAC. However, the exact molecular underpinning and cell-type of origin has yet to be defined in this inflammation-induced tumorigenesis. Traditionally, intestinal stem cells (ISCs) located at the base of colonic crypts, multicellular structures composed of ISCs that differentiate into intestinal epithelial cells (IECs), are widely predicted to be the cell-of-origin for this malignant transformation as the “Bottom-up model”. However, there is emerging interest in a “Top-down model”, which suggests the de-differentiation of terminally differentiated IECs in adenocarcinoma development. We hypothesize that the NF-κB inducing kinase (NIK) and its further activation of noncanonical NF-κB signaling is critical for maintaining gastrointestinal homeostasis by maintaining the proliferation-differentiation signaling axis within crypts. Our work shows that NIK regulates IEC regeneration and differentiation, therefore protecting against colorectal cancer. Conditional knockout mice with IEC-specific NIK deletion have increased susceptibility to cancer development following the chemical administration of AOM/DSS. Mechanistic studies evaluating crypts and organoids lacking NIK further revealed an imbalance of proper apoptosis and proliferation signaling. These colonic crypts have increased accumulation of mature, non-dividing IECs. Further, organoids derived from isolated ISCs exhibit stunted growth indicating diminished stem cell potential. Here, we suggest that terminally differentiated IECs are molecularly switched to de-differentiate into a stem-like state to compensate for the decreased regenerative capacity from the stem cell niche in this model for Top-down tumorigenesis. This finding is consistent with human CAC biopsy patients that also have attenuated noncanonical NF-κB signaling, including significant downregulation of NIK (MAP3K14) gene expression. Here, we present a novel role for NIK in regulating IEC regeneration and differentiation in protecting against CAC development. Information yielded here may be further utilized to improve the detection and treatment of CAC in human patients.

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Research, Virginia Tech Carilion Research Acceleration Program, and the Virginia-Maryland College of Veterinary Medicine.

Keywords: De-differentiation, Tumorigenesis, Organoids

1012

REFINING THE ENDOMETRIAL STEM CELL NICHE WITH SINGLE-CELL GENOMICS

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The lining of the uterus – the endometrium, is a highly heterogeneous and dynamic tissue which undergoes dramatic changes following a monthly hormonal cycle. Stem cells in the endometrium repair the tissue and proliferate extensively in the first part of the cycle in preparation for embryo implantation. Pregnancy absent, the tissue degrades and exits the body in the form of menstruation. Stem cells of the endometrium, despite their extensive repair and proliferation potential, have remained elusive. We have previously profiled different cell populations in the human endometrium over the monthly cycle and described the stem cell niche. Here we transcriptomically profile over one million cells from endometria of healthy controls and endometriosis patients by single cell- and single nucleus-transcriptomics. We refine the stem cell niche and describe the differences in its composition between healthy controls (during natural cycles and under hormonal therapy) and endometriosis patients.

Keywords: Endometrium, Genomics, Regeneration

1014

DECIPHERING THE ROLE OF INTERFERON SIGNALING ON THE ACQUISITION AND MAINTENANCE OF EPIGENETIC MEMORY AND ITS IMPACT ON INFLAMMATORY BOWEL DISEASE

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Inflammatory bowel disease (IBD) is a chronic inflammatory condition that alters the homeostasis of the gastrointestinal tract leading to life-threatening complications. Although genetic factors contribute to developing IBD, only 10% of disease variance can be explained by known IBD risk loci. One intriguing hypothesis of how chronic inflammation may arise relates to the process of tissues sensing environmental stress and attempting to protect themselves against future insults. Indeed, recent work has shown that following various stressors epithelial cells reprogram their epigenome to "remember" such events, a process termed inflammatory memory. Often, this involves the opening of chromatin at specific domains that remain accessible long after the resolution of inflammation. However, the molecular pathways that regulate inflammatory memory remain largely unknown. To further explore how inflammation alters the epigenetic landscape in IBD, we an-

alyzed single-cell ATAC-sequencing on intestinal biopsies from patients with Crohn's disease and healthy donor controls. Preliminarily, we observe increased chromatin accessibility in the promoters and enhancers of genes associated with interferon signaling in epithelial cells from Crohn's disease samples. In parallel, we developed a mouse model of inflammatory memory using the well-established Dextran Sodium Sulfate (DSS) protocol. Inflammatory cytokine analysis and immune profiling showed that three consecutive DSS cycles is sufficient to generate a chronic inflammatory state for over one month while allowing complete recovery once treatment is stopped. Intriguingly, we found that colon organoids derived from DSS treated mice showed an enhanced response to interferon signaling compared to naïve organoid controls. These results highlight a role for interferon as a candidate pathway associated with inflammatory memory in intestinal stem cells. We plan to leverage our organoid model to further dissect how interferon signaling may contribute to the acquisition and maintenance of inflammatory memory and whether this impacts tissue homeostasis in the context of IBD. The discovery of pathways and molecular mechanisms governing inflammatory memory will uncover novel therapeutic strategies to treat a broad spectrum of chronic inflammatory diseases.

Keywords: Inflammatory Memory, Inflammatory Bowel Disease, Epigenetics

TOPIC: GERMLINE AND EARLY EMBRYO

1016

ESTABLISHMENT OF HUMAN ALLOGENIC RECONSTITUTED TESTIS WITH IN VITRO-DERIVED HUMAN GERM CELLS

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Specification of germline cells, followed by survival, determination, self-renewal and meiosis in testis epithelial niches are essential for the production of spermatozoa and male fertility. In vitro gametogenesis (IVG) is a tool to that aims to recapitulate this process entirely in vitro. Xenogenic reconstituted testis (xrTestis) using mouse embryonic testicular somatic cells and human in vitro-derived Primordial Germ Cell-Like Cells (PGCLCs) result in modest survival and determination of hPGCLCs, but no further development, suggesting that this xenogenic system could be deleterious to efficient human gametogenesis due to species differences. Here, we evaluate hPGCLCs in human allogenic rTestis (arTestis). Single cell suspensions of human fetal testicular cells formed round and tight aggregates in floating culture for 2 days, with further morphological changes occurring with transfer to transwell membranes at the air-liquid interface (ALI) for an additional 14 days (D14). Using immunofluorescence staining at D14, human rTestis self-assembled and produced prominent tubular structures consisting of SOX9+ Sertoli cells surrounded by ACTA2+ peritubular myoid cells, HSD3B1+ Leydig cells and NR2F2+ interstitial cells. Furthermore, hPGCLCs, which were induced from human embryonic stem cells bearing SOX17-2A-tdTomato and TFAP2C-2A-eGFP (STAG) fluorescence reporters, were combined with human fetal testicular cells to generate arTestis. After floating culture, STAG double positive (STAG+) hPGCLCs were nicely recovered in the arTestis aggregates. Further analysis will be performed with additional culture on transwells at the ALI. Overall, these findings reveal that hPGCLCs and human testicular cells are able to self-assemble to form human-to-human testicular tissues in vitro, which could be a powerful system to enhance human IVG, as well as study human germ cell biology.

Keywords: Human allogenic reconstituted testis, Human male germ cells, In vitro gametogenesis

1018

GENERATION OF FUNCTIONAL GAMETES FROM UNIPOTENT MALE GERMLINE STEM CELLS THROUGH GERMLINE-DERIVED PLURIPOTENT STEM CELLS: NEW HOPE FOR MALE INFERTILITY

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Germline stem cells (GSCs) are unipotent stem cells that comprise only about 0.02–0.03% of all testicular cells that can be used in in vitro spermatogenesis or autologous transplantation in azoospermic men and childhood cancer survivors. Only a few studies have succeeded to improve their proliferative capacity during long-term culture, as well as direct their differentiation to haploid cells. GSCs can be dedifferentiated into germline-derived pluripotent stem (gPS) cells. gPS cells can give rise to cells forming all three germ layers and germ cells. Unlike embryonic stem cells and iPS cells, this approach does not rise any ethical concerns and require use of viral vectors or special manipulations for reprogramming. In this study, we showed for the first time that CD49f+ GSCs could be converted into gPS cells in the presence of GDNF, EGF, bFGF, IGF2, SCF, and mLIF; notably, those gPS cell colonies could be propagated for more than two months on >95% GATA4+ Sertoli cell feeders. GSC-derived gPS cells completed meiosis in vitro by introducing a TGF-β/Activin/Nodal pathway inhibitor, SB431542, followed by retinoic acid. Besides leptotene and zygotene spermatocytes, which were observed after SB431542 pre-treatment, a considerably higher number of spermatozoa were localized upon the combined action of retinoic acid, with a concomitant increase in Ddx4 expression. The resultant haploid male gametes were able to trigger blastocyst formation after intracytoplasmic injection. Following successful co-transplantation of GSC-derived gPS and Sertoli cells into germ cell-ablated mouse testes, the DDX4+ SYCP3+ cells per tubule were higher than those transplanted with only Sertoli cells. Sertoli cells were able to restore endogenous spermatogenesis by reforming the impaired niche, whereas exogenous gPS cells were able to undergo successful spermatogenesis in the presence of healthy niche cells. Overall, our approach shows that GSCs can be expanded in number and our GSC-gPS cell conversion method can upregulate the expression of pluripotent markers under defined culture conditions, gPS cells can undergo stepwise differentiation into functional sperm in vitro and can reinitiate spermatogenesis in vivo with somatic niche/Sertoli cell support. gPS cells offer great promise for patient-specific treatment of infertility.

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Keywords: Germline Stem Cell, Pluripotency, In Vitro Spermatogenesis

1020

HISTONE VARIANT H2AJ IS A HALLMARK OF HUMAN TROPHOBLAST FATE

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Trophoblast specification is the first cell-fate determination during embryogenesis in all eutherians. Past research has been focused on common mechanisms for trophoblast and placental development shared by all mammals. However, the molecular, cellular, and physiological characteristics, such as senescence and invasion into the endometrial tissues, are quite divergent among mammalian species. For example, human placenta tissues frequently migrate to endometrial tissues, where they become senescent, whereas such phenomena don't occur in mice. The lack of understanding of human placentation hampers the prognosis of placental diseases, such as pre-eclampsia. We discovered that the poorly understood histone variant H2AJ is enriched in the placentas of humans and large mammals but not in mice. Interestingly, we demonstrated that H2AJ is essential for human placental development in various cell culture models and for embryogenesis in large mammals, whereas it is dispensable in mice. We further demonstrated that H2AJ plays a critical role in DNA repair and regulates the expression of cytokines that are pivotal for pregnancy. This study discovers a novel epigenetic regulation during early embryogenesis and sheds the first light on how epigenetic mechanisms shape placenta development during mammalian evolution. Moreover, it reveals an unexpected role of genome integrity maintenance around the first cell-fate decision during early development.

Funding Source: Ludwig Family Foundation NIH R01 NICHD NIH R35 NIGMS

Keywords: Trophoblast, Epigenetics, Placenta

1022

UNCOVERING INITIATION MECHANISMS OF NEUROBLASTOMA VIA TRANSCRIPTIONAL PROFILING OF NEURAL CREST DEVELOPMENT

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Neuroblastoma, the most common extracranial pediatric solid tumor, is a neural crest cell (NCC) derived cancer of the peripheral nervous system with a widely varying clinical course: the five-year survival prognosis ranges from 50% to 90% for low-risk and high-risk groups. However, the exact stage of NCC development in which neuroblastoma occurs remains unclear, and it may vary between the groups with different outcomes. While most research has focused on studying neuroblastoma initiation in sympathoadrenal progenitors, chromaffin cells, and sympathetic neurons, recent studies by us and others suggest the initiation may occur earlier during NCC development. We hypothesize we can pinpoint the initiation mechanism of neuroblastoma by searching for matching transcriptional profiles between stages of normal neural crest development and neuroblastoma samples with different prognosis and stage of aggressive behavior. We have optimized protocols for differentiating human embryonic stem cells to early pre- and post-migratory NCC and their respective differentiating sympathetic neuron and chromaffin cell lineage specific derivatives in vitro, of which we have generated single cell and bulk RNA sequencing samples. To identify stage specific resemblance of NCC development and neuroblastoma, we compare developmental gene modules unique to each stage of NCC development to published single cell RNA sequencing data of neuroblastoma samples. Our results show that patient-derived neuroblastoma samples are not equal regarding their transcriptional profile reflecting embryonic developmental status. Even though all neuroblastomas are NCC derived, only some tumors demonstrate a NCC profile while others are more similar to neural stem cells. We suggest that these varying cancer profiles may be the key to understanding differences in neuroblastoma prognosis and outcome.

Keywords: Neuroblastoma, Neural crest, Cancer stem cells

1024

HUCMSC-DERIVED EXOSOMES PROTECT THE SPERMATOGONIAL STEM CELL NICHE THROUGH P38MAPK/ERK AND AKT SIGNALING PATHWAYS IN A HUMAN TESTICULAR DYSFUNCTIONAL MODEL

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Chemotherapy results in spermatogenic dysfunction and infertility in male cancer survivors. Testicular tissue cryopreservation being the only option, we leveraged the regenerative potential human Umbilical cord Mesenchymal Stem cells (h-UCMSCs) exosomes preserving the Spermatogonial Stem cell (SSC) niche as a fertility preservation approach. We used the human Sertoli cells (hSerC) as a testicular dysfunction model by inducing apoptosis using 250 µg/ml of cyclophosphamide (CP). Before inducing CP-induced damage, 1.50X109 h-UCMSCs exosomes were added to the hSerC cells for 24 hours based on the MTT assay. The CP-mediated cytotoxicity was induced for 24 h. The effects of exosomes on cell proliferation, DNA damage repair, antioxidant capacity, and apoptosis were assessed at the end of 24 h. The relative gene expression and immunoblot included i) markers of cell proliferation and survival (PCNA, AKT, Ki67, P21, CYC D1) ii) apoptosis (Cas3, Bcl-2, and Bax) iii) DNA repair (BRCA1 and RAD51) and iv) oxidative stress (catalase, SOD2, and SOD1) v) SSC proliferation, maintenance, and differentiation (hGDNF, NELL2, BMP3, BMP4, hFSHR). The exosome-pretreated group showed improved cell proliferation and cell viability ($p < 0.05$) compared to the CP group. Pretreatment with exosomes enhanced cell proliferation and reduced apoptosis whereas phosphorylated levels of ERK, AKT, and p38MAPK proteins were significantly reduced (($p < 0.001$) in CP-injured hSer cells. FACS analysis showed that the expression of SSC markers SSEA-4 and THY-1 were unaltered whereas CP-induced hSer cells had lower expression ($p < 0.01$). The relative gene expression of PCNA, AKT, Ki67, BMP4, IGF-1 in exosome treated group was significantly upregulated ($p < 0.001$) compared to the CP group with a concurrent downregulation of Cas3, Bax and SOD1, SOD2 suggesting anti-apoptotic, and antioxidative mechanisms exerted by exosomes. RNA sequencing revealed the differential upregulation of miR-17, miR-19a, and miR-17-92 clusters associated with MAPK/ERK in KEGG pathway analysis. Our preliminary observation suggests that exosomes could counteract the reproductive toxicity of CP and could potentially be considered a novel cell-free therapeutic option for preserving the SSC niche and thus increasing the chances of fertility in cancer survivor patients

Funding Source: The University of Chicago

Keywords: Testicular dysfunction, Exosomes, Sertoli cells

TOPIC: HEMATOPOIETIC, IMMUNE, AND ENDOTHELIAL

1026

UNPRECEDENTED EFFICACY OF RYTVELA IN REVERSING VASCULOGENIC DYSFUNCTION OF ENDOTHELIAL PROGENITOR CELLS IN OXYGEN-INDUCED RETINOPATHY THROUGH INHIBITION OF MIR-875

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Retinal vaso-obliteration is an initial key step in oxygen-induced retinopathy (OIR). During vascular development, endothelial Progenitor Cells (EPCs) promote vasculogenesis. However, the EPCs functional activity are impaired during OIR. We investigated the potential beneficial effects of rytvela (a new non-competitive allosteric interleukin (IL)-1 receptor inhibitor) on the post-transcriptional mechanism involved in both hyperoxia/IL-1b-induced EPC dysfunction during OIR. First, we found that EPCs subjected to hyperoxia or IL-1b exhibit a phenotype of early senescence, associated with a significant decrease of their migratory and vasculogenic properties; and that are antagonized by rytvela. To examine the post-transcriptional mechanisms associated with EPC dysfunction, we next profiled micro-RNAs (miRs) expression using next generation sequencing (NGS), in the same condition. Globally, NGS analysis reveal that 26 miRs are significantly modulated by both IL-1b and hyperoxia, and the expression of 9 of these miRs are recovered by rytvela. Interestingly we found that miR-875 – predicted to negatively regulate the expression of FOXF1, a key transcriptional factor for multiple pro-angiogenic factors including CXCR4 – to be upregulated. We next performed a gain-and-loss of function to study the precise role of miR-875 and the result confirms that overexpression of miR-875 in native EPCs leads to downregulation of FOXF1/CXCR4 signaling, that in turn decreases the migratory and vasculogenic properties of native EPCs. Conversely, inhibition of miR-875 protects the migratory and vasculogenic activity of EPCs against hyperoxic and IL-1b, believed to restore FOXF1/CXCR4 expression. Altogether, our results suggest that rytvela can protect vasculogenic properties of EPCs in OIR conditions through inhibition of miR-875. Bioengineering EPCs with rytvela or bilityr-875 based-therapy could provide a new potential strategy to preserve vessel integrity in ischemic retinopathies.

Funding Source: MITAC bility

Keywords: EPC, miRNA, inflammation

1028

TAZ PROTECTS HEMATOPOIETIC STEM CELLS FROM AN AGING-DEPENDENT DECREASE OF PU.1 ACTIVITY

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Hematopoietic stem cells (HSCs), which reside at the apex of the hematopoietic hierarchy, contribute to maintaining long-term repopulation and produce multilineage mature blood cells throughout life. During aging, age-associated functional defects in HSCs result in a decrease of regenerative potential and a myeloid-skewed immune system causing a substantial deterioration of immune response in the elderly. Although the loss of functionality, aged HSCs keep a certain level of stem cell function. It suggests aged HSCs have a protective mechanism to prevent the severe decline of regeneration capacity and adapt to an aging microenvironment. We find that TAZ, a transcriptional coactivator regulated by the Hippo signaling pathway is potently induced in HSCs during aging and maintains a minimal regenerative function of HSCs. We identify that TAZ-induced CLCA3A1 is a marker for “true” aged HSCs as well as a TAZ activity marker. As a result, CLCA3A1high LT-HSCs resemble old HSCs whereas CLCA3A1low LT-HSCs behave like young HSCs at the level of transcriptome and chromatin and in terms of function. Thus, we identify “young-like” HSCs in the old HSC pool. PU.1, a key regulator of HSC functionality is decreased in CLCA3A1high LT-HSCs. A noble mechanistic role of TAZ is to function as a transcriptional coactivator of PU.1 to activate its transcriptional activity. We unravel here that TAZ safeguards aged LT-HSCs to buffer the age-dependent decline of PU.1 activity. Our study would give a better understanding of the physiological features in old HSCs and assist to develop a future therapy for improving immune response in the elderly.

Keywords: Hematopoietic stem cell aging, TAZ, Hippo pathway, PU.1

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A HIGH FAT DIET IMPAIRS HEMATOPOIETIC STEM CELL FUNCTION AS A CONSEQUENCE OF FATTY ACID OXIDATION

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Stem cells and restricted progenitors have different metabolic signatures. This suggests that the stem cell state is metabolically regulated, though our understanding of stem cell metabolism remains limited. For example, little is known about how fatty acid oxidation regulates hematopoietic stem cell (HSC) function. In this study, we systematically characterized the role of fatty acid oxidation in hematopoiesis by genetically deleting in mice multiple enzymes that are necessary for different steps of fatty acid oxidation: CPT1a, VLCAD, and HADHA. We confirmed the metabolic effects of deleting these enzymes by performing metabolomics on HSCs and observing the depletion of metabolites at successive stages of fatty acid oxidation. We found that HSCs and restricted hematopoietic progenitors engage in fatty acid oxidation but that it is not necessary for HSC maintenance or steady-state hematopoiesis. When mice were administered a high fat diet, HSC function was impaired upon competitive transplantation into irradiated mice. However, this decline in HSC function was rescued by inhibiting fatty acid oxidation. This suggests that in the context of a high fat diet, fatty acid oxidation impairs HSC function. Overall, our results indicate that HSCs engage in fatty acid oxidation but that this is not necessary for HSC maintenance and that fatty acid oxidation can impair HSC function during HFD-induced obesity.

Keywords: Hematopoietic stem cells, Fatty acid oxidation, High fat diet

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INFLAMMATION-INDUCED AGING CHARACTERISTICS IN HEMATOPOIETIC STEM CELLS

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Hematopoietic Stem Cells (HSCs) are specialized master cells that give rise to all the blood cells in the body. During steady-state, they give rise to an unbiased lineage output but upon infection leading to inflammation, stress hematopoiesis is activated. This is inevitable as the HSCs are bound to be exposed to stressors throughout life. Hence, it is important to study these cells in order to prevent possible immune-related dysfunctions during organismal aging. To address the effects of inflammation on HSCs we used Glucose-6-phosphate isomerase (G6pi)-induced murine rheumatoid arthritis model. Flow cytometric sorting of bone marrow HSCs was performed 56 days post arthritic induction followed by RNA-Sequencing (RNA-Seq). Previously, we identified Clca3a1, a calcium-activated chloride channel accessory membrane protein as a novel true aged HSC (aHSC) marker by distinguishing the aHSCs to 2 sub-populations: Clca3a1hi having true aged transcriptome with functional defects and Clca3a1lo resembling young-like HSCs. Interestingly, around 50% of HSCs from young arthritic mice were Clca3a1hi, a phenotype that is not native to young HSCs. Principal component analysis from RNA-Seq data revealed that arthritic and control HSCs are transcriptionally distinct. The upregulated genes of arthritic inflammation-exposed HSCs (iHSCs) significantly overlapped with aHSCs despite their young age. However, the statistical significance in both upregulated and downregulated genes of iHSCs to aHSCs was higher when the iHSC population was phenotypically separated to Clca3a1hi and Clca3a1lo. Hallmark aHSC and myeloid-biased genes are among the significantly upregulated and genes related to differentiation are downregulated in young Clca3a1hi iHSCs. But, when naïve HSCs were exposed in vitro to IL-1b, a proinflammatory cytokine, no induction of Clca3a1 was found. This hints an involvement of complex niche-related in vivo signaling in both induction and maintenance of Clca3a1hi phenotype and transcriptome in young arthritic mice. In summary, inflammation, as a tool and a trigger, mimics aged-like HSCs both phenotypically and transcriptionally in young mice. Exploring further the contributions of niche could broaden our knowledge on key events involved in inflammaging of HSCs and to tackle them effectively in the future.

Funding Source: German Research Foundation (Deutsche Forschungsgemeinschaft – DFG)

Keywords: Aging, Inflammation, Hematopoietic Stem Cells (HSCs)

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MAINTENANCE OF HEMATOPOIETIC STEM CELLS WITH SMALL MOLECULE INHIBITORS

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Hematopoietic stem cells (HSC) produce mature blood cell types in the bone marrow. Due to their self-renewal ability, immunophenotypically primitive HSCs are ideal for studying blood disorders and clinical applications. It has been challenging to maintain the stemness of HSCs ex vivo. The mTOR signaling pathway is responsible for cell proliferation, lineage commitment and differentiation in HSCs. Over expression of the mTOR pathway has been found to exhaust HSC repopulation and loss of HSC stemness. The Wnt signaling pathway regulates cell homeostasis and self-renewal in HSCs. GSK-3 is known to inhibit β-catenin, which is essential for the activation of Wnt pathway. In this study, we investigated the effect of small molecule inhibitors on maintaining stemness in HSCs (CD34+, CD38-). Peripheral blood stem cells (PBSC) were treated with GSK-3 inhibitor (CHIR99021) and Rapamycin in a cytokine-free X-VIVO 15 medium. We maintained about 33% of HSCs (CD34+, CD38-), with over 90% still at a quiescent state after three days in culture. Ongoing experiments aim to determine the impact of this approach on DNA repair in HSCs. Current gene editing strategies rely on homology-directed repair (HDR), which mainly occurs in the S and G2 phases of the cell cycle, for successful insertion of the therapeutic gene. HSCs are relatively quiescent, so it is challenging to use gene editing strategies that utilize HDR. Since the non-homologous end joining (NHEJ) repair pathway is active throughout the cell cycle, homology-independent targeted integration (HITI) was developed as an NHEJ-based approach for donor insertion. Recent studies suggest that cell cycle arrest may improve HITI by eliminating DNA repair mechanisms in S and G2 phases. We hypothesized that prevention of cycling using CHIR99021 and Rapamaycin will also improve HITI. In addition, maintaining the stemness of HSC may further improve gene editing strategies by increasing the engraftment of edited HSCs.

Keywords: Hematopoietic Stem Cells, small molecule inhibitors, Gene editing

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LSD1/KDM1A AND GFI1B ORCHESTRATE HEMATOPOIETIC EMERGENCE FROM INDUCED PLURIPOTENT STEM CELLS

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Differentiation of induced pluripotent stem cells (iPSC) to hematopoietic lineages holds great promise to produce novel, or replace existing cellular advanced therapeutic and medicinal products. During embryogenesis, hemogenic endothelial cells give rise to hematopoietic stem and progenitor cells through a process called endothelial to hematopoietic transition (EHT). Understanding EHT from iPSC is crucial to establish hematopoietic cell production protocols. The transcription factor Gfi1b and its cofactor, the chromatin demethylase Lsd1 (Kdm1A), are essential for definitive wave EHT and functional hematopoietic stem and progenitor cells in mice. To study the role of GFI1B and LSD1 in EHT, we used human iPSC lines derived from patients expressing a dominant negative dysfunctional GFI1BQ287*, and we treated wild type iPSC with an irreversible LSD1 inhibitor. CD34+/CD144+/CD309+ Hemogenic endothelial cell formation during iPSC differentiation was unaffected in GFI1BQ287* patient lines and upon LSD1 inhibition. In contrast, hematopoietic commitment and hematopoietic progenitor yield was severely reduced in GFI1BQ287* iPSC and completely absent upon inhibiting LSD1. To uncover the molecular mechanisms underlying the affected hematopoietic commitment via EHT, we performed single cell RNA sequencing (scRNAseq) on the heterogeneous hemogenic endothelial population harvested from iPSC that were differentiated in absence or presence of a LSD1 inhibitor. scRNAseq revealed a dynamic expression of genes associated with EHT in vivo, and a complete absence of cells with a hematopoietic gene expression profile in LSD1 treated conditions. Furthermore, we identified gene modules that are specifically associated with LSD1 inhibition. GFI1B expression in hemogenic endothelial cells was not observed while GFI1B cofactors, such as LSD1 were expressed. Hematopoietic specification of CD34+ MACS sorted hemogenic endothelium was occurring independent of other accessory cells. These hemogenic endothelium cells were transduced with GFI1B constructs to influence EHT. Based on the results we show that the endothelial program during EHT is partly controlled by LSD1/GFI1B and not efficiently down-regulated upon functional impairment of LSD1, or upon expression of the dominant negative GFI1BQ287*.

Funding Source: Health Holland Symphony consortium product and process development cellular products, Dutch government, VWS.

Keywords: GFI1B, LSD1, hematopoiesis

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REGENERATION OF MOUSE ENDOTHELIAL CELLS WITHIN BONE MARROW NICHE AT SINGLE CELL AND CLONAL LEVEL

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Bone marrow endothelial cells (BM-Ecs) are the essential element of the bone marrow (BM) niche and support proper function of hematopoietic stem cells (HSCs). However, conditioning for HSCs transplantation causes significant damage to the recipients' BM-Ecs and may lead to transplantation-related morbidity. Our aim was to understand the cellular and clonal mechanisms of BM-Ecs regeneration after irradiative conditioning. To identify the potential endothelial progenitor subpopulations in BM niche, we performed single cell RNA sequencing (scRNA-seq) of mouse BM-Ecs and combined our data with published scRNA-seq datasets. The metanalysis Included 13,037 BM-eCs and revealed the subpopulation of BM-EC that expresses both sinusoidal and arteriolar markers along with Apelin receptor (referred to as trans-eCs). Imaging of the BM niche showed that trans-eCs localize between the sinusoidal and arterial eCs, linking the distinct eCs within the vascular network.Next, we developed single-cell BM-eCs in vitro assay to quantify the clonogenic potential of different BM-eCs fractions. Limiting dilution assay demonstrated that all prospectively isolated arteriolar, sinusoidal and trans-eCs showed similarly high clonogenic potential (1/4.3, 1/5.06, 1/5.23 of sorted cells, respectively). This indicates that a substantial fraction of each BM-EC subpopulation can re-enter the cell cycle. To verify whether high clonogenic potential of BECs observed in vitro contributes to regeneration of BM-eCs after irradiative conditioning we used rainbow mice, which showed random expression of fluorescent proteins in endothelial cells (Cdh5-CreER; R26VT2/GK3) upon induction. 21 days after irradiation we observed highly polyclonal pattern of BM-eCs, without any detectable oligoclonal regions. To

quantify the clonality of BM-eCs during regeneration in rainbow mice, we developed a method based on graph theory, local assortativity and machine-learning modeling. The data strongly suggest that 10% of surviving BM-eCs undergo, on average, one cell division to regenerate cell lose caused by irradiation. Concluding, our novel single-cell in vitro clonogenic assay and in vivo clonal tracking model suggest polyclonal regeneration of BM-eCs after irradiative conditioning.

Funding Source: Supported by Horizon Europe ERC Grant number: 101041737 and National Science Center grant Maestro no 2018/30/A/NZ3/00495.

Keywords: bone marrow niche, endothelial cells, hematopoietic stem cells transplantation

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LONG AND SHORT ISOFORMS OF MLLT3 BALANCE HUMAN HSC FATE DECISIONS

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MLLT3 is a key regulator of human hematopoietic stem cell (HSC) self-renewal whose expression declines in culture. Maintaining MLLT3 levels in cultured cord blood (CB) HSCs results in expansion of transplantable HSCs without transformation or differentiation bias. Analysis of RNA-seq data and epigenetic marks associated with the MLLT3 gene in human HSCs revealed a second TSS linked to a novel MLLT3 isoform (MLLT3-S). MLLT3-S encodes a truncated protein that retains the AHD domain responsible for protein-protein interactions, but lacks the chromatin binding YEATS domain. MLLT3-S overexpression (OE) in hematopoietic cell lines confirmed the generation of a stable protein that interacts with known MLLT3 protein partners DOT1L and superelongation complex, but cannot bind chromatin. While MLLT3-L OE in CB HSCs promoted their expansion, MLLT3-S OE suppressed it, implying distinct functions for the two isoforms in HSCs. Conversely, while knockdown (KD) of MLLT3-L in CB HSCs triggered premature differentiation, MLLT3-S KD resulted in relative expansion of immunophenotypic HSCs. However, although MLLT3-S deficient HSPCs appeared phenotypically normal in culture, they engrafted poorly in immunodeficient mice, showing that both isoforms of MLLT3 are necessary for proper HSC function. scRNA and bulk RNA seq of HSPCs with MLLT3-L and/or MLLT3-S OE and KD revealed that the two isoforms have opposing effects on expression of key HSC processes, such as mitochondrial biosynthesis and oxidative phosphorylation, translation, and splicing, as well as several HSC transcriptional regulators. This analysis also revealed that MLLT3-S OE suppresses IGFBP2, a regulator associated with HSC expansion and highly proliferative fetal HSCs, whereas MLLT3-L OE promotes its expression. Bulk RNA-seq and scRNAseq analysis of human developmental tissues revealed that, while MLLT3-L is expressed already in hemogenic endothelium in the embryo, MLLT3-S is induced in maturing fetal liver HSCs. This suggests that by suppressing MLLT3-L-driven expression of IGFBP2 and HSC expansion, MLLT3-S may promote HSC maturation in the fetal liver and their transition to homeostatic state. The interplay between long and short isoforms of MLLT3 in human HSCs may provide a mechanism by which mature HSCs balance between expansion and maintenance modes.

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Keywords: hematopoiesis, human, gene expression regulation

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TIE2 FORMS TWO DISTINCT CLASSES OF COMPLEXES TO REGULATE VASCULAR STABILITY

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The angiopoietin-Tie2 pathway regulates blood vessel stability, remodeling, and wound healing. Previously, we demonstrated that Ang1-like synthetic ligands at high oligomeric states accelerate cell migration and promote vascular stability, but how Tie2 performs these opposing cellular functions is unclear. In the present study, we show that Tie2 forms two classes of complexes: Tie2-α5β1 integrin and Tie2-tight junction complex. We show the Tie2-α5β1 complex promotes cell migration by forming focal adhesions and recruiting VE-cadherin out of the cell-cell junction.

We also demonstrated that the Tie2-α5β1 complex is critical to activating the pAKT/FOXO1 signaling axis to promote cell survival. In contrast, the Tie2-tight junction complex consisted of ZO1, claudin-5, andbilityrn that facilitate tight junction assembly for vascular stability. We further demonstrated that computationally designed Tie2 super agonists up-regulate tight junction expression in mature and developing endothelial cells. Tie2 super agonists accelerate junctional ZO1 and claudin-5 re-assembly after chemical disruptions in HUVECs, indicating that the Tie2 signaling directly modulates the assembly of tight junctions at the endothelial paracellular space. In conclusion, we illustrated the mechanism of Tie2-mediated cell migration and tight junction formation via two classes of Tie2 complexes to regulate angiogenesis. Understanding how Tie2 regulates angiogenesis can offer a new therapeutic target to combat diseases that exhibit vascular dysfunction.

Keywords: Angiogenesis, Tie2 signaling, Computationally designed protein

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UNVEILING THE BIOLOGICAL ROLE OF PERIPHERAL BLOOD HUMAN CIRCULATING HEMATOPOIETIC STEM AND PROGENITOR CELLS

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Although most hematopoietic stem/progenitor cells (HSPC) reside in the bone marrow (BM), few circulating HSPC (cHSPC) are also found in the peripheral blood (PB) at steady state. However, their biological role and relationship with BM-resident counterpart in humans remain still not fully elucidated. In the present study, we characterized cHSPC composition by multi-parametric

flow cytometry on 110 PB and 48 BM samples of healthy donors of different ages. These analyses were combined with cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq), in vitro and in vivo assays, and integration site (IS) analysis in 8 patients treated with HSPC gene therapy (GT) at >2 years-follow-up to investigate the functional and differentiation properties of PB vs BM HSPC. We observed that cHSPC count decreased over ageing and showed different composition than BM HSPC, suggesting diverse recirculation capacity of distinct HSPC subsets. cHSPC showed enriched differentiation- and adhesion molecules-transcriptional priming than BM counterpart and were endowed with BM homing and multilineage differentiation potentials in vitro and in vivo. However, cHSPC displayed reduced long-term engraftment, associated to the low content and transcriptional pre-activated state of the more primitive subset. Primitive and myeloid cHSPC populations were transcriptionally and functionally skewed toward erythroid lineages than BM-resident counterpart, while a substantial fraction of trafficking lymphoid HSPC displayed a signature of thymus seeding progenitor type 1 (TSP1). In line with this latter result, we found a higher IS sharing between PB mature lymphoid cells and steady-state cHSPC in our cohort of GT-patients, suggesting their contribution to steady-state lymphopoiesis. In summary, cHSPC are a peculiar steady-state reservoir of pre-activated hematopoietic progenitors endowed with BM homing and repopulation potential. The functional erythroid commitment of cHSPC suggests their supportive role in extramedullary erythropoiesis. Furthermore, our transcriptional and IS analyses suggest the key function of trafficking lymphoid HSPC in lymphoid organs seeding. Overall, our work represents one of the most comprehensive studies on cHSPC, unveiling fundamental insights on their biological role in humans.

Keywords: Circulating Hematopoietic Stem Cells, Human hematopoiesis, Extramedullary hematopoiesis

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HIGH-THROUGHPUT VASCULOGENESIS ASSAY USING IPSC DERIVED ENDOTHELIAL CELLS, PERICYTES, AND FIBROBLASTS

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Human iPSC can provide multiple lineage specific cells for regenerative therapy and drug screening. In vitro vasculogenesis assays are useful in various areas including anti-angiogenic drug screening, underscoring the need for long-term, stable capillary formation with a reproducible, high throughput assay. Here, we report an vasculogenesis assay using iPSC derived endothelial cells (eCs), pericytes, and fibroblasts in hydrogel system. IPSC lines were differentiated into vascular lineage using APEL2 differentiation medium supplemented with Activin-a, BMP4, VEGF, and CHIR99021 (2 days) followed by VEGF and SB431542 (5 days). On day 7 of differentiation CD31+ and CD31- cells were separated and CD31+ cells were expanded prior to cryopreservation. CD31- cells were further differentiated to pericytes in DMEM, 10% FBS, PDGF-bb and TGF-b for one passage and expanded in DMEM, 10% FBS. Fibroblasts were differentiated from pericytes in fibroblast medium. Fibroblasts, eCs, and pericytes were mixed in ratio of 12:6:0.6 million cells for 1 mL hydrogel consisting of gelatin, fibrinogen, and aprotinin. 10 uL of cell/gel mixture were added to each well of ibid 96-well plate. For the first week of vasculogenesis, medium included VEGF, aprotinin, angiopoietin-1, and bFGF then angiogenic growth factors were removed in following weeks. All cell components were cryopreserved and thawed for functional validation and assembly. eCs were confirmed to express CD31, CD34 and CD144 and differentiated from a GFP+ iPSC line to visualize capillary structures. Pericytes were confirmed to express PDGFR-b, NG2, and CD44. Fibroblasts expressed vimentin, connexin43, and collagen-IV. When fibroblasts, EC, and pericytes were assembled with 12:6:0.6 ratio, most robust capillary formation was observed within 24 hours and sustained for more than 3 weeks. With activation of alternative complement pathway, we observed degeneration of capillaries. Capillary area, branch points, vessel lengths and vessel widths were quantified by MATLAB software. Our results show that all cell components (EC, pericytes, and fibroblasts) are successfully differentiated from human iPSC with functional recovery after cryopreservation. This study provides the foundation for iPSC-derived 3D-tissue, drug screening and study model of immune cell reactions.

Keywords: Vasculogenesis, Angiogenesis, In vitro capillary formation

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UNBIASED LINEAGE TRACING OF LONG-TERM HSCS IN VIVO AT STEADY-STATE

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The mammalian blood system is comprised of over ten distinct cell types, all of which are derived from the hematopoietic stem cell (HSC). The hematopoietic system is essential for maintaining the health and longevity of the human body, and perturbations to the system cause an array of life-threatening diseases. The various cell types have previously been well characterized, but an important question that remains in the field is how the small population of long-term HSCs contribute to steady-state hemato-

poiesis. Investigating the lineage relationships of this system not only provides valuable information about normal development, but also for developmental disorders and malignancies. A current limitation to understanding this relationship is, in part, due to controversial lineage tracing models. Hoxb5 is the most precise functional marker to date for distinguishing long-term HSCs. With that information in mind, for this project we use hoxb5-CreERt2 mice to specifically label long-term HSCs; these mice are crossed with reporter mouse lines that harbor either LSL-tdTomato or LSL-rainbow. This model allows us to track daughter cells and perform stem cell clonal analysis at varying time points. Through these tracing studies, we have seen that hoxb5+ long-term HSCs have a limited contribution to steady-state hematopoiesis throughout the several time points. Interestingly, under stress by lymphocyte depletion, we have found that this does not expand the long-term HSC contribution to hematopoiesis. Instead, we believe that the lymphocyte population is regenerated by multipotent progenitors or cells further down the hierarchy. This data suggests that a greater disturbance to the system is necessary to illicit a response from the long-term HSCs. Further investigation of the lineage relationships that govern the hematopoietic system will continue to provide insight on development, as well as provide more precise research targets for drug therapies.

Keywords: Hematopoiesis, Lineage Tracing, Hoxb5

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MYCT1 MODERATES ENVIRONMENTAL SENSING VIA ENDOCYTOSIS TO PRESERVE HUMAN HSC SELF-RENEWAL

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Ex vivo expansion of functional human hematopoietic stem cells (HSC) would greatly improve the access to HSC transplantation therapies for blood disorders. However, the programs governing human HSC self-renewal and engraftment ability are poorly understood, and cannot be recapitulated in culture. We discovered a novel HSC regulatory gene, MYCT1 (MYC target 1), that is selectively expressed in endothelial cells (EC) and undifferentiated human HSPCs but becomes drastically downregulated during HSC ex vivo culture. Knockdown (KD) experiments revealed that MYCT1 is critical for human HSC expansion and engraftment. Single cell RNAseq in MYCT KD and OE (overexpression) HSC cultures indicated that MYCT1 governs transcriptional signatures associated with HSC identity, as well as biological processes essential for HSC stemness, such as tightly controlled mitochondrial activity. Whereas the loss of MYCT1 worsened these “stemness” signatures, restoring MYCT1 expression in cultured CB HSCs restored these dysregulated programs compared to control cells. Strikingly, maintaining MYCT1 expression improved ex vivo expansion of the most undifferentiated EPCR+ITGA3+ human HSPCs and enhanced engraftment ability upon transplantation to NSBGW mice. We discovered that MYCT1 localizes in the endosomal membrane and interacts with vesicle trafficking and signalling machinery essential for HSC and EC function. Loss of MYCT1 led to hyperactivation of endocytosis and exaggerated signaling responses to cytokines in the culture microenvironment, whereas

restoring MYCT1 expression in cultured human HSPCs was sufficient to balance abnormal endocytosis and restrain the excessive signaling responses. These data show that the moderation of environmental sensing in human HSCs through MYCT1-controlled endocytosis is essential for preserving human HSC stemness. As MYCT1 expression is downregulated in cultured HSPCs, our data imply that loss of the molecular machinery required for proper sensing of microenvironmental signals is a key contributor to the dysfunction of cultured human HSCs.

Keywords: hematopoietic stem cells, endocytosis, environmental sensing

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G9A INHIBITION PROMOTES LYMPHOID DEVELOPMENT AND T CELL DIFFERENTIATION FROM INDUCED PLURIPOTENT STEM CELLS

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Employing differentiation protocols that allow efficient generation of T cells from human induced pluripotent stem cells (iPSCs), we have performed genetic knockdown and knock-out screens and identified epigenetic control elements that are critical for in vitro T cell differentiation (Vo et al, Nature 2018). Using a novel stroma-free T cell differentiation protocol (Jing et al., Cell Stem Cell 2022), we screened a library of small molecules with known modes of action against chromatin-modifying enzymes, and discovered that inhibition of histone lysine methyltransferase G9a promotes T cell production from iPSC-derived hematopoietic stem and progenitor cells (HSPCs). Using human iPSCs and zebrafish models, we demonstrate that G9a normally acts as a repressor of lymphoid potential during hematopoietic development. Furthermore, by incorporating small molecule-mediated G9a repression into our pre-existing T cell differentiation protocol, we have generated iPSC-T cells with a molecular signature that closely resembles mature T cells derived from peripheral blood. We show that iPSC-T cells derived via G9a repression exhibit robust effector response and antitumor activity when engineered with an anti-CD19 chimeric antigen receptor (CAR). Our studies have established that combining in vitro iPSC differentiation with unbiased screening approaches leads to the discovery of novel mechanisms by which epigenetic regulators affect lymphoid development. Leveraging these new insights, we seek to exploit epigenetic modulators to facilitate the generation of mature, functional iPSC-T cells for adoptive immunotherapies.

Keywords: T cell differentiation, Epigenetic regulation, CAR T cell therapy

TOPIC: KIDNEY

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URINE-DERIVED STEM CELL-SECRETED KLOTHO PLAYS A CRUCIAL ROLE IN THE HK-2 FIBROSIS MODEL BY INHIBITING THE TGF-β SIGNALING PATHWAY

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Renal fibrosis is an irreversible and progressive process that causes severe dysfunction in chronic kidney disease (CKD). The progression of CKD stages is highly associated with a gradual reduction in serum Klotho levels. We focused on Klotho protein as a key therapeutic factor against CKD. Urine-derived stem cells (UDSCs) have been identified as a novel stem cell source for kidney regeneration and CKD treatment because of their kidney tissue-specific origin. However, the relationship between UDSCs and Klotho in the kidneys is not yet known. In this study, we discovered that UDSCs were stem cells that expressed Klotho protein more strongly than other mesenchymal stem cells (MSCs). UDSCs also suppressed fibrosis by inhibiting transforming growth factor (TGF)-β in HK-2 human renal proximal tubule cells in an in vitro model. Klotho siRNA silencing reduced the TGF-inhibiting ability of UDSCs. Here, we suggest an alternative cell source that can overcome the limitations of MSCs through the synergetic effect of the origin specificity of UDSCs and the anti-fibrotic effect of Klotho.

Funding Source: This research was supported by the Korean Fund for Regenerative Medicine (KFRM) grant funded by the Korea government (the Ministry of Science and ICT, the Ministry of Health & Welfare) (22C0701L1).

Keywords: Chronic kidney disease; Renal fibrosis, Urine-derived stem cells; Klotho, Mesenchymal stem cells

TOPIC: LIVER

1056

MONITOR AND CHARACTERIZE IPSC CULTURE AND DIFFERENTIATION USING ADVANCED FLOW CYTOMETRY AND LIVE-CELL IMAGING

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iPSCs are a valuable resource in many areas of research and clinical development, however, highly specific conditions are required to maintain their pluripotency and viability. Long term iPSC cultures can develop genotypic and phenotypic heterogeneity, so it is vital that methods for detecting these changes are developed. Increasing use of stem cells in both clinical and research settings necessitates fast, reliable, and inexpensive solutions for iPSC culture and characterization. During this study, iPSCs were grown in 2D and 3D under optimized or non-optimized conditions. Characterization of pluripotency surface markers was performed using the iQue ® Advanced Flow Cytometry Platform and morphological characteristics assessed using the Incucyte® Live-Cell Analysis System. Long-term hepatic differentiation was conducted in 2D using the same methods for characterization and monitoring with additional endpoint functional studies. Analysis of 2D-cultured non-optimized and optimized growth conditions revealed increased expression of differentiation marker SSEA-1 (+43.7%) and reduced expression of pluripotency markers SSEA-4 (-34.2%) and TRA-1-60 (-36.2%) in non-optimal iPSCs, with a similar trend observed in 3D-cultured iPSCs. Morphology analysis showed a marked difference between each growth condition. iPSCs in optimized conditions formed tightly packed colonies, while non-optimized iPSCs were more spread out and no longer formed colonies, resembling fibroblasts. Images were quantified for cell nuclear/cytoplasm ratio, which reduced in non-optimized conditions, from 0.6 to 0.4. During hepatic differentiation, pluripotency surface markers SSEA-4 (-96.6%) and TRA-1-60 (-96.4%) decreased while differentiation marker SSEA-1 (+33.3%) and hepatocyte marker CD99 (+47.9%) increased. CYP1A2 endpoint functional assay showed high levels of activity of enzyme in iPSC derived hepatocytes compared to no activity in undifferentiated iPSCs. These data illustrate the simplicity of using advanced flow cytometry and live-cell analysis in combination to monitor and characterize iPSCs during culture, scale up and differentiation studies.

Keywords: Differentiation, Cell Culture, Phenotyping

TOPIC: MESENCHYMAL STROMAL CELLS, ADIPOSE, AND CONNECTIVE TISSUE

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MESENCHYMAL STROMAL CELL EXTRACELLULAR VESICLES VARIATION FOR IMMUNOMODULATION AND TISSUE REPAIR

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The cell therapy field is focused on safety and functional relevancy of mesenchymal stromal cell extracellular vesicles (MSC eVs) due to limited clinical efficacy of hMSC based Phase II trials. Donor variation likely contributes to inefficient clinical trial success. Our work focuses on interrogating MSC heterogeneity by quantifying MSC derived EV clonal variability. It is critical to develop useful reproducible methods for effective evaluation/control of variation among MSC EV populations. We demonstrate an innovative approach to identify/rigorously characterize variation present in a single MSC donor population, the first step to establish criteria for performance-based selection/manufacture of functional MSC EV products. MSCs derived from femoral condyle marrow space of a single human donor were subcultured via limiting dilution in fetal bovine serum media for 8 days and imaged daily (phase contrast microscopy). 4 clonal colonies were picked from this donor and individually cultured to passage 5. 48-hour serum free media wash removed exogenous serum derived eVs. MSC eVs were isolated from media by ultracentrifugation and banked at -80°C (consistent with MISEV 2018). EV isolation confirmed by electron microscopy. 48 biochemical analytes were measured via Luminex multiplex on lysed/non-lysed eVs and residual non-EV secretome from each MSC clone population. Results showed differences in analyte levels for each clone (eVs/secretomes). Higher expression of Neuropilin-1 and IFN-α in lysed eVs, IL-1ra in whole eVs, and IL-6, IL-8, MMP-1, MMP-2, and MCP-1 in non-EV secretome fractions. Similar expression was seen between Neuropilin-1/IFN-α among eVs from different clones. IL-8/MMP-2/MIP-1α and M-CSF/MCP-1 were similarly expressed for non-EV secretomes from different clones. Initial results show clonal eVs/secretomes can be harnessed to signify unique biologically active cytokine distributions that may be important for consistency in immunomodulatory/tissue repair potency. Relevant differences between contents of eVs and non-EV secretomes derived from individual clonal populations may provide a new innovative way to optimize MSC therapeutics. Additional studies/analyses are key for identifying performance metrics for isolating/manufacturing MSC eVs with consistent clinical efficacy.

Funding Source: This work was made possible by the Clinical and Translational Science Collaborative of Cleveland, UL1TR0002548 from the National Center for Advancing

Translational Sciences (NCATS) component of the National Institutes of Health.

Keywords: Mesenchymal Stromal Cell, Extracellular Vesicle, Exosome

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AUTOMATION AND PERFORMANCE BASED SELECTION FOR IMPROVED REPRODUCIBILITY OF MSC MANUFACTURING

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Current generation of culture expanded human mesenchymal stem cell (MSC) populations is associated with tremendous donor-to-donor and batch-to-batch variation. Current methods for MSC fabrication are based on Competitive Expansion (CE), which selects for cells based solely on survival and proliferation and therefore compromises efficacy. Using the Cell X platform, we are proposing the alternative strategy of Performance Based Selection (PBS) to selectively expand monoclonal cell populations with attributes that correlate with the desired future performance. This study quantitatively measured and compared clonal colony attributes of founding progenitor cells and their clonal colony progeny within and between patients. Human bone marrow-derived cells were obtained from 6 patients undergoing hip arthroplasty and were cultured to allow colony formation. For each patient, 3 CE population were expanded to 25 doublings and up to 15 PBS clonal colonies were picked using the Cell X robot and expanded similarly. After harvest, large field of view imaging of PBS plate was performed daily to obtain time-lapse data of proliferating progenitor cells to identify monoclonal colonies. At Day 10, using the Cell X robot, selected clonal colonies were picked using atraumatic shear force aspiration. During expansion, CE and PBS plates were imaged every three days to estimate cell confluency. Colonyze, a customized automated large field of view Image Analysis software developed in our laboratory was used to define and quantitatively quantify the colony morphology attributes such as effective proliferation rate, cells per colony, colony area, cell density, cell aspect ratio. A total of 18 polyclonal CE populations were expanded and 98 PBS populations were picked, 26 of which were successfully expanded to Passage 5. Our study demonstrated extensive heterogeneity in the MSC clonal populations. We showed that CE populations and PBS monoclonal colonies have similar expansion kinetics. The combination of Cell X and Colonyze provides unprecedented ability to make quantitative measurement and selection in a complex heterogeneous cell environment and then to act on those to define highly documented reproducible and repeatable protocols to produce MSCs.

Funding Source: NIH SBIR grant #R44GM133291

Keywords: MSC, manufacturing, reproducibility

1062

INFLUENCE OF ADIPOSE TISSUE DEPOT HARVESTING SITE ON THE MULTILINEAGE INDUCTION CAPACITY OF MALE RAT ADIPOSE-DERIVED MESENCHYMAL STEM CELLS

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Recently, substantial attention has been raised towards the adipose-derived mesenchymal stem cells (AdMSCs) as an important and potential therapeutic option for employment in diverse tissue engineering and regenerative medicine applications. Moreover, the AdMSCs isolated from rats (r-AdMSCs) are frequently utilized. However, the influence of the adipose depot site on the multilineage differentiation potential of the r-AdMSCs is still ambiguous. Hence, the main objective of the present study is to explore the influence of the adipose tissue harvesting location on the ability of AdMSCs to express the stem cell-related markers, pluripotency genes, as well as their differentiation capacity in male Sprague Dawley (SD) rats for the first time. Herein, we have isolated r-AdMSCs from the inguinal, epididymal, peri-renal, and back subcutaneous fats. Cells from diverse depots have been compared in terms of their phenotype, immunophenotype, and expression of pluripotency genes (OCT 4, Nanog, Sox 2, Rex-1, and Tert) using RT-PCR. Besides, we have investigated their potential for multilineage (adipogenic, osteogenic, and chondrogenic) induction using special stains confirmed by expression of the related genes (AdipoQ, and CFD for adipogenesis, BMP2, OPN, and BSP for osteogenesis, and ACAN, CHM1, and Col2A1 for chondrogenesis) using RT-qPCR. All cells could positively express stem cell marker CD 90 and CD 105 with no significant in-between differences. However, they did not express the hematopoietic markers as CD 34 and CD 45. All cells could be induced successfully. However, epididymal and inguinal cells presented the highest capacity for adipogenic and osteogenic differentiation, followed by the peri-renal and subcutaneous cells. On contrary, the subcutaneous cells exhibited a superior potential for chondrogenic differentiation over the other sites. In conclusion, the adipose tissue depot harvesting site could influence the differentiation capacity of the isolated AdMSCs. Thus, it is necessary to consider the collection site selection to improve the outcomes of their use in distinct regenerative cell-based therapies.

Funding Source: The research was supported by a full scholarship of the Egypt-Japan Education Partnership (EJEP), Egypt and the support of the WISE proposal based project supported by MEXT, Japan.

Keywords: Adipose stem cells, Harvesting site, Differentiation

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PDGFRB-STAT5-IGF1 SIGNALING IN SKELETAL STEM CELLS DRIVES SKELETAL OVERGROWTH IN MICE

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Autosomal dominant PDGFRb gain-of-function mutations cause skeletal overgrowth in mice and humans with the rare genetic disease Kosaki overgrowth syndrome. However, the cellular basis and molecular mechanisms of these disorders are still being discovered. We previously showed that mice with a gain-of-function PDGFRb D849V point mutation on a Stat1-deficient background develop skeletal overgrowth. Skeletal stem cells (SSCs) isolated from these mice exhibit increased colony formation, suggesting overactive stem and progenitor cells as a cellular origin of overgrowth. Single-cell RNA transcriptomics with SSCs-derived colonies revealed increased expression and phosphorylation of signal transducer and activator of transcription 5 (STAT5) and overexpression of the STAT5 target gene, insulin-like growth factor 1 (IGF1). We hypothesized that PDGFRb causes skeletal overgrowth by activating local STAT5-IGF1 signaling in the skeletal lineage. To investigate the significance of STAT5 and IGF1 in the overgrowth phenotype, we conditionally deleted Stat5, Igf1, or IGF1 receptor Igf1r, from the axial skeleton of PDGFRbD849V;Stat1-/- mice. For conditional gene deletion, we utilized Prx1-Cre which targets mesenchymal cells in the early limb bud including bone, cartilage, muscle connective tissue and dermal fibroblasts, but not skeletal muscle. Longitudinal skeletal overgrowth of PDGFRbD849V;Stat1-/- mice was normalized by deletion of Stat5, Igf1, or Igf1r, suggesting the importance of STAT5 and IGF1 signaling. We propose that by directly phosphorylating STAT5 in Prx1-lineage skeletal cells, PDGFRbD849V hijacks the growth hormone (GH) signaling pathway to promote skeletal overgrowth. Our work establishes the cellular basis of skeletal growth disorders caused by PDGFRb mutations and gives new insight into a role of STAT5 in skeletal development and genetic disease.

Funding Source: This work was supported by grants from NIH/NHLBI (F32 HL142222 for H.R.K.), NIH/NIAMS (R01 AR073828 for L.E.O.), and the Oklahoma Center for Adult Stem Cell Research (OCASCR, a program of Tobacco Settlement Endowment Trust) (L.E.O).

Keywords: Platelet-derived growth factor receptor, Skeletal stem cells, Genetic disease

1066

THE ROLE OF ADAR1 IN MESENCHYMAL STROMAL CELLS CONTROLLING SENESCENCE AND IMMUNOMODULOTORY

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Mesenchymal stromal cell (MSC) is an ideal seed cell for stem cell therapy and its aging state seriously affects its treatment for immune diseases. Recent studies have shown that epigenetic change may be the main factor driving aging. RNA editing enzyme ADAR1 is one of the most abundant epigenetic transcriptome modifications. Its role in the senescence of MSCs and the regulation of their immune regulation ability has not been determined, and the specific mechanism needs to be clarified. In this study, we aim at clarifying the specific mechanism of ADAR1 regulating the mesenchymal stromal cell'' senescence and therapeutic effect. We use RNA-sequence (RNA-seq) technology and bioinformatics analysis, ADAR1 was screened to play an important role in cell senescence. Western blot (WB) and quantitative real-time PCR (qPCR) results show that ADAR1 is down-regulated in three cell senescence models (replication senescence, radiation-induced senescence, and chemotherapy-induced senescence). And MSC develops the senescence phenotype after knocking down ADAR1 by lentivirus interference (such as p16, p21 is increased). In addition, the MSC-shADAR1 by intramuscular injection had a higher cell clearance rate compared with the MSC-scramble group. And the ability to inhibit T cell activation, proliferation, and inflammatory secretion is weakened in MSC-shADAR1. The correlation analysis showed that the expression of ADAR1 was negatively correlated with the senescence of MSC and positively correlated with the immune regulation ability of MSC. The database of ADAR1 editing sites in MSC was established by using RNA-seq and DNA-seq techniques. We found CCL28, a chemokine related to immunocyte chemotaxis and cell senescence, was significantly up-regulated in MSCs knocked down ADAR1, and the expression of CCL28 was also significantly up-regulated in senescence cells. Lentivirus interference ADAR1 attenuates the therapeutic effect of MSC on Con A-induced liver injury in mice. To sum up, we prove that down-regulation of ADAR1 causes MSC senescence, reduces cell proliferation rate, and weakens immune regulation. These changes frustrate the excellent cell therapy ability of MSC. Our findings provide solid data for the subsequent use of small-molecule drugs to optimize the clinical treatment of MSC.

Funding Source: No

Keywords: Mesenchymal stromal cells, senescence, cell therapy

1070

MESENCHYMAL STEM CELLS DERIVED EXTRACELLULAR VESICLES PREVENT DNA DAMAGE CAUSED BY CHEMOTHERAPY AND OXIDIZING AGENT-INDUCED OXIDATIVE STRESS

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DNA damage induces various problems including mutation, tumorigenicity, and inducing apoptosis. Eventually DNA damage in cellular level leads to various irreversible/degenerative disorder. There are many reasons of DNA damage such as viral infection, radiation, carcinogen, chemotherapy reagent, and other oxidizing agent. Especially chemotherapy lead DNA damage through oxidative stress even in healthy tissue while treating cancer cells, lead several problems in cancer survivors such as infertility. Mesenchymal stem cells (MSCs) are a very promising source for regenerating damaged tissue through their differentiation potential, paracrine activity, and extracellular vesicle (EV) secretion. Espe-

cially, MSC-derived eVs are promising factors in tissue regeneration by restoring damaged cells through delivering proteins and miRNA. Although many study shows the therapeutic effect of MSC-derived eVs on damaged cells, the detailed mechanism is still not completely revealed yet. In our previous study, we found that MSC-derived eVs not only restore damaged cells but also protect cells against cytotoxic chemotherapy reagent cyclophosphamide. Based on the cytotoxic mechanism of cyclophosphamide, which induces DNA break, we hypothesized that MSC-derived eVs could prevent DNA damage. In this study, we treated MSC-derived eVs on human ovarian granulosa cells (hGrC1) prior to cyclophosphamide chemotherapy (CTx) or hydrogen peroxide (H2O2) induced cell damage. We evaluated DNA damage by analyzing double-strand break marker γH2AX protein. We found that MSC-derived eVs treated group shows significantly low population of damaged cells after CTx treatment (8.19±4.85%) while untreated group shows higher population of damaged cells after CTx (17.65±4.90%). Interestingly, H2O2 induced much severe DNA damage than CTx. Even this severe DNA damage induced by H2O2, MSC-derived eVs treated group showed lower population of damaged cells (22.43±1.74%) compared to higher damaged cell population in untreated group (49.61±6.94%). Our data demonstrate that MSC-derived eVs could prevent DNA damage induced by chemotherapy reagent and oxidizing agent. We conclude that MSC-derived eVs are a promising simple treatment option for protecting healthy tissue in various conditions which concerning DNA damage.

Funding Source: This study supported by start-up fund of the University of Chicago (AA).

Keywords: Mesenchymal stem cell, extracellular vesicles, DNA damage

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MECHANICAL CONDITIONING REJUVENATES MESENCHYMAL STEM CELLS FROM AGED PATIENTS

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Mesenchymal stem cells (MSCs) are an appealing therapeutic cell type for many diseases. However, patients with poor health or advanced age often have MSCs with poor regenerative properties. A major limiter of MSC therapies is cellular senescence, that is induced by poor donor health and/or by expansion in cell culture. In this study, we examined the hypothesis that mechanical loading can rejuvenate MSCs from aged patients and enhance their ability to expand in culture and therapeutic potential. We obtained MSCs from four aged donors (68-92 years old, two male/two female) and conditioned them with mechanical loading and/or an EGFR/ErbB-2/4 inhibitor. The mechanical loading applied was at 0.1 Hz with maximum strain of 7.5% with a physiological waveform for 4 hours per day for 7 days. Mechanical loading alone increased proliferation of the MSCs by 50-100% in comparison to non-treated MSCs. We found that mechanical loading increased phosphorylation of Akt by five-fold and Cyclin-D1 by six-fold in comparison to control MSCs. Following loading, we observed a long term, durable effect in enhancing proliferation, with control cells reaching cell cycle arrest after ~5 cumulative doublings whereas mechanically conditioned cells continued to grow for at least ~9 cumulative doublings. Mechanical load also increased the expression of SIRT1, SIRT6, and SIRT7, and phosphorylation of SIRT1, FoxO1, and FoxO4 in comparison to control cells. Loading improved the multipotency of aged MSCs in differentiating into adipogenic and osteogenic lineages, and reduced senescent cell phenotypes, indicated by reduced SA-β-galactosidase expression. Mechanical conditioning was also found to reduce reactive oxygen species buildup and enhance the recognition and repair of DNA damage in aged MSCs after only one day of treatment. Mechanistic studies using inhibitors demonstrated that the functional enhancements were mediated by oxidative stress and DNA damage repair signaling with crosstalk between Sirtuin proteins, DNA damage repair protein ATM, cell cycle regulatory protein Akt, and several antioxidant proteins. Taken together, these results demonstrate that physiological mechanical conditioning can rejuvenate the functionality of MSCs derived from aged patients and improve their prospects in cell-based regenerative therapies.

Funding Source: NIH R01 (Baker – PI). Project Title: Mechanical Conditioning of Mesenchymal Stem Cells for Enhanced Recellularized Vascular Grafts. Total Funding: $1,016,233; Project Period: 9/01/17-9/01/23

Keywords: Regenerative medicine, Senescence, Mechanobiology

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DEVELOPMENT OF AIRWAY EPITHELIAL CELLS MIMICKING STEPWISE DIFFERENTIATION USING HUMAN TONSIL-DERIVED MESENCHYMAL STEM CELLS

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Airway defects commonly caused by tumor removal, trauma, infection, etc. are difficult to treat because the airway has a complex multi-layered structure. The key to fully recovering these defects is the reconstruction of respiratory epithelium, the lining next to the cartilage. Autologous epithelial cells or progenitor cells are used to reconstruct the epithelium, but they are not suitable candidates for regeneration due to their difficult properties for expansion and differentiation in vitro. Here, we developed an alternative for respiratory epithelial regeneration using human tonsil-derived mesenchymal stem cells (TMSCs) to induce epithelial cells through stepwise differentiation. TMSCs were isolated from human tonsillar tissues of patients undergoing tonsillectomy and differentiated into airway epithelial cells following the human embryonic developmental process. To generate airway epithelial cells, TMSCs were exposed to various chemical agents or protein

combinations during four steps. We found that TMSCs can be induced into the definitive endoderm (DE) with a low concentration of activin A, which activator of the Nodal/ TGF-β signaling pathway. Next, a combination of growth factors regulating BMP, TGF-β, and WNT signaling induces the differentiation of DE-induced TMSCs into anterior foregut endoderm identified by upregulating gene expression of PAX7, SOX2, and GATA3. An environment rich in BMPs, WNT, and FGFs differentiated TMSCs into lung progenitor cells, as evidenced by increased gene expression of NKX2-1, an early lung progenitor marker. In the final step, the expression of Keratin 5, a basal cell marker, and FOXJ1, a ciliated cell marker, were increased in TMSCs-derived airway epithelial cells generated through air-liquid interface culture. Our results demonstrate that TMSCs-derived airway epithelial cells can be generated by stepwise differentiation and represent a potential alternative for treating functional recovery of respiratory defects.

Keywords: Tonsil-derived mesenchymal stem cells, Respiratory defect, Epithelial cells

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EVALUATION OF NEURAL GENE EXPRESSION IN DEDIFFERENTIATED FAT PROGENITOR (DFAT) SPHEROIDS AFTER CO-CULTURE WITH OXYGEN-GLUCOSE DEPRIVATED RAT HIPPOCAMPAL SLICES.

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Dedifferentiated fat (DFAT) progeny cells are a recently discovered and not fully understood subpopulation of cells isolated from adult adipose tissue. It has been shown that DFAT cells may be characterized by better adjuvant and plasticity abilities than standard adipose-derived mesenchymal stem/stromal cells (ASC). In our work, we decided to culture DFAT cells in the form of three-dimensional spheroids, and evaluate their neural gene expression potential, which has not been previously reported. First of all, we characterized spheroids obtained from DFAT cells and compared them to ASC spheroids. Next, with the use of an ex vivo model, we performed DFAT vs ASC co-culture with intact or injured by the oxygen-glucose deprivation rat hippocampal slice culture and assessed the gene expression of astrocyte, oligodendrocyte, and neuronal markers in both populations of cells. Our results indicated that both ASC and DFAT easily form spheroids on anti-adhesive surfaces and actively proliferate up to 72 hours of spatial culture. We also discovered that the 3D form of cell culture positively impacts all neural gene expression in comparison to standard 2D cultured cells. We showed that ASC-3D cells co-cultured with oxygen-deprived hippocampal slices (OGD) upregulate the expression of neural markers such as NESTIN and neuron markers such as MAP2 and β-III-TUBULIN. In ASC-3D after contact with OGD slices, we also observed the increased expression of astrocyte (GFAP and S100β) and oligodendrocyte (NG2) markers in comparison to control ASC-2D cells. What's more, we observed that the expression of neuronal markers in DFAT-3D cells co-cultured with OGD slices was significantly higher than in ASC-3D although there was no difference in gene expression between control DFAT-3D cells cultured without hippocampal slices. Comparing the neural differentiation abilities of cells cultured as spheroids or monolayer, we showed that: both ASC-3D and DFAT-3D cultured in the presence of injured neural tissue can direct the gene expression toward neuronal, astrocytic, and oligodendrocytic phenotype. Moreover, DFAT-3D possesses better neural differentiation potential compared to ASC-3D. These discrepancies may explain their different origins and may indicate their potentially different therapeutic capabilities.

Funding Source: Medical Research Agency grant no. 2020/ABM/01/00014 and National Science Centre, grant no. 2018/31/N/NZ4/03275.

Keywords: dedifferentiated fat progenitors, adipose derived mesenchymal stem/stromal cells, neural-lineage differentiation

TOPIC: MUSCULOSKELETAL

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MICRORNA-125B AS BIPOLAR PLAYER IN OSTEOGENIC PATHWAY

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Bone metabolism is highly regulated and recent studies showed that microRNAs (miR) can contribute to the deregulation of pathways needed to maintain healthy bone structures. Seeliger et al. already identify five circulating miR associated with osteoporotic fractures among which miR-125b. Overexpression of miR-125b is well-known to enhance osteoporosis and regulate osteogenic differentiation of human mesenchymal stem cells (hMSCs) via several pathways. Here we evaluated the involvement of the osteoporotic miR-125b in BMP pathway. In this work we aim to evaluate the potential of miR modulation for subsequent use in clinical approaches for bone diseases and defects. hMSCs were transfected with mimic of miR-125b or negative control combined or not with osteogenic differentiation. Two different protocols of differentiation were tested, one using dexamethasone (DEX) and another using calcium-enriched (Ca) medium. Exposure to DEX or Ca medium increased mineralization of hMSCs compared to basic medium and is accompanied by a decrease of miR-125b expression. Interestingly, this seems to be correlated with increased RUNX2, BMPR2 and SMAD4 levels. hMSCs transfected with miR-125b mimic and stimulated with DEX presented a higher mineralization compared to cells transfected with scrambled miR and this observation seems to be correlated with higher levels of RUNX2, SMAD4 and BMPR2. On the contrary, cells treated with Ca medium and transfected with miR-125b mimic showed a decrease in mineralization compared to scrambled miR. This result is supported by a decrease of RUNX2 and BMPR2 levels. Accordingly, inhibition of miR-125b in Ca-treated cells increased mineralization compared to scrambled miR and seems to upregulate BMPR2 and RUNX2 levels. Interestingly other osteoporotic related miRs as miR-21 and miR-100-5p are modulated when miR-125b expression is modified. We showed that miR-125b differentially modulates mineralization of hMSCs during DEX and Ca-induced osteogenic differentiation. This dissimilarity can be explained by the difference of the targeted pathways. Therefore, miR-125b affinity

for targets implicated in bone remodeling changes and induces an opposite final physiological effect. With this work we slightly open the door to possible therapeutic approaches for bone diseases such as osteoporosis.

Funding Source: This work is supported by the Province of Limburg, Limburg Invests in its Knowledge Economy (LINK)

Keywords: microRNA, Mesenchymal stem cells, Bone

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LINEAGE-SPECIFIC DIFFERENCES AND INFERENCE OF REGULATORY NETWORKS GOVERNING HUMAN CHONDROCYTE DIFFERENTIATION

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To address large gaps in our understanding of the molecular regulation of articular and growth plate cartilage development in humans, we used our directed differentiation approach to generate distinct cartilage tissues from human embryonic stem cells (hESCs). The resulting transcriptomic profiles of hESC-derived articular and growth plate chondrocytes were similar to fetal epiphyseal and growth plate chondrocytes, with respect to genes both known and previously unknown to cartilage biology. With the goal to characterize the regulatory landscapes accompanying these respective transcriptomes, we mapped chromatin accessibility in hESC-derived chondrocyte lineages, and mouse embryonic chondrocytes, using ATAC-sequencing. Integration of the expression dataset with the differentially accessible genomic regions revealed lineage-specific gene regulatory networks. A closer analysis of how gene expression variance was controlled by epigenetic accessibility revealed more biologically relevant information, including several TFs and TF families previously unknown in cartilage biology, whose role in chondrogenesis can now be further investigated. Transcriptomic profiles of hESC-derived cartilage across developmental time also revealed TFs that may play a role in specification of chondrocytes to these distinct lineages. We functionally validated binding of two transcription factors (RUNX2 in growth plate chondrocytes and RELA in articular chondrocytes) with their predicted genomic targets. The maps we provide thus represent a framework for probing regulatory interactions governing chondrocyte differentiation. This work constitutes a substantial step towards comprehensive and comparative molecular characterizations of distinct chondrogenic lineages, and sheds new light on human cartilage development and biology.

Keywords: cartilage, regulatory, development

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EFFICACY OF FISETIN AND RESVERATROL AS SENOTHERAPEUTICS TO AMELIORATE CELLULAR SENESCENCE OF CHONDROGENIC PROGENITOR CELLS DERIVED FROM KNEE OSTEOARTHRITIS PATIENTS

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Osteoarthritis (OA) is an age-related cartilage degenerative disease. Chondrogenic progenitor cells (CPCs), the stem cell-like cells with capacity of self-renewal found in the articular cartilage have been considered as the main cartilage repairer in OA. Our previous study showed that CPCs of osteoarthritic articular cartilage acquire cellular senescence. Senescent cells secrete a mixture of molecules, known as the senescence-associated secretory phenotype (SASP) and through the paracrine effects of SASP affect surrounding cell and might interfere their regenerative function. Flavonoids, are emerging as potential therapeutic agents to mitigate senescence. We hypothesized that senotherapeutics fisetin and resveratrol would mitigate senescence of CPCs. The CPCs isolated from OA knee cartilage were characterized based on phenotypic expression of stem cell markers (CD90+, CD105,+ CD73,+ CD29,+ CD49e,+ CD45low/-), clonogenicity, and tri-lineage differentiation assays. Pre-treatment with increasing doses of fisetin and resveratrol (5μM-100μM) for 24 hours was given. Fisetin significantly decreased senescence index (SI) by 42.93% at 100μM (p=0.01) while resveratrol decreased it by 30.21% at 50μM (p< 0.05).This was further supported by significant decline in expression of cellular senescence markers p53 and p38MAPK at same dose (50μM) of both drugs. Additionally after treatment with fisetin and resveratrol, secretion of SASP comprising of pro-inflammatory cytokine IL-1β and matrix-degrading enzymes MMP-9 and MMP13 from the senescent CPC were also significantly downregulated. Only resveratrol significantly downregulated the expression of hypertrophy marker, COL-10 at 50μM. Additionally after 21-day chondrogenic differentiation, pre-treatment with resveratrol resulted in significantly increased expression of cartilage synthesis marker collagen type 2. In summary, fisetin and resveratrol ameliorated senescence of CPCs by downregulating the p53 effector protein and SASP. Therefore, these natural drugs can act as potent Serotherapeutic and can enhance the regeneration power of CPCs in knee OA.

Keywords: Chondroprogenitor cells, senotherapeutics, Fisetin, resveratrol

1084

MAKING AND BREAKING THE SKELETAL MUSCLE STEM CELL NICHE

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Skeletal muscle stem and progenitor cells including those derived from human pluripotent stem cells (hPSCs) offer an avenue towards personalized therapies. Here we demonstrate both stem and progenitor cells readily fuse to form human-mouse myofibers, but skeletal muscle progenitor cells (SMPCs) from hPSCs inefficiently take up position in human-mouse stem cell niches. In contrast, SMPCs expressing the transcription factor PAX7 were 50-fold more likely to associate with a subset of immature human myofibers that resembled fetal niches, 1-2 months after transplantation. We hypothesized lack of SMPC engraftment into the muscle stem cell niche was due to cell competition with endogenous mouse SCs occupying SC niches. Thus, we generated an inducible diphtheria toxin SC ablation mouse model compatible with human engraftment to test this hypothesis. Interestingly, ablation of mouse SCs only increased numbers of immature human myofibers and both PAX7+ SMPCs and SCs now predominantly formed niches with human-only myofibers, instead of residing in chimeric mouse niches, suggesting that cell competition is not the prevailing driver of niche formation. Thus, we profiled SC ablated mice with single nucleus RNA-Seq and identified the absence of a transient myofiber subtype, expressing the fetal actin isoform Actc1, that could support Pax7 cells during regeneration. Similarly, the transplanted immature human myofibers strongly expressed ACTC1, and we used spatial RNA-seq to identify key factors driving de novo human niche formation, including sarcomere assembly and biosynthesis of fatty acids, improving our ability to support human PAX7 cell repopulation. To demonstrate ACTC1+ myofibers are essential to supporting PAX7 SMPCs, we used CRIPSR/Cas9 to insert a FKBP12-Caspase9 fuse gene in the 3' end of ACTC1 in hPSCs that were then differentiated into SMPCs and transplanted i' vivo. Upon Caspase-induced apoptosis, we found a 90% reduction in ACTC1+ myofibers and over a 100-fold decrease in PAX7 cell numbers compared to non-induced engrafted controls. As opposed to other niche systems, where niches are formed by stem cell homing to empty niches, we found that transient regenerating human skeletal muscle is essential to emerging niche formation in vivo to support PAX7 cells.

Funding Source: CIRM Quest, NIH NIAMS, MDA, ICTS KL2

Keywords: Emerging niches, Muscle stem cells, Spatial RNA sequencing

1086

RIBONUCLEOTIDE REDUCTASE M2B (RRM2B) IN INTERPLAY BETWEEN STEM CELLS AND NICHE VIA MODULATING STEM CELL FATE IN SKELETAL MUSCLE

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Decline of regeneration capacity is one of the critical causes for dysfunctional skeletal muscle in elderly. The balance among quiescence, differentiation, and self-renewal of skeletal muscle stem cells (MuSCs) is tightly regulated by their intrinsic and extrinsic properties from the niche. Ribonucleotide reductase M2B (Rrm2b), constitutively expressed in proliferating and non-proliferating cells, modulates MuSC quiescence/differentiation in muscle in response to injury. RRM2B is demonstrated to directly correlate to muscle dysfunction in patients with mitochondrial depletion syndrome. Rrm2b knockout in myofibers led to weakness of muscles, such as a loss of muscle mass and strength. After muscle injury, damaged myofibers were more efficiently repaired in the Rrm2b myofiber-specific knockout mice than the control mice, but these myofibers were thinner and showed weak functioning. Rrm2b-deleted myofibers released several myokines, which trigger MuSCs to differentiate but not re-enter the quiescent stage to replenish the stem cell pool. Overall, Rrm2b in the myofibers plays a critical role in modulating the MuSC fate by modifying the microenvironment, and it may lead to a possible strategy to treat muscle disorders. This study can lead to establish a therapeutic method targeting skeletal muscle disorders in humans and has a beneficial impact on the quality of life of the elderly.

Keywords: Muscle stem cell, niche, mitochondria

1088

OPTIMIZATION OF FIBRONECTIN CONCENTRATION FOR MAINTENANCE OF PORCINE SATELLITE CELLS.

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'Cultured meat' or 'artificial meat' has emerged as an alternative food that could solve overpopulation, livestock gas and animal ethics. Satellite cells which are the major material of cultured meat cannot be maintained in vitro. Fibronectin is one of extracellular matrix (ECM) which were widely used in cell culture. Fibronectin affects biological process such as cell adhesion, differentiation and migration. However, research on cell maintenance and proliferation according to long-term culture of porcine satellite cells cultured on various concentration of fibronectin are unclear. In this study we first investigated cell proliferation, cell cycle, expression level of PAX7 and MYOD1 gene when porcine satellite cells were cultured on 20μg/ml fibronectin-, gelatin- and non-coated

dishes at long-term culture. We found that 20μg/ml fibronectin had lowest doubling time at short-term and long term culture which indicates that fibronectin had most effective proliferation compared to gelatin coated dishes or non-coated dishes. Furthermore, expressed Pax7 and MyoD1 by immunocytochemistry (ICC) were higher in porcine satellite cells cultured on fibronectin coated dishes than in gelatin or non-coated dishes. As many studies showed that mixture of ECM had positively affected various types of cells by mimicking in vivo niche. We next analyzed the proliferation of gelatin and fibronectin mixture, however, there were no significant effect on porcine satellite cells. Next we determined the optimal concentration of fibronectin for proliferation and maintenance of porcine satellite cells at long term-culture. The porcine satellite cells cultured on 5μg/ml and 20μg/ml had lowest doubling time compared to 1μg/ml at long-term culture. The porcine satellite cells cultured on 1μg/ml and 5μg/ml of fibronectin coated dish had maintained well by expressing high level of Pax7. However, 20μg/ml of fibronectin had lowest the expression level of Pax7 and MyoD1 gene at long-term culture. Comprehensively, our result indicate that porcine satellite cells cultured on fibronectin with concentration of 5μg/ml could maintain and proliferate well at long-term culture. Our results may provide another way to maintain porcine satellite cells for long-term culture.

Funding Source: This work was supported by IPET through 'High Value-added Food Technology Development Program', and was funded by the Ministry of Agriculture, Food and Rural Affairs(MAFRA) (322006-05-1-CG000)

Keywords: Porcine Satellite cells, Fibronectin, Maintenance

Clinical Trial ID number: Proliferation

1090

IDENTIFYING PROGENITOR-LIKE CELLS IN THE NUCLEUS PULPOSUS CONTRIBUTING TO THE DEVELOPMENT AND MAINTENANCE OF A HEALTHY INTERVERTEBRAL DISC

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In Intervertebral Disc Disease (IDD) the functional capacity of the disc decline leading to impaired reparative capacity of the intervertebral disc (IVD) tissues in the spine. Loss of progenitors of the nucleus pulposus (NP) in the IVD could be the underlying cause. Knowledge of the heterogeneity of cells in the NP, identity and molecular characteristics of NP progenitors, and their lineage origins is limited. Developmental lineage studies in mice show that NP cells are derived from the embryonic notochord. Two distinct cell types populate the NP, large vacuolated notochordal-like cells (NCLs) and smaller chondrocyte-like cells (CLCs). In humans many NCLs are present in the NP after birth but these reduce rapidly with age and few remain during adolescence. In adult NP, CLCs are the predominant cell type. The reduction of NCLs and appearance of myofibroblast-like cells in human NP coincides with the age-onset of IDD. In contrast, NCLs persist in animals that are more resistant to IDD, such as the mouse. NCLs could therefore represent a pool of NPC progenitors that serve to maintain NP function and homeostasis and protect against IDD. In adult tissues, infrequently dividing cells (slow cycling, sCy) are long-lived "stem" cells that generate rapidly dividing (fast cycling), short-lived progenitor cells. Both fast cycling and slow cycling stem cell populations can also produce unique differentiated lineages. We determined the gene expression signatures of single fetal, postnatal day 10 and 8 week old NP cells. We found considerable cell heterogeneity in the adult mouse NP, with both fast cycling and slow cycling cells being present. We tested for sCy cells (label retaining cells, LRCs) in mice by H2B-GFP retention analysis in which dilution of H2B-GFP with cell division is used as an indicator of the speed of cell division and the GFP signal is retained preferentially in LRCs. Comparing mouse and human datasets, cycling NP cells expressing mitotic genes were present in both, suggesting cell proliferation occurs in adolescent NPs. By lineage tracing in mice we identified a population of cycling Tagln+ cells located in the periphery of the NP (PeriNP cells) which are potential progenitors that contribute to the whole NP. Tagln+ cells and their descendants were diminished in aged NP or puncture-induced disc degeneration.

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Keywords: Slow cycling progenitors, Intervertebral disc progenitors, Lineage tracing

TOPIC: NEURAL

1092

USING HUMAN IPSCS TO EXPLORE THE MECHANISMS BY WHICH GLUCOCEREBROSIDASE (GBA) MUTATIONS CONTRIBUTE TO PARKINSON'S DISEASE PATHOGENESIS

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The early phases of neurodegenerative diseases such as Alzheimer's disease may be influenced by changes in endocytic trafficking and lysosomal function. In this study, I will be using human iPSC-neurons to investigate the molecular and cellular pathogenic mechanisms of synucleinopathies, such as dementia, with Lewy bodies (DLBs). I hypothesize that endosomal and lysosomal structure and functions are impacted in the human DLB-iPSC model and alpha-synuclein plays an important role in inducing endosomal and lysosomal dysfunction in this model. To study the cellular and molecular pathways involved in DLB pathogenesis, I will use iPSC-derived neurons from 3-4 human DLB patients. This will also include, 3-4 iPSC-derived neurons from healthy age-matched controls. For this experiment(s) to be carried out, data will be collected using live cell imaging techniques to examine mitochondria, endosomes, lysosomes, and autophagosomes. I will quantitate and analyze the results to establish: 1) if endocytic trafficking is defective 2) if autophagic/lysosomal activities are reduced 3) if mitochondria dynamics and functions are altered

in DLB cells 4) if alpha-synuclein is abnormally accumulated in human DLB neurons with the collected data, I will compare various sizes, structures, motility, and functional asset between cell cultures obtained throughout the experiment. By the end of the proposed experiments, the evidence gathered, will give me the ability to support or refute my hypothesis concerning changes in the endosomal-lysosomal pathways and their potential contribution to DLB pathogenesis. The significance of my research is that my findings will shed critical light on the relationship between endo-lysosomal dysfunctions and DLB pathogenesis.

Keywords: Alzheimer's disease, iPSC, Dementia with lewis bodies (DLB)

1094

THE EFFECT OF INTRANASAL ADMINISTRATION OF HUMAN NEURAL CREST-DERIVED NASAL TURBINATE STEM CELLS BY AMELIORATING INFLAMMATORY RESPONSE IN REPEATED HEAD TRAUMA MODELS

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The aims of the study were: 1) whether intranasal administration of Human neural crest-derived nasal turbinate stem cells (hNTSCs) works in a repeated head trauma model; 2) whether the effect of intranasal administration of hNTSCs is similar to that of PLX3397. Male C57BL/6 mice (6-8 weeks old) were used for in vivo experiments. A 30 g steel weight with a flat-end was dropped from a height of 8 cm onto the center of the head. Experimental mice were divided into three groups; TBI mice without any treatment, TBI mice treated with PLX3391, and TBI mice administered with hNTSCs intranasally. PLX3397 (290mg/kg) was administered via an intraperitoneal route for 21 days following the start of weight-drop experiments. Intranasal administration of hNTSCs was done once following TBI. Repeat TBI produced no obvious brain damage after the last injury. Brain edema did not differ between TBI models and treatment groups. An increase of Iba1+ and GFAP+ cells was observed in cortex of TBI models. Iba1+ cells were not observed in PLX3397-treated TBI models as well as TBI models administered with hNTSCs. GFAP+ cells were remarkably deceased in PLX3397-treated TBI models as well as intranasally administered TBI models, compared with TBI models. Cellular disorganization in hippocampus was noted following repeat TBI. Iba1+ cells were increased in hippocampus of TBI models, but not observed in PLX3397-treated TBI models as well as intranasally administered TBI models. GFAP+ cells were remarkably deceased in PLX3397-treated TBI models as well as intranasally administered TBI models. PLX3397 aministration for 21 days ameliorated a reduction of neurobehavioral ability in repeated TBI models. The similar effect was observed in TBI models administered with hNTSCs. Through our study, we found that intranasal administration of hNTSCs has the microglia depletion effect and administered hNTSCs moved to remote regions such as hippocampus. We assume that the observed protective effects of hNTSCs on repeat TBI models dampen neuroinflammation and could be mediated through paracrine signaling on astrocytes and microglias.

Keywords: stem cell, microglia, head trauma

1096

CD44 INTERACTIONS WITH C1Q AND HYALURONIC ACID IN NEURAL STEM CELLS

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Spinal Cord Injury (SCI) is a devastating condition with no effective therapeutics. Neural stem cells (NSC) have been investigated for transplantation, showing promise in preclinical studies and early clinical trials. NSC may contribute to recovery by differentiating into new neurons and oligodendrocytes, as well as reducing inflammation or providing neuroprotective signaling to spared tissue. Recently, we have shown a novel role for the immune molecule, C1q, as an extrinsic factor affecting NSC transplantation efficacy through interactions with the receptor CD44. Additionally, hyaluronic acid (HA) has been shown to exhibit a molecular weight (MW) dependent (MW) bioactivity, mediated through CD44, in NSC. We investigated the interactions of high and low MW HA, C1q, and CD44 and their effect on NSC bioactivity. Further, we compared fetal and embryonic stem cell derived NSCs since these two NSC types have been shown to behave differently after transplantation. Bioactivity was characterized by viability and apoptosis, proliferation, and concentrations of secreted inflammatory and trophic factors using ApoLive-Glo™, EdU, and Luminex assays, respectively. Preliminary results show that both 40kDa HA and C1q significantly increase the ratio of viable to apoptotic cells compared to untreated NSC and NSC treated with 1000kDa HA. Given previously published CD44-C1q and CD44-HA interactions in NSC, we hypothesize that high, but not low MW, HA will decrease C1q signaling in NSC by blocking C1q-CD44 interactions. Since HA interacts with CD44 in a MW-dependent manner, investigating the effects of HA on C1q-CD44 interactions is likely to provide further insights that can be leveraged to improve NSC transplant efficacy.

Keywords: Neural Stem Cells, CD44-Hyaluronic Acid Interactions, CD44-C1q Interactions

1098

HUMAN NEURAL STEM CELL-DERIVED EV REVERSES COGNITIVE DECLINE, NEUROINFLAMMATION AND SYNAPTIC LOSS FOLLOWING RADIATION- AND CHEMO-THERAPY FOR BRAIN CANCER

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Clinical cranial radiotherapy (CRT) and adjuvant chemotherapy (temozolomide, TMZ) for the treatment of primary and metastatic CNS cancers often lead to unintended and debilitating cognitive impairments and neuroinflammation. This is particularly concerning for the childhood survivors of brain cancers who show reductions in I. Q. up to 3 points per year. Despite recent progress in understanding the inflammatory mechanism of such a progressive degenerative condition, no clinical recourse is available yet. We investigated regenerative interventions designed to prevent or reduce the adverse neurocognitive sequelae following CRT. Past studies showed long-term consequences of acute CRT, including cognitive decline, loss of neuronal architecture, spine integrity, and neuroinflammation. We posit that human neural stem cell (hNSC)-derived extracellular vesicle (EV) can ameliorate CRT-induced adverse neurocognitive and inflammatory sequelae. We showed that hNSCs or hNSC-derived EV reverse experimental acute CRT-induced cognitive impairments, neuron and spine damage, and, neuroinflammation. Retro-orbital vein injections of hNSC-eVs imparted long-term neuroprotection. We identified candidate miRNA within the EV cargo, miRNA-124-3p, that reversed acute CRT-induced cognitive deficits and neuroinflammation. To increase the translational relevance of our approach, we tested the effectiveness of GMP-grade hNSC lines that have shown neuroprotective efficacy in neurodegenerative conditions and are now poised to undergo clinical trials for TBI and spinal cord injury. GMP-grade hNSC-derived eVs were tested against fractionated CRT (8.67 Gy, 3 doses) in combination with adjuvant TMZ treatment for brain cancers. IV injections of EV improved learning and memory, anxiety, and memory consolidation process in the CRT-TMZ-exposed animals. eVs reversed CRT-TMZ-induced microglial activation, astrogliosis, and loss of synaptic integrity. Importantly, hNSC-EV were equally effective in reversing a cognitive loss in a brain cancer model (astrocytoma) receiving CRT-TMZ, asserting its neuroprotective impact in a clinically-relevant model. Thus, stem cell-derived eVs show promising outcomes that can be translated into clinics to thwart this unmet medical need impacting thousands of cancer survivors.

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Keywords: radiation therapy, chemotherapy,stem cell-derived extracellular vesicles, cognition, neuroinflammation, brain cancer

1100

SINGLE-CELL TRANSCRIPTOMIC ATLAS OF THE MAMMALIAN NEUROGENIC NICHE REVEALS EXERCISE AS A COUNTERMEASURE FOR BRAIN AGING

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The hippocampus dentate gyrus serves as a microenvironment niche to support the self-renewal and differentiation of neural stem cells throughout life. Yet, the generation of neurons, glia, and overall hippocampus function deteriorates with age through unclear mechanisms. We have previously found neural stem cells undergo early cellular aging in the mature brain and that exercise slows this process. Here, we investigate molecular aging and exercise interventions on neurogenic niche cell types at the systems level. 7431 cells from the dentate gyrus of young and mature mice plus upon long-term running and short-term running were analyzed by mid-depth single-cell RNA sequencing. Systematic bioinformatics approaches identify mechanisms by which multiple niche cells exhibit early molecular aging and how exercise rescues these aging effects. Remarkably, other niche cells remain resistant to early aging, continue to undergo development in the adult brain, and benefit from exercise through aging-independent mechanisms. We additionally interrogated the cross-talk interactions between neural stem cells and their niche components in young, aging and exercise contexts to derive a comprehensive cell-cell communication network of the neurogenic niche. Our findings provide a valuable systems-level resource to explore early aging in the neurogenic niche, deconvolve complex inter-

cellular communications, and reveal potential therapeutic targets of how exercise slows the aging process.

Keywords: Neurogenic niche, exercise, Single-cell transcriptomics

1102

ASSESSING THE FUNCTION OF SMAD1 AND SMAD5 IN THE SPECIFICATION OF DORSAL NEURON IDENTITY USING MOUSE EMBRYONIC STEM CELLS AS A MODEL SYSTEM

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Spinal dorsal interneurons (dIs) integrate and relay somatosensory information from the periphery to the brain. Individuals with impaired sensory circuitry are unable to interpret external stimuli from the environment, and as a result do not feel pain, touch, or properly modulate motor output. dIs are generated endogenously when discrete domains of neural progenitor cells (NPCs) arise along the dorsal-ventral axis of the spinal cord, as a consequence of signaling from the roof plate, and paraxial mesoderm. The roof plate secretes growth factors, such as Wnts and bone morphogenetic proteins (BMPs), that direct progenitor cells to proliferate, differentiate, and migrate laterally to form different populations of dIs. BMP signaling is translated by R-Smads, Smad1 and Smad5, which act as transcriptional regulators. Upon BMP binding, the R-Smads are phosphorylated and translocate into the nucleus where they regulate transcription. Two R-Smads, Smad1 and Smad5, are present during dorsal spinal cord development, however their precise roles directing dI specification have remained unresolved. Using our mouse embryonic stem cells (mESC) directed differentiation protocol for dIs as a model system, we have assessed the hypothesis that Smad1 and Smad5 differentially transduce BMP signaling. Using CRISPR/Cas9 approaches, we have compared the differentiation potential of Smad1 and Smad5 knockout mESC lines to control lines. Our findings suggest that R-Smads have distinct, rather than redundant activities specifying dI fates. Future work will assess the specific genomic targets of Smad1 and Smad5 during dI specification. These studies will shed light on the mechanisms needed to generate pure populations of dIs for future cellular replacement therapies and drug screen platforms.

Keywords: Mouse Embryonic Stem Cells, BMP signaling, Smad1 and Smad5

1104

THE ROLE OF TOX IN REGULATING THE PROLIFERATION OF MULLER GLIAL CELLS IN THE RETINAL DEGENERATION MOUSE

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Age-related macular degeneration (AMD), retinitis pigmentosa (RP), diabetic retinopathy (DR), and glaucoma, which are major retinal diseases that cause blindness, are still problems to be solved. Zebrafish undergo reprogramming events for retinal regeneration upon retinal injury, but in mammals, their proliferative and regenerative capacity is severely limited. Yap expressed in Müller glial cells gradually inhibits its activity in the damaged retina, resulting in limitations in proliferation and regeneration.The sustained activity of Yap showed the potential for reprogramming in the mammalian retina. Tox, which plays an important role in proliferation, cell development, and formation, has been mainly studied in the fields of the immune system, tumors, and neurons, and it has recently been found that Tox inhibits proliferation in some tumor cells. The role of Tox in the retina, which has yet to be explored, has to be confirmed. Tox is expressed in Müller glial cells and some retinal cells in the mouse retina. It was found that the expression of Tox in Müller glial cells increased as the retinal damage progressed. On the other hand, Cyclin D1 started to decrease in the damaged retina on the 4 days, where the expression of Tox was significantly increased, and the Yap decreased on the 6 days. Cell proliferation was inhibited when Tox was overexpressed in mouse Müller glial cells, and proliferation and cell cycle genes were reduced when overexpressed with Tox compared to cells overexpressing just Yap. Knockdown of Tox in Glast-CreERT2; Rosa26 rtTA/EGFP mice upregulated Yap and Cyclin D1. Tox suppresses the proliferation of MGs and reduces cell cycle genes. This leads to inhibition of Yap and downregulation of cell cycle genes. Ultimately, this data suggests that Tox may be a critical gene in the limit of mammalian retinal regeneration. Furthermore, if Tox KO can induce reprogramming in the damaged retina of mammals, this study is valuable as a prior study on gene therapy using Tox shRNA.

Keywords: Müller glial cell, Retinal Reprogramming, Tox

1106

TARGETED DELETION OF PAX3 ALTERS DIFFERENTIATION OF SACRAL NEURAL CREST PROGENITORS THAT FORM PELVIC AUTONOMIC INNERVATION

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Contraction of the urinary bladder to accomplish emptying is mediated by motor neurons in the pelvis that are part of the autonomic nervous system. In rodents, these neurons are aggregated into

major pelvic ganglia (PG) just dorsolateral to the bladder neck. PG originate from sacral neural crest whose migration paths into the fetal urogenital sinus have been recently described. However, knowledge of molecular mechanisms that regulate PG neurogenesis is lacking. To identify candidate factors during development of PG, we assessed transcription factor expression patterns in lower urinary tracts of fetal mice. This analysis identified a prominent pulse of Pax3 expression during PG neurogenesis and subsequent hybridization chain reaction in situ identified Pax3 mRNA in differentiating PG neurons. Pax3 is widely expressed during embryogenesis and global loss of this gene causes perinatal lethality that precludes postnatal analysis. To circumvent this challenge, we devised crosses using a conditional allele Pax-3tm1.1Sjc (Pax3flox, after cre Pax3KO) to specifically delete Pax3 from neural crest progenitors that give rise to pelvic autonomic neurons. By incorporating a cre line that is prominently expressed in neural crest progenitors (Sox10-cre) we aimed to test whether neural crest expression of Pax3 is required for normal development of PG, bladder innervation, and bladder function. During fetal stages, we found that Pax3 KO/NC-cKO mice initially have normal-sized aggregates of sacral NC progenitors that colonize the fetal urogenital sinus. However, differentiation of PG neurons is delayed in fetal Pax3 KO/NC-cKO mice and postnatally the PG of these mutants are smaller, with fewer fascicles extending from the PG to accessory ganglia at the bladder neck. Pax3 KO/NC-cKO mutants survive to adulthood and functional studies of bladder contractility using electrical field stimulation on bladder muscle strips found reduced contractility in these mutants. These studies demonstrate that Pax3 plays an essential role in differentiation of sacral neural crest that form the PG. Further study of the developmental cues that promote differentiation and innervation of LUT tissues by PG neurons will benefit efforts to compensate for damaged pelvic innervation and will aid efforts to innervate artificial bladder scaffolds.

Funding Source: R01 DK078158

Keywords: neural crest, autonomic neurogenesis, lower urinary tract innervation

1108

COMBINATORIAL ACUTE PLG BRIDGE + CHRONIC HUMAN NEURAL STEM CELL TRANSPLANT AS A THERAPEUTIC APPROACH FOR SPINAL CORD INJURY

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Human neural stem cell (hNSC) transplantation is a promising therapeutic strategy for spinal cord injury (SCI), a devastating condition that affects ~7 million individuals globally. While regeneration in the injured spinal cord is limited by physical and chemical barriers, we have previously shown that acute implantation of a multichannel poly(lactide-co-glycolide) (PLG) bridge promotes robust axonal regrowth through the injury and motor recovery. However, these regenerated axons remain largely unmyelinated (< 10%), therefore limiting functional repair. Here, we test the hypothesis that chronic hNSC transplantation following SCI coupled with acute PLG bridge implantation will promote oligodendrogenesis, myelination of regenerated axons, and increase functional motor recovery. Indeed, we demonstrate a robust capacity of donor hNSC to migrate into PLG bridge channels along regenerating axons and integrate into the host spinal cord as myelinating oligodendrocytes and synaptically integrated neurons using pseudorabies viral tracing. Lastly, chronic hNSC transplantation coupled with acute PLG bridge implantation elicited increased locomotor recovery in comparison to hNSC only and PLG bridge only control groups. Taken together, these data identify a successful novel strategy to enhance neurorepair via chronic hNSC transplantation and acute PLG bridge implantation to promote regeneration and motor function following SCI.

Keywords: Neural stem cells, Biomaterials, Spinal cord injury

1110

DORMANT STATE OF QUIESCENT NEURAL STEM CELLS LINKS SHANK3 MUTATION TO AUTISM DEVELOPMENT

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Autism spectrum disorders (ASDs) are common neurodevelopmental disorders characterized by deficits in social interactions and communication, restricted interests, and repetitive behaviors. Despite extensive study, the molecular targets that control ASD development remain largely unclear. Here, we report that the dormancy of quiescent neural stem cells (qNSCs) is a therapeutic target for controlling the development of ASD phenotypes driven by Shank3 deficiency. Using single-cell RNA sequencing (scRNA-seq) and transposase accessible chromatin profiling (ATAC-seq), we find that abnormal epigenetic features including H3K4me3 accumulation due to up-regulation of Kmt2a levels lead to increased dormancy of qNSCs in the absence of Shank3. This result in decreased active neurogenesis in the Shank3 deficient mouse brain. Remarkably, pharmacological and molecular inhibition of qNSC dormancy restored adult neurogenesis and ameliorated the social deficits observed in Shank3-deficient mice. Moreover, we confirmed restored human qNSC activity rescues abnormal neurogenesis and autism-like phenotypes in SHANK3-targeted human NSCs. Taken together, our results offer a novel strategy to control qNSC activity as a potential therapeutic target for the development of autism.

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Keywords: quiescent neural stem cells, autism development, human qNSC

1112

TRANSPLANTED STEM-CELL DERIVED HUMAN PHOTORECEPTORS DO NOT ENGAGE IN CYTOPLASMIC MATERIAL TRANSFER WITH THE RECIPIENT MOUSE RETINA

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Transplanted mouse photoreceptors have been shown to preferentially sit at the site of injection and transfer cytoplasmic material with host photoreceptors, rather than migrating and integrating with the host circuitry. Bidirectional intercellular communication between photoreceptors, termed material transfer, has the potential to be harnessed therapeutically, to provide diseased host photoreceptors with healthy cargo. However, it is currently unknown if human photoreceptors will also engage in material transfer, and if there is a selectivity for the type of cargo transferred, as syngeneic mouse transplants have tracked material transfer using GFP as a proof-of-concept protein. To ask if material transfer is stage-specific in human photoreceptors, we transplanted stem-cell derived photoreceptors purified from retinal organoids at different ontological ages (week 10, 14, and 20), into wild type, Nrl-/-, and immune deficient (NSG) mice. We took advantage of human-specific antibodies to selectively identify human mitochondria and human recoverin, a photoreceptor protein. Unexpectedly, regardless of donor cell age or mouse recipient model, transplanted human photoreceptors did not transfer human cytoplasmic material to host photoreceptors. In contrast, human mitochondria+ and human recoverin+ cells were associated with human nuclei in the subretinal space, away from the mouse photoreceptor cell layer, or in rare cases (< 5% of donor cells), migrated into the mouse photoreceptor cell layer. In a flow cytometric in vitro assay, we observed that human photoreceptors transferred mitochondria in vitro, suggesting that a species difference may inhibit this process in vivo and that xenograft models are not a tractable system to study material transfer of human photoreceptors.

Funding Source: We are grateful for funding support for this research: Medicine by Design, Natural Sciences and Engineering Research Council of Canada, and the Vanier Canadian Graduate Scholarship.

Keywords: Retinal Organoids, Cell Transplantation, Cytoplasmic material transfer

1114

DIRECT AND FAST SOMATIC TO NEURAL STEM CELL FATE REPROGRAMMING WITHOUT REJUVENATION PATTERNS

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Cell fate conversion by ectopic expression of transcription factors provides strategies for cell-based basic and medical research. Induced neural stem cells (iNSCs) are promising cell sources for neurological disease modeling and regenerative medicine. They have the potential to bypass the shortcomings of pluripotent cells such as tumorigenicity, tedious protocols and a neonatal phenotype and postmitotic neuron without expandability and stemness. Here, we describe a robust and efficient iNSC reprogramming approach using an engineered transcription factor. With this approach, we are able to directly and quickly generate iNSCs from both mouse and human somatic cells. These iNSCs can self-renew and are multipotent to give rise to mature neuronal subtypes and glial cells. By contrasting the routes towards pluripotent versus neural stem cells using sensitive lineage reporters and genome-wide analysis, we defined iNSC reprogramming roadmaps without going through a pluripotent intermediate state. iNSCs derived from aged cells show aging-related genomic DNA methylation patterns indicating the aging-related epigenetic signatures are retained during iNSC reprogramming. We will discuss our efforts to apply iNSCs to model neurodegenerative diseases. iNSCs directly generated from patients demonstrate authentic next-generation stem cell models that truly represent the age of the donors to study pathology associated with aging 'in a dish'.

Funding Source: the National Natural Science Foundation of China (Grant No. 31771454) the Research Grants Council of Hong Kong General Research Fund (Grant No. 17128918 and 17101120)

Health and Medical Research Fund (Grant No. 06174006 and 08192886)

Keywords: direct reprogramming, induced neural stem cells, Aging signatures

1116

IN VIVO CONTEXT-DEPENDENT DIFFERENTIATION OF TRANSPLANTED HUMAN GLIAL PROGENITOR CELLS

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Neither rodent models nor in vitro studies of human cells fully reflect the molecular regulation of human glial progenitor cell (hGPC) expansion, differentiation and myelination in vivo. To this end, we used scRNA-seq of more than 60,000 human cells to characterize pluripotent stem cells (PSCs) and their derived hGPCs, as produced from either hESCs (WA09) or iPSCs (C27), at three different stages: undifferentiated stem cells; GPCs in vitro, following their induction and differentiation in culture; and in vivo, 20 weeks after their neonatal transplantation into immunodeficient and myelin-deficient shiverer mice. In culture, hGPCs were transcriptionally non-overlapping with their parental PSCs and comprised wholly of GPCs, astrocytic lineage cells, and a minority of neural progenitor cells with no residual PSCs. Strikingly, transplantation of these cells into shiverer mouse corpus callosum resulted in substantial context-dependent differentiation, such that when the human cells were extracted back from the 20-week-old chimeric brains, they were found to be comprised solely of well-differentiated astrocytes, oligodendrocytes and their direct progenitors. We noted that each of these phenotypes was characterized by a transcriptional state more mature than that achieved in vitro, while the overall human population was devoid of earlier neural or pluripotent cells. To identify transcription factors (TFs) active in the regulation of this context-dependent maturation, we used a combination of gene co-expression, motif enrichment, and extrapolation of cell-trajectories, which confirmed the critical roles of SOX9 and SREBF1, and of SOX10 and NKX2-2, for astrocytic and oligodendrocytic maturation, respectively. Focusing on the latter, we then generated a transcriptional network governing both early and late stages of myelination, which highlighted the network importance of the TFs TFEB and NKX6-2, among others. By this means, we have identified pathways whose targeting may permit the therapeutic modulation of human glial specification and maturation in vivo. In addition, our data suggest that the context-dependent differentiation of human glial progenitor cells potentiates their terminal phenotypic differentiation after in vivo delivery, further suggesting their safety as a therapeutic vector.

Funding Source: Supported by NINDS, CNS2, Inc. and Sana Biotechnology.

Keywords: scRNA-Seq, Glial progenitor cell, Oligodendrocyte

1118

THE CONTRIBUTION OF THE GUT MICROBIOME TO BRAIN AGING AND REJUVENATION

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Normal brain aging involves cellular and structural changes including inflammation, a reduction in adult neurogenesis and synaptic plasticity, and a loss of brain vasculature along with its functional decline. Coincidentally, the gut microbiome, the population of microorganisms in the gastrointestinal tract, undergoes a community shift with aging. Dysbiosis has been reported in brain disorders, suggesting that the gut microbiome could play a role in physiological processes in the central nervous system, including aging. Aging has always been considered irreversible. However, research over the last 40 years has challenged this assumption, and it is becoming evident that systemic manipulations can partially counteract the age-related loss of plasticity in the brain. Based on this evidence, we hypothesized that depleting the dysbiotic microbiome could rejuvenate the aged brain. We treated young and old mice with antibiotics (ABX), using a protocol already shown to deplete the gut microbiome efficiently. Single-nucleus RNA sequencing of cortices and hippocampi revealed that ABX treatment triggered changes in vasculature plasticity and a shift in the expression of genes involved in myelination in both young and old mice. Analysis of old mouse brain vasculature confirmed these findings, highlighting a higher blood vessel density and a significant rise in endothelial cell tight junction protein expression in different brain regions after ABX treatment. Consistent with the RNA seq data, the abundance of proteins involved in myelination was also enhanced in microbiome depleted old mice. Interestingly, the effect on young mouse brains was the opposite, showing a decrease in blood vessel density and myelin after ABX administration. Moreover, a preliminary study on adult neurogenesis indicated that ABX treatment impaired neurogenesis in young mice and enhanced the proliferation of neural stem cells in the old brains. This work is the first unbiased, brain-focused comparative study on the effect that microbiome has on the aging central nervous system. Our results suggest thus far are consistent with the intriguing possibility that manipulation of the gut microbiota might be one of the most effective of the increasing number of treatments capable of improving the structure and function of the aging brain.

Funding Source: This work was supported by the Simons Foundation (Collaboration on Plasticity in the Aging Brain), NIH/NIA 1R01AG072086, and a generous gift to the Harvard Stem Cell Institute from the Vranos Family Foundation.

Keywords: Brain Aging and rejuvenation, Gut-brain axis, Neural stem cells

1120

IGFBP4 NEGATIVELY REGULATES PANCREATIC ISLET SIZES BY INHIBITING THE ACTIVITY OF ISLET PROGENITORS

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Pancreatic islet sizes and beta cell mass are critical for glucose homeostasis. The mechanism that controls islet sizes remains unclear. Here, we demonstrate that a secreted protein—Insulin-like Growth Factor-Binding Protein 4 (Igfbp4) is critical for the size and the function of the islets. Our previous study establishes an islet organoid culture system using Procr+ islet resident progenitors. In this study, we found that the progenitors isolated from neonatal mice formed significantly larger organoids in vitro than those isolated from adult mice. Interestingly, we found that the expression of Igfbp4 is silent in neonatal islets and is increased in adult. Consistently, addition of Igfbp4 protein in the organoid culture resulted in smaller organoids, while knockdown of Igfbp4 leads to larger organoids. In genetic mouse models, deletion of Igfbp4 exhibits increased islet size and improved islet function. Single cell RNA sequencing reveals that Igfbp4 is specifically expressed in Procr+ islet progenitors, which is further verified by western blot and qPCR analyses. Mechanistically, Igfbp4 binds to Protein C (PC), the ligand of Procr, and inhibits the PC-Procr interaction, thereby blocking the formation of Procr-Hsp90aa1-Src complex. The action of Igfbp4 decelerates the proliferation of the progenitors. Our data suggest Igfbp4 as an inhibitor for the islet progenitors and islet sizes, reveal a novel Igfbp4-PC competition mechanism, and provide new molecular insights into islet growth and diabetes prevention.

Keywords: Pancreatic islet progenitor, Procr, Igfbp4

1122

STANDING OUT FROM THE CROWD: STEM CELL-DERIVED ISLET CELLS FUNCTION INDEPENDENT OF CLUSTERING WHEN IMPLANTED INTRAMUSCULARLY

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Therapeutic implantation of human pluripotent stem cell-derived islet (SC-islet) cells has the potential to provide a functional cure for type I diabetes (T1D) without the supply limitations of cadaveric islets. Islets function biologically as clusters of distinct cell types which aggregate upon differentiation; therefore SC-islet cells are historically implanted as islet clusters. While recent clinical evidence validates this approach, cryopreservation of islet clusters often leads to poor post-thaw recovery. Implantation of SC-islet cells without aggregation potentially eliminates processing steps and simplifies cryopreservation. However, this approach has been largely unexplored due to unknowns regarding physiological function of transplanted cells. Here, we report that SC-islet cells implanted without aggregation are highly functional in vivo following implantation into the muscle. In vivo studies were conducted using male NOD scid gamma mice 7-8 weeks old, into which SC-islet cells were intramuscularly implanted using standard clusters (n=8), clusters disaggregated prior to implantation

(n=8), or as cells differentiated without aggregation (n=5). A subset was dosed with streptozotocin post-implant to induce diabetes, and blood glucose (BG) levels measured over time. All mice transplanted with non-aggregated SC-islet cells showed comparable in vivo function to that of mice transplanted with standard clusters, as demonstrated by glucose-stimulated human c-peptide production between 500 and 2000 pM for more than 20 weeks post-implant. Chronic and acute BG control following induction of diabetes were also comparable between groups. Our findings demonstrate the feasibility of SC-islet cell implantation without aggregation which has potential manufacturing and supply chain implications that are advantageous compared to clusters. Moreover, the results show the promise of the muscle as an implantation site for SC-islets. Such advantages, if translatable to the clinic, would further the potential of SC-islets as an accessible treatment for many with T1D.

Keywords: Diabetes, Beta islet, Cell transplantation

1124

3D RECONSTRUCTION & MICROANATOMICAL ANALYSIS OF HUMAN PANCREATIC PRECANCERS REVEAL NOVEL STRUCTURAL & MORPHOGENETIC PHENOTYPES

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Pancreatic intraepithelial neoplasia (PanIN) is the predominant premalignant precursor to pancreatic cancer, a lethal disease with a dismal prognosis. Despite being a critical step in early pancreatic carcinogenesis, the origin and development of these microscopic precancers remain poorly understood as traditional histological analysis of discrete 2D tissue sections cannot accurately assess PanIN connectivity, global structure, or spatial relationships within the human pancreas. Here, webilityrizee the complex 3D architecture of human PanINs with CODA, a novel machine-learning pipeline for quantitative 3D reconstruction of large volumes of serially-sectioned H&E-stained tissue at single-cell resolution. By applying CODA to 39 samples of grossly normal pancreas containing a total of 899 separate lesions, we reveal that PanINs comprise two novel 3D structural phenotypes: a tubular form that mimics the normal pancreatic ducts, and a lobular form that appears as dense clusters. We also identify several morphologically and molecularly distinct epithelial subtypes that coexist within the neoplastic epithelium and train a machine-learning annotation algorithm was to map these histological classifications onto the 3D architecture. We find that tubular and lobular 3D structures are associated with specific epithelial subtypes that resemble foveolar or pyloric cells in normal gastric glandular morphogenesis and mucosal metaplasia. This work serves as a platform for further mapping of genetic and phenotypic heterogeneity within PanINs by integrating CODA-guided 3D microanatomy with immunohistochemistry, spatial transcriptomics, and multi-region sequencing. Our findings raise the intriguing possibility that gastric gland-like developmental programmes play a role in PanIN pathogenesis and demonstrate the potential of artificial intelligence-driven 3D image analysis to direct mechanistic studies of cancer initiation and progression in human tissue.

Keywords: pancreatic precancer, gastric metaplasia, machine-learning 3D image analysis

TOPIC: NO TISSUE SPECIFICITY

1126

IMPROVED METHODS FOR CRISPR EDITING AND HOMOLOGY-DIRECTED REPAIR (HDR) IN IPSC

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CRISPR-based editing and homology-directed repair (HDR) has become a widely used tool for introducing precise changes in the genome. However, it's utility in various cell types such as iPSC is often impeded by the lack of reliable reagents and protocols. Here, we report optimized CRISPR reagents and design parameters to improve HDR in various cell types including iPSC. Using Alt-R™ S.p. Cas9, Alt-R guide RNAs and Alt-R donors oligos, we present efficient and precise insertion of donor template at multiple target sites measured by Next Generation Sequencing (NGS). HDR rates can be further boosted by the use of small molecule compound, Alt-R HDR enhancer V2. In addition, we present the performance of an improved small, protein-based HDR enhancer as an additional reagent to increase HDR rates when co-delivered with Cas9 RNP complex. We also feature rhAmpSeq™ CRISPR Analysis System that provides a streamlined workflow for high throughput, targeted NGS based screening of editing outcomes and HDR rates in any cell type of interest.

Keywords: CRISPR-Cas9, genome editing, analysis tool, HOMOLOGY-DIRECTED REPAIR (HDR), iPSC

1128

NON-DESTRUCTIVE MONITORING OF ADIPOGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS BASED ON CELLULAR METABOLISM

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Stem cell-based therapies have emerged in the area of regenerative medicine and tissue engineering because of their ability to treat various incurable diseases and disorders. However, the ability to differentiate stem cells into specific cell types (e.g., bones, fats, cartilage, and neurons) in a highly selective manner to assay the maturation of stem cell differentiation plays a pivotal role in utilizing the full potential of stem cell-based applications. Differ-

entiation of stem cells into mature cell types is guided by growth factors or hormones, but recent research suggests that metabolic shifts occur during differentiation and modulate the differentiation process. In this work, we have developed a novel methodology using electrochemical detection to 1) investigate the real-time monitoring of adipogenesis and 2) assess the maturation of adipocyte differentiation of human adipose-derived mesenchymal stem cells (ADMSCs) without any labeling or pre-treatment. The following results are compared with the standard techniques such as Oil Red O staining, fluorescence microscopy, and qPCR that are conventionally used to evaluate adipogenic differentiation. Moreover, the dynamic shifts in cellular metabolism during adipogenesis are electrochemically detected, which is involved in mitochondrial respiration and metabolic activities, and were monitored non-destructively. Therefore, this developed method is highly useful for the monitoring of adipogenic differentiation and its implementation in biomedical and clinical applications.

Funding Source: National Research Foundation of Korea (NRF) grant funded (Grant Nos. NRF-2022R1A4aaA2000776, NRF-2019M3A9H2031820, NRF-2022R1A2C4002217) and Korean Fund for Regenerative Medicine (Grant number RS-2022-00070316).

Keywords: Stem cell differentiation, Adipogenesis, Electrochemical detection

1130

SERUM ALBUMIN MAINTAINS WNT WATER-SOLUBILITY AND ACTIVITY

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Wnt signaling is recognized as the most important cell signaling pathway in adult stem cell-derived organoid culture. Currently, Wnt is added to cells as FBS-containing conditioned medium (CM) or CHAPS-containing purified protein. However, using FBS makes it hard to control lot-to-lot variation and is prone to contamination, and using detergent on in vitro experiments may be harmful to cells. In this reason, there has been a growing body of research that investigates on how to create a serum-free and detergent-free active Wnt preparation for adult stem cell-derived organoid culture. Prior study has addressed Afamin (AFM), a type of glycoprotein, as a serum factor that solubilizes Wnt3a in CM. However, we found that AFM cannot solubilize Wnt3a by itself and that serum albumin (SA) is an indispensable co-factor for Wnt solubilization in Wnt3a-AFM CM. We have proven this by showing Wnt3a-AFM CM alone cannot maintain adult stem cell-derived organoid proliferation, while adding SA to Wnt3a-AFM CM effectively supports the growth and expansion of organoids. Our results also show that SA does not need AFM to solubilize and stabilize purified Wnt3a protein in in vitro experiments. Therefore, we introduce a novel SA-based method for high-performance Wnt preparation that does not need AFM nor CHAPS.

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Keywords: Wnt3a, Serum Albumin, Organoids

1132

TRACKING TRANSPLANTED LIMBAL EPITHELIAL CELLS IN AN ANIMAL MODEL OF LIMBAL STEM CELL DEFICIENCY USING NANOPARTICLES TO UNCOVER CORNEAL EPITHELIAL REGENERATION.

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The corneal epithelium, which covers the front surface of the eye, has the ability to self-renew and regenerate. However, physical or chemical trauma to limbal epithelial stem cells (LESCs) that reside at the border between the cornea and sclera can result in limbal stem cell deficiency (LSCD), causing vision loss due to corneal opacification, inflammation, conjunctivalization, and neovascularization. Autologous or allogenic LESCs have shown to be a suitable sources for cell-based therapies aimed to treat LSCD, but their exact role in corneal regeneration remains unclear. To better understand the role of LESCs in corneal regeneration, we synthesized custom mesoporous silica-coated gold nanoparticles (R-Au-MSNs) to label and track transplanted primary human limbal epithelial cells in a LSCD animal model. The aim of this study was to assess the feasibility of using R-Au-MSNs as a tool for non-invasive visualization and tracking of the transplanted cells in vivo over time. Through multimodal imaging, we observed sparse distribution of R-Au-MSNs in the peripheral cornea and limbal area. However, we did not only detect R-Au-MSNs in transplanted cells but also in surrounding tissue, indicating that R-Au-MSNs may not be an effective tool for long-term tracking of transplanted LESCs in vivo. Despite this limitation, our study also revealed that LESC transplantation improved therapeutic outcome in rabbits, including reduced corneal opacification and partial corneal epithelial reconstruction. However, the small number of transplanted cells found in the corneal epithelium and limbus suggests that these cells did not repopulate the corneal epithelium. These results point to a more complex role of transplanted LESCs in corneal regeneration, highlighting the need for further research to gain a deeper understanding of their instructive role and regulatory effects.

Funding Source: This work was supported by the research program ZonMw TOP (Grant 91217058,VISION).

Keywords: Nanoparticles, Cornea, cell-based therapy

1134

HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED ARTERIAL ENDOTHELIAL CELLS FOR REVASCULARIZATION THERAPIES IN TISSUE ENGINEERING

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As the field seeks to scale up implant size and improve therapeutic potential, tissue engineering presents a critical need for neovascularization in both implant and host ischemic tissues. Implantation of endothelial cells, either directly into the native tissue or incorporated into the implant, has demonstrated efficacy in improving vascularization and tissue perfusion. Primary endothelial cells are difficult to obtain, often from allogenic sources, and offer limited replicative potential. However, the large proliferative capacity and accessibility of human induced pluripotent stem cell-derived endothelial cells (iPSC-eCs) make them a promising tool in revascularization of ischemic tissues. This study describes a method for chemically defined, monolayer differentiation of vascular endothelial cells using a cardiac mesoderm intermediate. Subsequent magnetic-activated cell sorting (MACS) with vascular endothelial cadherin (CD144) provided an average 97% purity with defined arterial, venous, and capillary subpopulations and thbilityty to preferentially drive arterial or venous phenotype ratios. HiPSC-eCs show the expected cobblestone morphology, surface markers by flow cytometry, and gene and protein expression. Cell viability and proliferative capacity was high, assessed by MTT assay. Functional assessments demonstrated angiogenic potential in vitro via microvascular network formation on Matrigel and defined substrates and sprouting assays both innately and in response to proangiogenic factor supplementation. Sacrificial alginate fiber-derived channels were lined with hiPSC-eCs, forming a vessel endothelium that became confluent with culture and demonstrated the cells' ability to both recapitulate native vasculature and support in vitro vessel patterning of engineered tissues. Collectively, these findings demonstrated the ability to derive phenotypically defined, functional arterial/venous endothelial cells from iPSCs for revascularization therapies, providing a means to both overcome current thickness-based diffusion limitations of engineered tissue fabrication and revascularize ischemic tissue in vivo.

Keywords: Revascularization, HiPSC derived endothelial cells, Tissue Engineering

1136

HEAD AND NECK TISSUE STEM CELL DYSREGULATIONIH AGE AND CANCER

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Advancing age and tobacco use are major risk factors for cancers of the head and neck (HNC). Management of HNC involves multimodal treatment strategies that are relatively ineffective, have high rates of recurrence, and cause devastating harm to speech, swallowing, and overall quality of life. Despite these severe clinical sequalae, genes and pathways contributing to HNC development in males and females across the lifespan are understudied and poorly understood, leaving a dearth of interventions that can interrupt HNC promoting mechanisms. Our research takes an integrative approach to reveal novel mechanisms contributing to HNC initiation and progression across lifespan, and to test the hypothesis that HNC initiation is driven by the age-related dysregulation of tissue function and acquisition of (epi)genetic mutations in tissue stem cells of the head and neck, both of which are significantly influenced by biologic sex and accelerated following carcinogenic exposure. Our data demonstrate dysregulation of head and neck tissue stem cells with increasing age, and following exposure to the chemical carcinogen, and tobacco mimetic, 4NQO. In young (4-6mo), middle-aged (16-18mo), and aged (24mo) C57BL/6 mice, muscle satellite cell (SC) frequency declines with age in the tongue ($p<.0001$), pharynx and esophagus ($p=.0008$), and neck ($p<.0001$), mesodermal progenitors (Sca1Ps) of the neck are reduced in female mice ($p=.0297$), and quiescent ($p=.0141$) and proliferating ($p=.0012$) epithelial stem cells (EpSCs) and epithelial progenitors ($p=.0294$) are differentially impacted by age. Following 4NQO exposure, gross lesions are observed on the tongue at higher frequency in middle-aged and aged male mice ($p<.001$), and a reduction was observed in the overall frequency of neck SCs ($p=.016$) and of pharyngeal and esophageal Sca1Ps ($p=.016$), while an increase in frequency was observed in EpSCs ($p<.001$). Our data demonstrates age-, sex-, and carcinogen-dependent dysregulation in head and neck tissue stem cells. Work to determine mechanisms underlying these alterations and their potential role in oncogenic transformation is ongoing. Overall, this innovative and translationally-oriented work will fill critical knowledge gaps that are a clinical priority for the prevention, early identification, and treatment of HNCs.

Funding Source: F32AG071208, DP1OD025432, Harvard Stem Cell Institute,

Keywords: aging, cancer, head and neck

1138

HMGB1/RAGE/TLR4 SIGNALING IS A POTENTIAL TARGET FOR TREATMENT OF RADIATION DAMAGED SALIVARY GLANDS WITH EFFECTIVELY CONDITIONED MONONUCLEAR CELLS (E-MNC)

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We have recently developed a new therapy using E-MNC and demonstrated its effectiveness against radiation-damaged salivary glands (SGs). Then, we found immunomodulatory (class A scavenger receptor Msr1+/CD11b+) M2 macrophages in E-MNC brought therapeutic effects due to their phagocytic elimination of extracellular HMGB1, which acts as damaged-associated molecular patterns (DAMPs). Therefore, to clarify its deeper mechanism, we first investigated how significant resolving sterile inflammation via inhibition of HMGB1/RAGE/TLR4 signaling is as a therapeutic target for the radiation-damaged SGs. Mouse E-MNC was induced from peripheral blood mononuclear cells in culture using a serum-free medium supplemented with 5 specific recombinant proteins for 5-6 days. Then, after E-MNC transplantation, clearance of extracellular HMGB1 and inhibition of RAGE/TLR4 pathway in radiation-damaged SGs in mice were assessed. Moreover, SGs of TLR4 KO mice were irradiated and then examined whether tissue injury via sterile inflammation progresses or how HMGB1, released from injured SG cells, affects the damage progression. In parallel, cultured SG epithelial cells were exposed to culture supernatant containing extracellular HMGB1 obtained from SG cell culture after irradiation. After irradiation to SGs, released HMGB1 was increased in damaged tissues and expression of RAGE in macrophages and TLR4 in ductal cells was recognized. Then, pro-inflammatory genes were upregulated in damaged tissues via NF-kB pathway. However, E-MNC treatment could inhibit HMGB1 release and TLR4 expression in damaged tissue. Meanwhile, we found the onset of radiogenic salivary hypofunction was substantially delayed in TLR4 KO mice and RAGE-positive cells were alternatively increased. Culture supernatant containing extracellular HMGB1, derived from irradiated SG cells, induced TLR4 expression in cultured SG epithelial cells without irradiation and promoted their cell death. HMGB1/RAGE/TLR4 signaling might contribute the progression of SG damage after radiation therapy and be a potential target for E-MNC therapy.

Keywords: Cell-based Therapy, Radiogenic salivary gland dysfunctioin, Peripheral blood mononuclear cells

TRACK: MODELING DEVELOPMENT AND DISEASE (MDD)

Session 3: Odd

1:30 PM – 2:15 PM

TOPIC: CARDIAC

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INDUCED HEART FAILURE WITH PRESERVED EJECTION FRACTION USING MINI-HEART ENGINEERED CARDIAC TISSUE PLATFORMS CAPTURE KEY FUNCTIONAL AND TRANSCRIPTOMIC FEATURES OF HFPEF

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Heart failure with preserved ejection fraction (HFpEF) is a significant and growing health problem in which patients present with elevated left ventricular diastolic stiffness despite a relatively normal ejection fraction. Therapies that reduce mortality and morbidity in Heart Failure with reduced Ejection Fraction (HFrEF) have little effect in HFpEF, as the etiology and the clinical course are different. Therefore, as encouraged by the FDA Modernization Act 2.0, there is an urgent need to develop advanced human-specific assays for understand the underlying processes, and to develop targeted therapies for HFpEF, as animal models fail to adequately mimic this human pathophysiology. Novoheart has created the first in vitro human models of HFpEF with its mini-Heart cardiac tissue platforms. HFpEF phenotype was induced by endothelin-1 (ET-1) and transforming growth factor-β1 (TGF-β1) in human ventricular cardiac tissue strips (hvCTS) and cardiac organoid chambers (hvCOC). Compared to healthy controls, the HFpEF hvCTS and hvCOC exhibited significantly higher passive tension and tissue stiffness, and slower contraction and relaxation kinetics, with no significant change in developed force or EF. To assess the fidelity of the models, contractile data and bulk RNA

sequencing of the models were compared to a HFpEF patient database. Of the differentially expressed genes (DEG) between HFpEF and healthy subjects, 29% and 33% of these overlap with the HFpEF-hvCTS and HFpEF-hvCOC models, respectively. Comparative functional analysis revealed that HFpEF-hvCOC more closely mimicked patient hearts. Of note, SERCA2a—one of the highest DEG in the calcium signaling pathway—was the most significantly downregulated in HFpEF patients and the induced HFpEF models. Forced expression of SERCA2a by AAV1 transduction rescued the disease phenotype in HFpEF-hvCTS by restoring the slowed contractile kinetics. These preclinical human HFpEF hvCTS and hvCOC models thus recapitulate characteristic HFpEF phenotypes. With predictive engineered HFpEF models, we demonstrated investigation of HFpEF mechanisms, discovery of a novel therapeutic target, and the feasibility of clinical-grade AAV1-SERCA2a as an effective HFpEF treatment. These findings lead to an open IND from the FDA for a related Phase 1 gene therapy clinical trial.

Keywords: Disease Modeling, Human Bioengineered Cardiac Tissue Models, HFpEF

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DISCOVERING CELL SIGNALING INTERACTIONS DIRECTING HUMAN VENTRICULAR CARDIOMYOCYTE DIFFERENTIATION IN MULTILINEAGE STEM CELL IN VITRO MODELS

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Heart failure is a decline of cardiac function in part due to cardiomyocyte (CM) death. Human pluripotent stem-cell-derived cardiomyocytes (hPSC-CMs) can be utilized to develop cell-replacement therapies for heart repair, but to generate specific cardiomyocyte sub-types, it is essential to understand the cell fate decisions and developmental cues that allow pluripotent cells to become a particular cell type. A cell's development is highly dependent on its environment within a tissue, particularly through cell-cell interactions between distinct cell types. To understand how CMs are influenced by their environment, we examined the differentiation states of CMs in a 2D (monolayer) versus 3D (embryoid body) cellular environment and found that ventricular CMs (vCMs) develop more efficiently in the 3D environment. We further investigated the developmental states of vCMs differentiated between these environments using single-cell RNA sequencing analysis, and found that the 3D environment contained higher proportions of non-CM cell types, and the 3D-derived CMs from this system exhibited a more developmentally mature cellular state. Examining the signaling events from non-CM to CM specific cell types, we discovered 14 3D-specific pathways, 7 2D-specific pathways, and 20 shared pathways. By conducting CRISPR-Cas9 knockout and activation screens on the differentially identified cardiac receptors and ligands, we identified diverse signaling pathways that impact the differentiation of vCMs. Finally, we show that the generation of vCMs can be modulated pharmacologically by altering these pathways, through either activation of signals derived from the 3D system or inhibition of signals derived from the 2D system. This study reveals how distinct non-CM cardiac cell types may impact the generation of vCMs through cell-type specific non-cell autonomous signaling cues, thereby enabling the generation of pure populations of in vivo-like vCMs that can be used for heart failure treatment.

Funding Source: UCSD Genetics Training Program from the National Institute for General Medical Sciences, T32 GM008666, and National Heart, Lung, And Blood Institute of the National Institutes of Health, F31HL163996

Keywords: ventricular cardiomyocytes, cell signaling, development

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ESTABLISHMENT OF 3D MATURE CARDIAC TISSUE PLATFORMS FOR MODELING DMD CARDIOMYOPATHY

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The major cause of mortality in patients with Duchenne muscular dystrophy (DMD) or Becker muscular dystrophy (BMD) is cardiomyopathy. Our research aims to find genes whose expression can modify a disease-related phenotype of DMD-cardiomyopathy and compensate the partial effect of abbreviated/truncated dystrophin gene replacement therapies. The patient iPSC-derived in vitro disease model is useful for analysis of pathological mechanisms and development of therapeutics; however, the major limitation of current models is the lower expression of components of dystrophin associated protein complex (DAPC) due to immaturity of iPSC-CMs, resulting in lack of disease phenotypes. To improve the maturation of DMD iPSC-CMs, we generated cardiac organoid or engineered heart tissues (EHT) by 3D co-culture with epicardium. In addition, the 3D cardiac organoids or EHT were stimulated with palmitate, PPARα agonist, dexamethasone and T3 to shift whose metabolism from glycolysis to fatty acid oxidation. To elicit pathological state, DMD-EHT was subjected to continuous mechanical loading. The mature cardiac organoid displayed adult-like gene expression profiles with regard to the genes associated with DAPC and calcium handing. DMD-EHT displayed impaired electrical coupling capacity under rapid pacing. Significant contractile dysfunction was also displayed in DMD-

EHT under the presence of continuous mechanical loading. In the meanwhile, marked cardiomyocyte loss was observed partly due to apoptosis of cardiomyocytes. These phenotypes in DMD-EHT precisely recapitulate the pathological development of DMD-cardiomyopathy. Our 3D mature cardiac tissue platforms provided insights into the mechanistic pathways leading to DMD-cardiomyopathy and useful to evaluate novel experimental therapeutic agents as a "pre-clinical trials in a dish".

Keywords: Duchenne muscular dystrophy (DMD), cardiomyopathy, engineered heart tissue

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A CARDIOID-BASED PHENOTYPIC, AUTOMATED, HIGH-THROUGHPUT PLATFORM FOR CARDIAC DRUG DISCOVERY AND CARDIOTOXICITY EVALUATION

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Cardiovascular Disease (CVD) is the leading cause of death accounting for 32% of global mortality. A major reason for this high death toll is the lack of effective new breakthrough therapies for CVD, which is in stark contrast to other disease indications. We postulate that a key reason for the continuous decline in CVD drug discovery is the lack of reproducible and representative human heart chamber in vitro models that can adequately predict human disease and are amenable to high-throughput primary screening. Here we have developed a fully automated high-throughput platform capable of culturing induced pluripotent stem cells (iPSC) and differentiating them into 3D self-organizing cardiac chamber organoids (Cardioids). iPSC and Cardioid quality and reproducibility are ensured through automated organoid handling and continuous image-based monitoring. This enabled efficient upscaling and maturation of Cardioids in screening-ready 384-well plates with minimal human intervention and very low Cardioid variability (< 10%). High-throughput, automated confocal imaging and simultaneous functional imaging of 384-well plates (FLIPR® Penta High-Throughput Cellular Screening System, Molecular Devices) was used as a phenotypic screening set-up. We have employed it to model disease (genetic cardiomyopathies) and assess different aspects of cardiotoxicity by subjecting iPSC or Cardioids to several genetic alterations and drugs. Crucially, Cardioids showed different chamber-level phenotypes reminiscent of cardiomyopathies. Additionally, Cardioids showed drug-induced structural and functional toxicity when exposed to oncological drugs or other cardioactive substances stimulating different pathways and targeting a variety of ion channels. Overall, we expect this platform and data to highlight the utility and biological relevance of using iPSC-derived 3D Cardioids as a promising new model for CVD drug discovery and toxicity screening while also showcasing the importance of quality-controlled, reproducible production and analysis in a high-throughput manner.

Keywords: 3D cardiac chamber organoids, automated screening platform, phenotypic screening

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HIPSC MODEL REVEALS M1 MACROPHAGES CAUSE ATRIAL ARRHYTHMIA CORRELATED TO ELECTROPHYSIOLOGICAL REMODELLING OF ATRIAL CARDIOMYOCYTES IN 2D AND 3D ENGINEERED HEART TISSUE

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Atrial fibrillation (AF) is the most common sustained arrhythmia with an estimated prevalence of 1.5–2%. Current treatments lack effectiveness and do not prevent recurrence. Inflammation is reported in AF patients, however, a causal connection remains elusive. In this study, we investigated the arrhythmic effects of pro-inflammatory macrophages (M1) on human induced pluripotent stem cell (hiPSC)-derived atrial cardiomyocytes (aCM), to better understand their role in AF. Macrophages are known to play a role in cardiac electrophysiology, but their role in arrhythmia is unclear. Three healthy hiPSC lines were differentiated to aCM and M1. Multi-electrode array recordings of isogenic, 2D cocultures of M1 and aCM showed a significant increase in beat rate irregularity compared to control conditions ($P < 0.001$). Furthermore, spike amplitude of aCM and M1 cocultures was significantly decreased ($P < 0.001$). Arrhythmias occurred only after activation of macrophages in the coculture. Addition of anti-inflammatory glucocorticoids significantly reduced beat irregularity in aCM and M1 cocultures compared to vehicle (1.9-fold decrease for Hydrocortisone, $P < 0.005$) further supporting macrophage mediated inflammation to be the cause of the arrhythmia. RNA sequencing of aCM and M1 cocultures revealed aberrant expression of arrhythmia associated cardiac sodium and atrial potassium ion channels (SCN5A, KCNA5). Normal expression was restored through hydrocortisone addition. In addition, inflammation-induced arrhythmia was observed in 3D engineered heart tissues containing hiPSC derived aCM, M1 and cardiac fibroblasts. Tissues showed a significant increase in beating irregularity ($P < 0.005$) compared to controls and had a significant, median reduction (>30%) of contraction amplitude ($P < 0.05$). Our findings suggest a causal relationship between M1 and the occurrence of atrial arrhythmia. The reduction of arrhythmia using glucocorticoids correlates to clinical observations, that show their use being linked to reduced post operative AF burden. These results strongly support the relevance of the proposed hiPSC coculture model and elucidate a new potential AF mechanism.

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Keywords: Atrial fibrillation, Inflammation, 3D Tissues

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ENGINEERED CRISPR-ACTIVATION HUMAN STEM CELL LINES UNDERGO SILENCING DURING CARDIOMYOCYTE AND ENDOTHELIAL DIFFERENTIATION

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Human pluripotent stem cells serve as opportune models to study the regulatory factors and pathways underpinning development/differentiation and the functional states of specialized cell types. More importantly, stem cells and their derivatives can be perturbed using gain- or loss-of-function approaches to examine gene function during these processes. CRISPR activation (CRISPRa) is advantageous for activating transcription of endogenous genomic loci by using a nuclease-dead Cas9 (dCas9) to recruit transcriptional activators to targeted sites. Here we sought to study gene function by applying the CRISPRa system to induced pluripotent stem cells (iPSC) and derivatives such as cardiomyocytes (iPSC-CMs) and endothelial cells (iPSC-Endos). We first generated a constitutively active CRISPRa stem cell line, using dCas9 fused to VP64, p65, and Rta (dCas9-VPR) targeted to the AAVS1 safe harbor locus and driven by the CAG promoter. These stem cell lines expressed dCas9-VPR transcript and upregulated both a reporter assay and multiple endogenous target genes (up to ~100s-fold). However, upon differentiation, this dCas9-VPR activity was lost in both iPSC-CMs and iPSC-Endos. To test whether silencing was locus-dependent, we targeted additional safe harbor sites (hROSA and CLYBL) and again observed strong activity in pluripotency and silencing during differentiation. Loss of transgene activity resulted from loss of transcription, which in turn correlated with gain of promoter CpG DNA methylation levels. Next, we tested whether silencing was promoter-specific by introducing muscle-specific promoters, but again, there was no dCas9-VPR activity in iPSC-CMs. Finally, we tested whether silencing was dependent on the dCas9-VPR cassette by exchanging it for mCherry. This resulted in iPSC lines that retained fluoroprotein expression during differentiation. These findings demonstrate that the dCas9-VPR cassette induces silencing of multiple promoters in multiple safe harbors during iPSC differentiation to CM and Endo lineages. This highlights a need to design and test new genome engineering strategies, with considerations for gene(s) being targeted, promoter activity, and safe harbor site targeting, for applications in stem cells and differentiated cell types.

Keywords: genome engineering, CRISPR activation, transgene expression

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LENVATINIB INDUCED INHIBITION OF THE VEGF RECEPTOR INCITES CYTOTOXICITY TO CARDIAC ENDOTHELIAL CELLS

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Human induced pluripotent stem cells (hiPSCs) enable high-throughput, patient-specific, pre-clinical evaluation of cancer drug cardiotoxicity. Tyrosine-kinase inhibitors (TKIs) have revolutionized the field of precision oncology, but despite their clinical efficacy, small molecule TKIs often have off-target effects, due in part to the numerous signaling pathways they inhibit. Lenvatinib is a small molecule TKI that inhibits the vascular endothelial growth factor (VEGF) pathway and was approved for treatment of differentiated thyroid cancer (DTC), renal cell carcinoma (RCC), and hepatocellular carcinoma (HCC) in 2015. As Lenvatinib has been shown to induce high rates of hypertension in patients, it is suspected that the drug is causing damage to vascular endothelial cells, which line the blood vessels of the cardiovascular system and highly express the signaling VEGF pathway . Patients taking Lenvatinib have also exhibited drug-induced QT interval prolongation, leading to cardiac rhythm disturbances. Thus, we surmise that Lenvatinib may also alter cardiomyocyte electrophysiology. To determine how Lenvatinib affects the heart and cardiovascular system, drug dose response/time course cytotoxicity and functionality experiments were conducted in 2D hiPSC-derived cardiomyocytes (CMs), endothelial cells (ECs), and advanced multi-lineage 3D models (cardiac spheroids, microfluidic organ-chips). Following treatment with Lenvatinib, hiPSC-CMs exhibited minimal cytotoxicity, but calcium imaging and multielectrode arrays revealed contractile dysfunction. Lenvatinib treatment on hiPSC-ECs caused cytotoxicity in 2D and 3D model systems. These studies demonstrate the utility of 2D and 3D hiPSC-derived cardiovascular model systems in unveiling the mechanisms of drug cardiotoxicity.

Keywords: cardiotoxicity, TKI, cancer

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TRANSCRIPTOMIC ANALYSIS OF HUMAN ENGINEERED HEART TISSUES CREATED BY USING CARDIAC MYOCYTES DIFFERENTIATED FROM INDUCED PLURIPOTENT STEM CELLS CULTURED IN 5% VERSUS 20% OXYGEN

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Human induced pluripotent stem cell-derived cardiac myocytes (iPSC-CMs) in 2D culture exhibit immature structural and functional phenotypes with fetal-like characteristics that remain a key barrier for their utility in generation of physiologically relevant adult disease models. 3D engineered heart tissues (EHTs), that better recapitulate adult cardiac tissues in structure and function, can be generated from iPSC-CMs. Robustness and reproducibility of EHTs can be impacted by the quality of cells used to construct them. We hypothesized that 5% O2 condition and the substrate used to maintain iPSC cultures and support CM differentiation (recapitulating early embryonic mesodermal induction and cardiac cell specification) enhance EHT maturation. We generated atrial-like EHT models using patient-specific iPSCs by reprogramming peripheral blood mononuclear cells of patients with atrial fibrillation. EHTs were generated by combining iPSC-CMs in a fibrin-based hydrogel suspended between flexible posts. We evaluated differentially expressed genes of EHTs constructed from iPSC-CMs that were grown in 5% vs. 20% O2 conditions, using different matrices (Matrigel, Geltrex). Successful EHT generation was independent of O2 level or substrate used for maintaining iPSCs and CM differentiation. EHTs maintained spontaneous beating. Electron microscope studies showed that sarcomeric structure of EHTs is more similar to adult atrial tissues than that of CM monolayers. Transcriptomic analysis indicated significant upregulation of genes and pathways associated with sarcomere, ion channels, calcium handling, energy production, and fatty acid metabolism in 5% O2 compared to 20% O2. EHTs generated from iPSC-CMs differentiated from iPSCs grown in 5% O2 (vs. 20%) culture exhibited more mature gene expression and greater resilience (with upregulation of DNA break repair genes and pathways). Optical mapping of voltage, Ca2+ cycling, and contractility are being used to evaluate functional maturity of EHTs. EHTs respond to mechanical cues that promote structural, metabolic, and contractile maturation. EHTs can be used as physiologically relevant preclinical models to understand pathophysiological mechanisms of cardiac diseases and to evaluate pharmacological agents tailored to the needs of individual patients.

Funding Source: This work was supported by grants from the National Institutes of Health (P01 HL158502) and the American Heart Association (18SFRN34170442).

Keywords: Human Induced Pluripotent Stem Cells, Cardiac Myocyte Differentiation, Engineered Heart Tissue Maturation

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INDUCING LUNG DIFFERENTIATION IN HUMAN HEART-FORMING ORGANOIDS TO FORM COMPLEX LUNG-HFOS

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Heart-forming organoids (HFOs) derived from human pluripotent stem cells (hPSCs) represent a complex, highly structured in vitro model for heart, foregut and vasculature development. Specifically, HFOs are composed of a myocardial layer lined by endocardial-like cells and surrounded by septum-transversum-like anlagen; they further contain spatially and molecularly distinct anterior versus posterior foregut endoderm (AFE versus PFE) tissues and a vascular network. The architecture of HFOs closely resembles aspects of early native heart anlagen prior to heart tube formation, which is known to require an interplay with foregut endoderm development. In the embryo, the AFE gives rise to different organs such as the lung, esophagus and thymus. In HFOs, the

AFE is located in the inner core and represents immature tissue, which expresses markers for AFE-derived organs on the RNA but not the protein level, as revealed by single-cell RNA sequencing versus immunofluorescence staining. To further advance the current HFO model towards embryo-like multi-tissue complexity, we here combine our HFO differentiation protocol with directed lung differentiation to induce lung epithelium formation. We show via flow cytometry and immunofluorescence staining that cells expressing the lung progenitor marker NKX2.1 form in the inner core, specifically within the epithelium lining the endodermal cavities. Notably, the overall 3D structure and established pattern of HFOs remain intact, despite the increase in tissue complexity. Thus, the first step towards a combined, self-organized heart-lung-vasculature model has been achieved, opening new perspectives for investigating human diseases in vitro, as well as advanced teratogenicity assessment and drug discovery approaches to replace animal experiments efficiently.

Keywords: Multi-tissue organoid, Heart development, Lung development

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ISOGENIC IPSC-BASED 3D VESSEL-ON-CHIP MODEL OF CADASIL DISEASE REVEALS VASCULAR SMOOTH MUSCLE CELL PHENOTYPIC SWITCHING UPON HETERO-CELLULAR INTERACTION

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CADASIL is a hereditary small vessel disease caused by NOTCH3 mutations leading to an accumulation and deposition of NOTCH3 protein around vascular smooth muscle cells (VSMCs). The major pathogenic feature of CADASIL is the progressive degeneration of VSMCs of small and middle-sized cerebral arteries leading to vascular dysfunction. Here, we established three CADASIL and isogenic gene-corrected lines from two CADASIL patients. By using a set of standard 2D assays, we observed comparable levels of NOTCH3 and contractile proteins such as SM22 between the VSMCs derived from CADASIL and isogenic iPSCs. In addition, CADASIL iPSC-VSMCs showed reduced contraction responses upon vasoactive stimulation when compared to isogenic iPSC-VSMCs while intracellular Ca2+ release dynamics where comparable. As endothelial cell (EC) expression of NOTCH3 ligands, such as Jagged-1, is required for activation of NOTCH3 in VSMCs, we recapitulated EC-VSMC interactions by using an iPSC-based engineered 3D vessel-on-chip (VoC) model. In this system, CADASIL iPSC-VSMCs showed an elongated morphology and higher proliferative behavior when compared to isogenic iPSC-VSMCs while microvascular structure was comparable between the two conditions. Immunofluorescent analysis revealed increased levels of NOTCH3 and SM22 in CADASIL iPSC-VSMCs. Importantly, we confirmed that CADASIL iPSC-VSMCs present slower intracellular Ca2+ kinetics upon vasoactive stimulation in the VoC. Finally, CADASIL iPSC-VSMCs morphological and phenotypical differences manifested in the VoC were rescued upon inhibition of NOTCH3 cleavage and activation with the y-secretase inhibitor DAPT. By enabling EC-VSMC interaction in the 3D VoC model, we revealed that CADASIL iPSC-VSMCs result in profound phenotypical changes that were not evident in conventional 2D assays. This study highlights the significance of patient iPSC-based VoC for modeling CADASIL and developing therapeutic strategies.

Funding Source: Netherlands Organisation for Health Research and Development (ZonMw); the Million Dollar Bike Ride Grant Program; the Novo Nordisk Foundation Center for Stem Cell Medicine; The Netherlands Organ-on-Chip Initiative (NOCI).

Keywords: disease model, vascular disease, CADASIL

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MODELING MYOCARDIAL INFARCTION USING MULTI-CELLULAR CARDIAC ORGANOIDS FROM HIPSCS

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Myocardial infarction (MI) is an irreversible myocardial injury resulting in high morbidity and mortality. Many cell-based cardiac in vitro models have been reported as a complementary approach to preclinical research, however, most approaches are not able to mimic human MI pathology. In this study, we generated a self-organizing cardiac organoid (COs) from human induced pluripotent stem cells (hiPSCs) with cardiomyocytes, fibroblasts, and endothelial cells (multi-cellular COs) that recapitulates cellular compositions of human heart. To model MI, the multi-cellular COs were cultured in the presence of cobalt chloride (CoCl2), a hypoxia mimetic agent, and we confirmed the increased expression of MI markers at the mRNA and protein levels. Furthermore, the cardiac damage by the treatment of CoCl2 resulted in the defective calcium influx and contractility of the COs with cardiac fibrosis. These results have important implications for the application of in vivo-like 3D heart and disease modeling, and these MI-CO models might provide a promising alternative to an animal experimental model for studying cardiac disease, as well as a drug screening system for finding therapeutic targets.

Keywords: Cardiac organoid, Human induced pluripotent stem cells, Disease modeling

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HUMAN MULTILINEAGE PRO-EPICARDIUM/ FOREGUT ORGANOIDS SUPPORT THE DEVELOPMENT OF AN EPICARDIUM/ MYOCARDIUM ORGANOID

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Recapitulation of embryonic development through the generation of human pluripotent stem cell-derived organoids has succeeded for several tissues, such as for the brain and the intestine However, the recreation of early stages of heart development in vitro has been particularly challenging. One important structure crucial for embryonic development of the heart is the pro-epicardial (PE) organ, a transient structure that develops next to septum transversum mesenchyme (STM) and liver bud. This structure comprises the epicardial progenitors that migrate towards the developing heart to form the outer epicardial layer that covers the myocardium. Recapitulation of PE development in vitro has not been reported so far. Therefore, the motivation of the present work was to recreate in vitro the embryonic development of the human PE through modulation of hPSC-derived mesendoderm progenitors in a in vivo-like 3D environment. As result, we achieved a self-organized multilineage hPSC-derived organoid that recreates PE/STM populations and posterior foregut/hepatic primordium development, with three clearly identifiable main regions: 1) an outer layer of WT1+/LHX2-/+low cells identified as PE-like cells, 2) an epithelial-like structure comprising CDX2/AFP positive endoderm gut tube cells and AFP/HNF4 positive hepatoblast-like cells, and 3) an intermediate population between the outer layer and the epithelium that is WT1+low/LHX2+, identified as STM-like cells. By co-culturing these organoids with cardiomyocytes in a 3D environment, we generated a epicardium-myocardium heart organoid model that self-organizes in a structure comprising a well-defined WT1+ epicardial-like layer that completely surrounds the myocardium-like tissue. These heart organoids recapitulate the impact of epicardial cells on promoting embryonic myocardium growth and cardiomyocyte structural and functional maturation. Therefore, the human heart organoids described herein open a clear path to advancing knowledge on how myocardium-epicardium interaction progresses during heart organogenesis in healthy or diseased settings, and represent a tool for embryonic heart developmental toxicity studies.

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Keywords: Human epicardium-myocardium heart organoid, Human pro-epicardium organoid, hPSC-derived multilineage organoid

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CELLULAR AND MOLECULAR MECHANISMS OF ALVEOLAR CELL FATE DIFFERENTIATION AND PATTERNING IN THE DEVELOPING HUMAN LUNG

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In the developing human lung, alveolar development requires highly organised, spatiotemporal interactions between subsets of differentiating progenitor cells of epithelial and mesenchymal lineages. Extensive studies have been performed to understand the underlying mechanisms which organise alveolar differentiation and cell patterning in the developing alveolar niche in human. However, it remains elusive. In this study, we have generated a multiomic cell atlas of human lung development that combines single-cell RNA and ATAC sequencing, high-throughput spatial transcriptomics, and single-cell imaging. The atlas identifies alveolar-fated epithelial progenitors in late-stage distal lung regions that can be modelled as self-organising organoids in vitro. We functionally validate that spatiotemporal cell-cell interactions between the alveolar-fated progenitors and surrounding populations of mesenchymal cells shapes spatial alveolar patterning in the developing alveolar niches. We also identify a Wnt-NKX2.1 axis controlling alveolar cell fate determination and differentiation and model fetal alveolar-type-2 cells during culture. Furthermore, we have found alveolar fate-promoting chromatin features that facilitates alveolar differentiation of human lung progenitor cells. Our single cell analysis in combination with lung organoid system revealed key aspects of human fetal lung stem cell biology, allowing mechanistic experiments to determine the cellular and molecular regulation of human alveolar development.

Keywords: human lung development, organoids, alveolar differentiation

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A MODEL OF PATIENT DERIVED AIRWAY EPITHELIAL BASAL CELLS REVEALS THE ROLE OF STAT3 SIGNALING IN AGE-RELATED RESPIRATORY SYNCYTIAL VIRUS INFECTION

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Respiratory syncytial virus (RSV) infection is a major cause of severe respiratory illness in infants, but usually causes only mild symptoms in adults. Apart from the immature immune system that may contribute to age-related RSV infection in infants, the role of airway epithelial cells, the primary cell of contact for RSV during infection, in age-related severity of RSV infection is poorly understood and warrants investigation. Here, we first derived airway basal stem cells (BSCs) from tracheal aspirate (TA) samples of neonates and adults. We compared the transcriptome of BSCs by age and found that age imprints BSCs manifested by differences in gene expression. In addition, we generated well-differentiated airway epithelial cells using air-liquid interface (ALI) cultures of neonatal and adult BSCs. Employing ALI cultures as a model for RSV infection, we further found that RSV infection induces significant epithelium damage and elicits more robust viral replication and chemokine/cytokine expression in neonatal ALI culture than adult ALI culture. We further found that STAT3 signaling is at a higher level in adult airway epithelium than neonatal airway epithelium at baseline. Mechanistically, STAT3 signaling blocks RSV-induced apoptosis to limit infection in human airway epithelial cells. Taken together, in this study we have successfully established a human BSCs-derived epithelial model for RSV research. Our model reproduces age-related cytotoxicity of RSV infection in humans and demonstrates the critical role of airway epithelial cell in RSV severity.

Funding Source: This work was supported by a NIH R21(AI 156597) grant to Paul H. Lerou.

Keywords: Basal stem cell, Airway epithelial cell, Respiratory syncytial virus

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DECIPHERING HUMAN LUNG SQUAMOUS CELL CARCINOMA TUMORIGENESIS IN VITRO

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Tumorigenesis is a complex process that involves the acquisition of genetic mutations, resulting in the dysregulation of epithelial cell proliferation and differentiation. Lung cancer is the leading cause of cancer deaths worldwide, with lung squamous cell carcinoma (LuSSC) being the second most frequent. However, the molecular mechanisms regulating the stepwise progression of LuSSC tumorigenesis remain to be elucidated due in part to the difficulties in repeatedly sampling human early cancer lesions and the lack of useful in-vitro/vivo platforms that fully recapitulate human LuSCC tumorigenesis. To overcome these issues, we sought to recapitulate the stepwise process of LuSCC tumorigenesis in vitro by introducing critical gene mutations into human airway basal cells, the cells of the origin of LuSCC. First, we analyzed the gene mutational profiles of low-grade premalignant lesions, carcinoma in situ (CIS), and invasive carcinoma to identify candidate driver genes of the major steps of LuSCC tumorigenesis using human patient samples. Gene mutation analyses revealed that TP53 mutations are the most frequently detected gene mutations at the early stages of LuSCC. Therefore, we knocked out TP53 in iPSC-derived human basal cells (iBCs) in vitro by CRISPR/Cas9 gene editing. Notably, TP53 KO iBCs displayed: 1) higher basal cell proliferation and yield in 3D culture conditions; and 2) lineage biases in basal cell differentiation towards secretory cell fates in 2D air-liquid interface cultures at the expense of basal cell fates, as indicated by reduced expression of basal cell markers such as TP63. However, given that LuSCC highly expresses basal cell markers and not secretory cell markers, these results suggest that TP53 mutation alone is insufficient to initiate the full LuSCC phenotype in basal cells, and additional gene mutations present in early lung cancer are likely required for LuSCC oncogenesis.

Keywords: carcinogenesis, stem cell-based disease modeling, iPS cell-derived airway basal cells

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BIOLOGICALLY RELEVANT SUPPORT FOR PRIMARY HUMAN KERATINOCYTES, MELANOCYTES AND FIBROBLASTS, UNDER XENOFREE- AND CHEMICALLY DEFINED CONDITIONS WITH BIOLAMININ-521

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Autologous skin cultures are one of the first in vitro expanded tissue types used in cell therapies. The pioneering research was published by J. G. Rheinwald and H. Green, in 1975. Their method of expanding human keratinocytes is based on murine feeder cells and bovine serum enriched medium, a method revolutionizing the treatment of severe wounds at the time and still much in use even Today. However, the use of animal components within cell-based therapies is one of the major factors restraining the treatment of wounds to only the severest cases. Protocols for pluripotent-based cell therapies has in the past decade transferred from animal-components to xenofree-and defined settings, one component in this transfer has been the development of recombinant laminin proteins as coating substrate. Laminins are an extracellular matrix protein family that is highly expressed within the basement membrane (BM) in all epithelial tissues, including the skin. The BM is a vital part of the stem cell niche supporting the basal keratinocytes in tissue homeostasis and in wound healing. The BM within the skin contains multiple laminin isoforms, where laminin-332, -331, laminin-511, -521, laminin-421 and -411 are the major components. Although the distinct role of the different laminin isoforms is still largely unknown, the essential role in maintaining healthy skin is demonstrated in Epidermolysis bullosa, a severe genetic skin disease caused by a mutation in laminin 332, leading to fragile skin and chronic wounds. Here we demonstrate that the use of recombinantly produced laminins, Biolaminins, as cell culture substrate can provide biologically relevant support to the major cell types of the skin, allowing for complete xenofree and defined expansion of primary adult keratinocytes, melanocytes, and fibroblasts. We demonstrate that the effect is laminin isoform dependent and provide superior support to the proliferative cells within the skin, also from aged donors, compared to current methods. For example, the use of Biolaminin-521 in expanding human primary keratinocytes resulted in 14 cumulative doublings compared to 8 with standard conditions after 30 days in cultures. In conclusion, we believe that this will allow for a new generation of safe- and efficient in vitro expansion of human keratinocytes, melanocytes, and fibroblasts.

Keywords: laminin, primary keratinocytes, wound

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LAMELLAR BODY FORMATION AND SURFACTANT SECRETION IN LRRK2 -/- DISTAL LUNG ORGANOIDS

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Neonatal respiratory distress syndrome (nRDS) occurs in infants born less than 28 gestational weeks, making it a leading cause of neonatal morbidity and mortality. It results from a gestational deficiency of surfactant, a molecule that is stored within lamellar bodies (LB) of alveolar type two (AT2) cells and reduces surface tension. The treatment for nRDS is intubation and exogenous surfactant therapy. Incidentally, recent studies in animal models have discovered that the inhibition of the leucine-rich repeat kinase 2 (LRRK2) gene increased the production and size of the surfactant-containing LBs. Studies in human model systems have not confirmed these findings. We propose to use LRRK2 knock out (KO) induced pluripotent stem cell (iPSC) derived distal lung organoids (DLOs) to elucidate the mechanism of LB development and surfactant secretion. LRRK2 functions in autophagy and vesicle trafficking and its impact on LB development may yield a new therapy for nRDS. The LRRK2 KO line was generated by deleting 5 bp at the G2019S site within the LRRK2 gene with CRISPR/Cas9 in iPSCs. We generated 3 different in vitro human model systems of wild type (wt) and LRRK2 KO iPSC derived DLOs including 1) 3D DLOs embedded in matrigel; 2) 3D DLOs dissociated into monolayers and 3) 3D DLOs dissociated onto trans wells in an air-liquid-interface (iALI) system. We evaluated the protein expression of alveolar type II markers including HTII-280 and pro-SPC along with the lamellar body marker, LAMP3. Nile red, a molecule that targets lamellar bodies, had higher expression in the 3D DLOs compared to the dissociated 3D DLO. Furthermore, flow cytometry analysis for HTII-280, showed that the 3D DLO dissociated iALI system had higher yields of AT2 cells compared to the 3D DLOs. Our future aims are to determine the size of the lamellar bodies in the wt and LRRK2 KO DLOs. Additionally, we will use ATP stimulation to determine the amount of surfactant secreted and elucidate the gene transcription profiles of lamellar body development in the LRRK2 KO DLOs. These findings will promote the understanding of lamellar body and surfactant development to potentially be translated into a therapeutic intervention for babies born premature.

Keywords: LRRK2, surfactant, nRDS

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IPSC-DERIVED ORGANOID-BASED MODEL OF INTESTINAL FILOVIRUS INFECTION

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Affected Ebola virus disease (EVD) patients lose copious amounts of fluids in a matter of days, rapidly deteriorating into hypovolemic shock and death. Similar intestinal manifestations were also reported for Marburg virus (MARV) disease, another filovirus. At present, available animal models insufficiently recapitulate the gastrointestinal symptoms of EVD patients. To fill this gap, we have established an induced pluripotent stem cell (iPSC)-derived human intestinal organoid (HIO) model that can be primed towards proximal (small intestine) or distal (colonic) intestinal lineages. Three-dimensional tissue-specific organoids recapitulate the heterogeneity, architecture, and cellular functions of the primary tissue, thus representing a powerful tool to study development and disease. The generation of a hiPSC CDX2-GFP reporter line highlights the role of CDX2 as a marker for the emergence of hindgut intestinal progenitors during our differentiation protocol. This platform can facilitate the study of late-stage EVD gastrointestinal symptoms, including diarrhea. We employed the generation of these organoids to study the effects of filovirus infection on intestinal epithelial integrity. Successful robust EBOV and MARV infections of iPSC-derived HIOs, affecting mostly epithelial CDX2+ enterocytes was achieved. The infected cells showed signs of cell damage. Transcriptomics analysis indicated the modulation of cell junction pathways and a set of ion transporters known to play a role in the induction of diarrhea. Taken together, these data suggest that EBOV and MARV compromise barrier integrity of the intestinal epithelium and cause abnormal ion flux as the basis for gastrointestinal dysfunction and diarrhea.

Keywords: iPSC-dervied HIOs, filoviruses, intestinal

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MORPHOLOGICAL AND PROTEIN EXPRESSION ALTERATIONS IN APICAL MICROVILLI OF RETINAL PIGMENT EPITHELIUM DERIVED FROM OCA1A PATIENT IPS CELLS

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Oculocutaneous albinism type 1A (OCA1A) is caused due to mutations in TYROSINASE gene (TYR) and results in pigmentation defects of the skin, hair, and eyes. OCA1A is associated with abnormal development of the fovea, a retina structure important for high-acuity and central vision. It is still unknown how the lack of pigmentation in retinal pigment epithelium (RPE) and choroid affects the neighboring neural retina during development. RPE cells exhibit apical-basal polarity with microvilli occupying the apical landscape, and mediating interactions with the photoreceptors. Our study investigates the morphological and molecular properties of the apical microvilli of OCA1A patient derived RPE monolayer tissue via induced pluripotent stem cell (iPSC) technology. Three unaffected control (CTRL) and two OCA1A iPSC lines used in the study have been reported earlier (George et al., 2022). Directed differentiation of iPSCs towards iRPE was performed as described earlier (Sharma et al., 2022). Morphology of apical microvilli was studied using electron microscopy (SEM & TEM) and immunofluorescence staining. Relative protein levels were studied using Western blotting (WB). RPE derived from OCA1A patients and TYR knockout iPSC exhibited pigmentation defects. TEM revealed reduced presence of apical microvilli in some areas of OCA1A-iRPE but not throughout the monolayer, whereas scanning electron microscopy did not reveal any observable defects as compared to CTRL-iRPE. Immunofluorescence staining for EZRIN protein and F-Actin staining, which plays an important role in formation of apical processes, was significantly reduced in all OCA1A-iRPE as compared to CTRL-iRPE. However, protein levels for SLCO1A2, which is localized at the apical microvilli and plays an important role in cellular uptake of all-trans-retinol in RPE, was significantly increased in OCA1A-iRPE, as observed by immuno-fluorescence staining and WB. Our data show that RPE from OCA1A, a form of albinism, exhibits altered morphological organization and protein expression pattern compared to pigmented RPE. How RPE apical microvilli interact with the developing neural retina postnatally is poorly understood, and whether these microvilli processes could be involved in shaping the neural retina is worth investigating.

Funding Source: NIH Intramural

Keywords: Oculocutaneous Albinism, Retinal pigment epithelium, Induced Pluripotent Stem Cells

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2D and 3D GASTRULOID MODELS FOR INVESTIGATING MAMMALIAN ENDODERM DEVELOPMENT

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Gastrulation is a dynamic developmental process where pluripotent cells undergo specification into the three germ layers - ectoderm, mesoderm, and endoderm - that will anticipate a future body organization. The endoderm will give rise to respiratory, and digestive tract and their respective organs, such as the lungs liver and pancreas. It initially forms a sheet of epithelial cells that subsequently transforms into a (gut) tube, comprising three distinct regions - foregut, midgut, and hindgut – along its Anterior-Posterior axis. Endoderm development encompasses a series of sequential of steps; from the specification of cells with an endoderm identity, the patterning of this progenitor tissue, and the region-specific emergence of nascent endodermal organs. To investigate endoderm development, we are using mouse and human pluripotent stem cell-based models that upon exposure to specific growth factors differentiate to form the three germ layers: (1) adherent cell-based 2D gastruloids, and (2) non-adherent cell aggregate-based 3D gastruloids. 2D gastruloids are scalable and highly reproducible, and they amenable to quantitative image-based analyses. This model is being used to quantify the impact of perturbations in gene regulatory networks on endoderm specification. In parallel, we are optimizing protocols to generate 3D gastruloids containing a gut tube-like structure comprised of endoderm cells. This model is being used to determine the factors driving endoderm tissue patterning under normal conditions and with perturbations. Furthermore, human gastruloids are being compared to mouse gastruloids (cross-species comparison), and gastruloids are being compared to correspondingly-staged mouse embryos (in vitro vs. in vivo comparison).

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Keywords: Gastrulation, Gastruloids, Endoderm

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REUTERI AND ITS METABOLITES PROMOTE INTESTINAL MATURATION AND REGENERATION IN HUMAN INTESTINAL ORGANOIDS AND MICE

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Little is known about the regulatory capacity of the microbiota in early gut development. In addition, the intestinal maturation and therapeutic efficacy of microbial metabolites are not well understood. We investigated various intestinal models that respond to gut microbial metabolites based on human pluripotent stem cell-derived human intestinal organoids (hIOs): physiologically relevant in vitro fetal-like intestine, intestinal stem cells, and intestinal disease models. We found that a newly isolated Limosilactobacillus reuteri strain DS0384 accelerated maturation of the fetal intestine using 3D hIO with immature fetal characteristics. Comparative metabolomic profiling analysis revealed that the secreted metabolite N-carbamyl glutamic acid (NCG) is involved in the beneficial effect of DS0384 cell-free supernatants on the intestinal maturation of hIOs. Experiments in an intestinal stem cell spheroid model and hIO-based intestinal inflamed model revealed that the cell-free supernatant from DS0384 comprising NCG promoted intestinal stem cell proliferation and was important for intestinal protection against cytokine-induced intestinal epithelial injury. Seven-day oral administration of DS0384 and cell-free supernatant accelerated intestinal development in neonatal mice. Moreover, NCG exerted protective and therapeutic effects on experimental colitis in mice. The metabolite NCG showed intestinal epithelial wound healing in a human intestinal epithelial cells (hIECs) model. These results suggest that DS0384 and its metabolite NCG are valuable agents for probiotic applications and treatment for disorders of early gut development and for preventing intestinal barrier dysfunction.

Funding Source: This research was supported by the Korean Fund for Regenerative Medicine(KFRM) grant funded by the Korea government(the Ministry of Science and ICT, the Ministry of Health & Welfare, 21A0404L1).

Keywords: human intestinal organoid, Limosilactobacillus reuteri, N-carbamyl glutamic acid

TOPIC: GERMLINE AND EARLY EMBRYO

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EVALUATING HUMAN BLASTOIDS TO MODEL EARLY HUMAN DEVELOPMENT

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The generation of in vitro human blastoids that recapitulate the human blastocyst is a transforming achievement that would enrich our understanding of early human development. While a multitude of protocols have been established for generating the blastoids, the authenticity of these blastoids in modelling human blastocysts remains unclear. In this study, we established a cell-identity reference map of human blastocysts at peri-implantation to early post-implantation stage through integration and characterisation of single-cell transcriptomics data generated from human blastocysts grown ex vivo. This molecular cell atlas can serve as a reference for benchmarking the fidelity of in vitro blastoids for modelling the natural blastocyst. Using this reference map, we have assessed the compendium of cell types and their transcriptomic signatures of blastoids generated from pluripotent stem cells and induced pluripotent stem cells. In addition, the molecular roadmap of blastocyst development gleaned from the cell atlas provides useful guidance to future generation of high-quality human blastoids that best recapitulate the in vivo counterparts.

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Keywords: blastoid fidelity, in vitro models, computational benchmarking

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MODELING DEFECTS IN EXTRAVILLOUS TROPHOBLAST DIFFERENTIATION IN THE SETTING OF TRISOMY 21

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Trisomy 21 (T21) pregnancies are known to have a higher risk of miscarriage due to abnormal trophoblast differentiation, the epithelial cells of the placenta. Within the placenta, trophoblast stem cells (TSC) differentiate to form either multinucleated, hormone-producing syncytiotrophoblasts (STB), or invasive extravillous trophoblast (EVT), cells involved in vascular remodeling and establishing blood flow to the placenta. Only limited studies of T21-affected EVT have been done, due to a lack of an optimal in vitro EVT model. Our lab has recently established hESC-derived TSC, with a significantly improved EVT differentiation protocol. Therefore, in this study, we utilized this newly-established protocol to investigate T21-affected EVT. Three T21-affected and two disomy hESC lines were converted to TSC, following a 4-day treatment with bone morphogenetic protein 4 (BMP4) and WNT inhibitor IWP2, and then transitioning the cells into TSC media. TSC establishment was confirmed by flow cytometry to ensure uniform surface expression of EGFR. Subsequently, EVT differentiation of the hESC-derived TSC was performed, using a recently-optimized EVT differentiation protocol. EVT differentiation was assessed by morphology, as well as flow cytometric analysis for surface expression of HLA-G (an EVT marker). When EVT differentiation was initiated, the morphology of T21-affected cells was significantly different: T21-affected EVT showed an ovoid morphology with slower cell growth, and did not display the elongated morphology that is characteristic of normal EVT. T21-affected EVT also showed an 8.7 fold decrease in surface expression of HLA-G by flow cytometry, when compared to disomy-hESC-derived EVT. Our results show reduced EVT differentiation of T21-hESC derived TSCs, based on both morphology and marker expression. This is the first report showing an abnormal EVT phenotype in T21-affected hESC-derived trophoblast. Additional evaluation of gene expression by qPCR, cell proliferation, and function (invasive capacity and secretome) are required to further assess EVT formation in the setting of T21.

Funding Source: California Institute of Regenerative Medicine

Keywords: Trophoblast differentiation, Trisomy 21, Placenta

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DERIVATION OF INDUCED TROPHOBLAST STEM CELLS FROM UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS FROM PATIENTS WITH PREECLAMPSIA

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Studying pregnancy complications requires renewable cell model systems. Our lab recently developed a disease-in-a-dish model system to study a hypertensive pregnancy disorder called preeclampsia (PE) using patient umbilical cord mesenchymal stem cells (UC-MSC)-derived induced pluripotent stem cells (iPSC). PE is characterized by abnormal formation or function of placental cells, called trophoblast. We converted the MSC-derived iPSCs into human trophoblast stem cells (hTSC) and found that PE-affected iPSC-TSC showed abnormal trophoblast differentiation and response to environmental stressors, when compared to healthy iPSC-TSC. A more recent publication demonstrated that a transient fibroblast intermediate generated during reprogramming to iPSC can directly generate induced trophoblast stem cells (iTSC). However, there is no assessment of the ability of these iTSC to model disease, such as PE. Therefore, we propose to derive iTSC from the same healthy and PE-affected UC-MSCs, comparing these to previously-derived iPSC-TSC, in order to perform in-depth comparison of the two methods. We used UC-MSCs derived from placentas of PE and non-PE patients, and

reprogrammed them using standard Yamanaka factors (OSKM). On day 8 post-transduction, reprogramming intermediates were transitioned onto mouse embryonic fibroblast (MEF) feeder layers with hTSC media, and cultured under 5% oxygen, following the recently published protocol. Cell characterization was performed by morphological assessment and by flow cytometry to measure surface expression of hTSC markers. Soon after the transition into hTSC media, we noted significant morphological changes of reprogramming intermediates, with cuboidal epithelial-like morphology. These cells further formed distinct hTSC-like colonies after several passages. Flow cytometric analysis showed increased expression of an early trophoblast marker, ENPEP, by passage 10. Our characterizations suggest that iTSC resembles hTSC and could be useful in modeling disease. Our next step is to determine whether the iTSC derived from PE-patients have a similarly-abnormal phenotype when compared to both iTSC derived from healthy patients, and the previously-established iPSC-TSC, in order to identify the best in vitro model system to study PE-affected trophoblast.

Funding Source: California Institute for Regenerative Medicine

Keywords: Trophoblasts, Reprogramming, Preeclampsia

TOPIC: HEMATOPOIETIC, IMMUNE, AND ENDOTHELIAL

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BIOENGINEERED IPSC-DERIVED MODELS OF HUMAN BONE MARROW FOR STUDIES OF INJURY AND DISEASE

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The human bone marrow (BM) is one of the most complex and critical tissues in the adult, functioning as the site for blood and immune cell production in homeostasis, injury, and disease. The marrow acts as an incredibly diverse stem cell niche, containing stromal and blood cells that help support the maintenance and differentiation capacity of hematopoietic stem and progenitor cells (HSPCs). The cell-cell and cell-matrix interactions within the niche help trigger blood cell production in response to injury, and to harbor downstream changes that may persist in the hematopoietic system during disease, such as in cancer metastasis or leukemias. As the development of human organs-on-a-chip (OoC) platforms has emerged over the past decade, there has been an increased relevance of using human BM models to study human- and patient-specific immune interactions in vitro. Here we report the development of a patient-specific bioengineered model of the BM, derived entirely from induced pluripotent stem cells (iPSCs) and its use in studies of radiation toxicity, blood malignancies, and systemic interactions. The engineered model of human BM (eBM) is derived from iPSC-derived osteoblasts, mesenchymal stem/stromal cells, and endothelial cells within a decellularized bone scaffold. This model was developed to be modular, with ability to include either healthy cord blood-, iPSC-, or BM-derived HSPCs. We validated the model with histological staining, flow cytometry, colony forming assays, and single-cell RNA sequencing, demonstrating maintenance and differentiation of blood progenitors and progeny, and describe here applications in acute radiation injury, leukemic infiltration, and systemic, multi-tissue interactions. Notably, eBMs were able to maintain donor acute myeloid and B-/T-lymphoblastic leukemias better than in liquid cultures, as well as recapitulating drug responses seen clinically and in vivo, as compared to traditional liquid cultures. Further, we linked the eBM model to the cardiac tissue by vascular perfusion to demonstrate the recruitment of monocytes from eBM in response to acute cardiac injury in a systemic, multi-tissue platform. We propose that this novel model system can be used to study malignant transformation, systemic immune responses, and the development of personalized therapeutics.

Funding Source: Funding supported by NIH NRSA 1F31CA275733-01, NSF GRFP 1644869, NIH P41EB027062, NIH R01 CA249799, and Alex's Lemonade Stand.

Keywords: Organ-on-a-chip, Bone marrow, Tissue engineering

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AN ENGINEERED HUMAN IPSC-BLOOD VESSEL WITH ORGANIZED SPIRAL-ORIENTED SMOOTH MUSCLE LAYERS AS A NEW FUNCTIONAL MODEL FOR CARDIOVASCULAR DISEASES

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Cardiovascular diseases (CVD) account for about 1/3 of the death in the world and shorten healthy life expectancy. Despite significant progress in the field, curative treatments have yet to be developed directly targeting the dysfunctional phenotypes observed rather than treating secondary symptoms that CVD gives rise to such as hypertension and diabetes. Since models using mouse vascular tissue are unable to fully recapitulate human pathophysiology observed in the clinic, due to the differences in

gene expression from that of humans, human iPSC-derived tissue-engineered blood vessels (TEBV) will serve as a more robust model to elucidate the mechanisms leading to CVD. Although some TEBVs with vascular smooth muscle cells (VSMCs) and endothelial cells (ECs) have already been established in the field, these models have caveats in their ability to mimic the physiology of the human artery. For example, the contraction of these vessels remains weak due to the lack of circumferential alignment of VSMCs and the lack of flexibility in the scaffolds. These two characteristics are important to address because it has been well established that contraction and dilation play a crucial role in vascular diseases. Here we propose a novel rolling-up molding method to form a physiologically deformable TEBV from human iPSCs. First, a VSMC fiber was formed with a 3D-printed microchannel mold and metallic anchors. The iPSCs-derived VSMCs suspended in a collagen hydrogel spontaneously contracted about 95% in volume and aligned axially in 24 hours. This fiber is rolled up by a 3D-printed inclined mold to spontaneously form a spiral shape. Finally, rolled-up VSMCs were embedded into collagen hydrogel with perfusion connectors, and ECs were seeded inside to establish the TEBV. As a proof of concept, our TEBV was also challenged to undergo other physiologically-relevant conditions, including maintenance under pulsatile perfusion, treatment with U46619, a thromboxane A2 agonist, and normalization with contraction by KCl. The relative contraction ratio was 78.0 ± 5.1%, similar to previously reported ex vivo experiments. Taken together, we anticipate that our model will serve as a more physiologically-relevant model to investigate the mechanisms behind CVD and thus will lead to therapeutic discoveries.

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Keywords: tissue-engineered blood vessel, vascular smooth muscle, contraction

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IPSC-DERIVED MACROPHAGES AS A PREVENTATIVE TREATMENT FOR KNEE POSTTRAUMATIC OSTEOARTHRITIS

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Post-traumatic osteoarthritis (PTOA) is a degenerative cartilage disease that occurs in relatively young patients following injury like ACL tear, causes pain, disability, and economic burden. The iPSC-derived macrophages have the potential to prevent the inflammatory insult to the joint and PTOA induction. Non-surgical model of PTOA using a noninvasive ACL tear mimics the human condition as close as possible. Immunogenicity poses challenges for development of human stem cell therapies in animal models. To study the potential iPSC-derived treatments on PTOA, immunocompromised Nude and wild type Sprague Dawley (SD) rats were used to study immune reactivity to human iPSC-derived cells. Since the joint is considered immune privileged site, we hypothesized that SD rats would not have a greater immune reaction compared to Nude rats when human iPSC-derived cells were injected into the injured knee. Human iPSCs were differentiated to macrophages using an optimized protocol, characterized via flow cytometry, and confirmed functionality through phagocytic assay. SD and Nude rats went noninvasive ACL injury and were injected with human cells 2 weeks later. Serum was collected during the day of injury, injection day, day 3, 7 10 and 14 post injection. We observed no significant difference in IgM levels and CD8+ cells between Nude and SD rats, demonstrating no immune reaction in immunocompetent rats after human cell injection to the joint. To further characterize the PTOA model, SD rats underwent non-invasive ACL injury and were followed for 16 weeks. Biobehavioral tests of rats with knee injury demonstrated an increase in left and right stride length, a decrease in sway width and a significant increase in left paw angle and a trend of increasing right paw angle. Rats were sacrificed after 18, 20 or 25 weeks, the knee joints were processed for histology and used for OARSI score, showing development of knee OA as early as week 18 post injury. This confirmed the feasibility of noon-invasive ACL tear to model PTOA and a feasibility of human stem cell therapy development. Future steps of this study will include introducing

differentiated iPSC-derived macrophages into an ACL injured rat model to modify the inflammatory environment and hinder OA onset, progression, and associated pain.

Funding Source: I would like to acknowledge CIRM for funding my internship.

Keywords: PTOA, iPSC, macrophages

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DIFFERENTIAL RESPONSES OF THE NLRP3 INFLAMMASOME PATHWAY TO AMYLOID BETA AND ALPHA-SYNUCLEIN IN IPSC-DERIVED MICROGLIA

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Neuroinflammation in Alzheimer's disease (AD) and Parkinson's disease (PD) is primarily driven by the brain resident immune response system, i.e., microglia. Under pathological conditions microglia induce several inflammatory pathways including the NLRP3 inflammasome, which can be induced by aggregated proteins such as amyloid beta (a-beta) in AD and alpha-synuclein (alpha-syn) in PD. Targeting of aberrant NLRP3 activation is currently being pursued for therapeutic intervention. To better understand the mechanism of NLRP3 inflammasome activation in both AD and PD, we developed an in vitro stem-cell based model of microglia (iPSdMiG) that closely resembles native microglia. Microglia exclusively exhibit the canonical NLRP3 inflammasome pathway, which is based on a two-step process of priming via NF-kappa-B nuclear translocation and a subsequent activation step leading to the formation of ASC specks. These key targets of the NLRP3 pathway were assessed in response to a-beta or alpha-syn exposure. Our data suggest unique roles of a-beta and alpha-syn in the induction of the canonical NLRP3 pathway. Specifically, a-beta alone was not sufficient to induce priming via NF-kappa-B nuclear translocation, but lipopolysaccharide (LPS)-primed microglia that were subsequently activated by a-beta led to the formation of ASC specks. Conversely, alpha-syn induces priming via NF-kappa-B nuclear translocation but does not play a role in the following activation step. Therefore, alpha-syn primed microglia require a subsequent activation, for example by the microbial toxin nigericin, to induce the formation of ASC specks. Additionally, the quantity of ASC speck formation differed between the two stimuli. LPS-primed microglia that were further activated by a-beta exhibited ASC specks in an average of 5 % cells at 30 minutes post activation. However, alpha-syn primed microglia followed by nigericin activation lead to an induction of ASC specks in an average of 27 % of cells at 3 hours post activation. Our in vitro cellular model of human microglia with validated inflammasome pathway-based read-outs will provide mechanistic insights required for deciphering inflammasome dynamics in response to disease-relevant stimuli such as a-beta and alpha-syn, and thus support therapy development.

Keywords: iPS cell-derived microglia, NLRP3 inflammasome, aggregated proteins

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REVERSAL OF MALIGNANT ADAR1 SPLICING PROMOTES NORMAL HEMATOPOIETIC STEM CELL RETENTION AND PREVENTS LEUKEMIA STEM CELL PROPAGATION

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Acute myeloid leukemia (AML) is characterized by a clonal proliferation of malignant myeloid precursors that harbor a reduced capacity for differentiation. Adenosine deaminase acting on RNA 1 (ADAR1) is an RNA editing enzyme that catalyzes the conversion of adenosine bases to inosine, which can alter protein function. ADAR1 has been shown to drive cancer stem cell (CSC) generation and therapeutic resistance in multiple malignancies. Treatment with 17S-FD-895 (Rebecsinib), a selective small molecule splicing modulator that targets the splicing factor 3b subunit 1 (SF3B1) component of the spliceosome, has been shown to reverse malignant splice isoform switching, inhibit activation of the interferon-inducible ADAR1p150 splice isoform, and significantly reduce leukemia stem cell (LSC) maintenance in vitro and in preclinical patient-derived AML mouse models. However, the effectiveness of Rebecsinib versus current therapies for the treatment of AML and the effects of Rebecsinib treatment on normal bone marrow stem cells has not yet been elucidated. Here, we established humanized mouse models using CD34+ stem cells isolated from the peripheral blood of adult secondary AML (sAML) patients or bone marrow of normal aged donors (63-79 years old). Strikingly, sAML-engrafted mice treated with Rebecsinib survived significantly longer compared to those treated with vehicle, Fedratinib (an FDA-approved JAK2 inhibitor), and the combination of Rebecsinib and Fedratinib. Interestingly, aged normal bone marrow engrafted mice treated with Rebecsinib (10mg/kg, biweekly for 2 weeks) had an increased number of human CD34+ cells in their bone marrow. Collectively, this data suggests that inhibition of ADAR1p150 may provide a competitive advantage for normal hematopoietic stem cells compared with leukemia stem cells in the bone marrow. These findings lay a foundation for developing

Rebecsinib as a clinical ADAR1 antagonist that promotes normal hematopoietic stem cell retention in the bone marrow niche.

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Keywords: acute myeloid leukemia (AML), aged normal bone marrow (aNBM), Adenosine deaminase acting on RNA 1 (ADAR1)

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ROLE OF APOBEC3 ENZYMES IN NORMAL HEMATOPOIETIC STEM AND PROGENITOR CELL MAINTENANCE AND MALIGNANT TRANSFORMATION IN MYELOPROLIFERATIVE NEOPLASMS

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Dysregulation of inflammatory cytokine responsive APOBEC3 cytosine deaminases has been shown to be a contributing factor in cancer evolution, presenting as gene expression changes and inclusion of distinct C-to-T mutation patterns. However, the context specificity and mechanisms by which APOBEC3 enzymes regulate hematopoietic stem cell function and the role they play in cancer initiation and progression require further elucidation. Lentiviral overexpression of APOBEC3C and an editase deficient APOBEC3C mutant in healthy cord blood, bone marrow and myeloproliferative neoplasm patient hematopoietic stem/progenitor cells (HSPCs) allows us to study the effects of innate immune deaminase dysregulation in the hematopoietic niche. By FACS sorting individual stem and progenitor cell populations, we can examine the distinct role of RNA and DNA editing in both normal hemopoietic stem cell fate determination as well as malignant transformation into cancer stem cells. We are focusing on the upregulation of APOBEC3C and adenosine deaminase acting on RNA1 (ADAR1), as we have previously shown them to be contemporaneously upregulated in the high-risk myelofibrosis (MF) stem cell population compared to normal aged bone marrow. By performing whole genome and whole transcriptome analysis we can compare these novel differential gene expression changes, RNA hyper-editing sites, and DNA mutation signatures induced by APOBEC3 mutagenesis to abnormalities seen in both hematopoietic malignancies and solid tumor cancers. Gene set enrichment analysis (GSEA) performed on this dataset has exposed numerous deregulated pathways brought on by exaggerated levels of APOBEC3, including changes in splicing pathways. Long term, we aim to use these findings to identify predictive biomarkers and druggable targets of leukemic initiation and progression.

Keywords: Hematopoiesis, Deaminase, RNA editing

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A TOOLKIT TO STUDY MICROGLIA STATES USING STEM CELL-DERIVED MODELS

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Single-cell transcriptomic studies reveal diverse microglial states in patients and mouse models. Moreover, emerging genetic studies of late-onset Alzheimer's Disease (LOAD) and other neurodegenerative diseases implicate microglia, the brain's resident macrophages. Yet, we know little about the underlying biology or how myeloid cells contribute to pathogenesis. Moreover, we lack tools to track these states and understand the impact of environmental challenges or genetic susceptibility. To gain insights into these questions, we turned towards stem cell-derived models to broadly characterize human microglia in vitro and understand their impact on the other cell types in xenograft models, allowing us to gain biological insight into disease pathogenesis. Our lab has developed a novel human stem cell-based platform (https://doi.org/10.1101/2022.05.02.490100) that induces stem cell-derived microglia (iMGLs) to take on diverse transcriptional signatures similar to those found in the human brain in response to exposure to brain-relevant substrates. Moreover, we conceived a lentiviral protocol that allows for efficient genetic manipulation of microglia (>90% transduction). Building on these systems, we generated reporters of microglia state to track the expression of key microglial genes and adapted iPSC "villages", or pooled cultures, that combine iPSC cell lines in the same dish. Using these tools in vitro and in xenograft models, we identified genetic regulators and functional changes of microglia states in late-onset Alzheimer's disease. Together, these tools represent a broad toolset that will allow us to answer some of the most pressing questions, such as when and how in disease these states are formed, what is their impact on disease progression, and how plastic they are. These tools will be invaluable to understanding the role of microglia in AD and the brain in general.

Keywords: Microglia, neuro-immune interraction, Alzheimer's disease

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ABERRANT EARLY HEMATOPOIETIC PROGENITOR FORMATION MARKS THE ONSET OF THE HEMATOPOIETIC DEFECT IN SHWACHMAN-DIAMOND SYNDROME

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Shwachman-Diamond Syndrome (SDS) is an inherited disorder, usually presenting with bone marrow failure and pancreatic insufficiency. Approximately ninety percent of SDS patients carry mutations in the ribosome associated gene, SBDS. Reduced blood cell counts are commonly seen in early childhood, but the cellular and molecular events that initiate and accompany this process are unknown. We hypothesized that the onset of SDS hematopoietic defects occurred at a specific embryonic hematopoietic developmental stage. To evaluate this, we generated SDS and control transgene-free human-derived induced pluripotent stem cells (iPSCs). SDS iPSCs recapitulated the SDS hematological phenotype. Detailed evaluation of definitive hematopoietic differentiation by flow cytometry revealed defects that started at the early emerging hematopoietic progenitor (EHP) stage after mesoderm and hemogenic endothelium were normally induced. Hematopoietic colony formation from EHPs were also markedly reduced, and introduction of SBDS in SDS iPSCs improved colony formation. Transcriptome analysis revealed reduced expression of ribosome and oxidative phosphorylation-related genes, in both, undifferentiated and differentiated iPSCs. However, certain pathways (e.g. DNA replication) and certain genes (e.g. CHCHD2) were either exclusively or more severely dysregulated in EHPs, compared to undifferentiated iPSCs and to more mature hematopoietic cells. These results offer insight into the embryonic onset and characteristic of the hematopoietic defects in SDS. Disruption of specific ribosome and oxidative phosphorylation genes may explain overall reduced cell growth; however, the hematopoietic defect likely starts due to disruption of additional genes.

Funding Source: Canadian Institute of Health Research, the Butterfly Guild, and Nicola's Triathlon for Kids.

Keywords: Shwachman-Diamond Syndrome iPSC model, Definitive hematopoiesis, Early emerging hematopoietic progenitors

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INVESTIGATING HYPER-STABLE COMPUTATIONALLY DESIGNED TIE2 BINDERS TO PROMOTE VASCULAR STABILITY

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Diabetics are at increased risk of vascular diseases resulting from glucose-driven misregulation of vascular permeability. Currently, medical intervention relies on metabolic regulation, with no way to mitigate vasculopathy once it begins. The Ang-Tie2 pathway is known to regulate vascular stability. Recent studies show that inhibition of the Tie2 signaling axis results in increased atherosclerosis, and that hyperglycemia increases expression of inhibitory Ang2, suggesting that strong activation of this axis could help mitigate diabetic vascular instability. We used computationally designed protein scaffolds conjugated with the Ang1 F-domain (FDS) at a range of valencies and geometries to evaluate Tie2 signaling output and tight junction formation. We found that FDS with various combinations of F-domains exhibit Ang1-like activity, upregulating pAKT, and inducing migration and tube formation. Cells treated with FDS recruit junction factors Integrin, VE-Cadherin, ZO1, Occludin, and Claudin-5, and show accelerated ZO1 reassembly after chemical disruption. Interestingly, FDS revealed two functionally distinct Tie2 clusters, one that recruits integrin for pAKT/FOXO regulation, and another that regulates junctional permeability. To assess the potency of our FDS in promoting endothelial identity and maturity, we evaluated them in iPSC-endothelial (iENDO) differentiation. FDS were able to drive Tie2 receptor superclustering during endothelial maturation, accelerating endothelial maturity. Importantly, we observed that Tie2 receptor clusters recruited TJP1 to the membrane to enhance tight junction formation at a very early stage of differentiation (d8) and 15-fold higher than controls. Finally, sc-Seq of d14 iENDOs showed that FDS-treated cells were overrepresented in clusters with mature transcriptional signatures and exhibited enriched expression of

ZO1 compared to controls. To assess whether computationally designed FDS could rehabilitate diabetic vascular phenotypes, we used a diabetic vascular organoid model treated with FDS. We observed that FDS were able to reverse the diabetic phenotype, reducing collagen secretion, relocalizing pericytes, and repairing tight junctions. Our data suggest that computationally designed FDS could be used to stabilize vascular permeability.

Keywords: endothelial, tight junctions, vascular stability

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CELLULAR BARCODING OF THE LEUKEMIC NICHE REVEALS AN APELIN-MEDIATED CLONAL EXPANSION OF NICHE ENDOTHELIAL AND MESENCHYMAL STROMAL CELLS

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Hematopoietic stem and progenitor cells (HSPCs) reside in niches that provide regulatory signals for their function. HSPC clones have been examined by cellular barcoding, but the clonality of endothelial (ECs) and stromal cells (SCs), the cellular components of the niche, is unknown. We hypothesized that perturbations such as acute leukemia alter EC and SC clones to support leukemogenesis. We developed a new zebrafish model of acute erythroid leukemia (AEL) by overexpressing human CMYC under the blood promotor draculin (drl). drl:CMYC marrows demonstrated an expansion of progenitors (fc=4.8;p< 0.000001) overexpressing the erythroid master regulator gata1a (fc=1.4;p=0.01) and fetal hemoglobins hbbe1.1/2 (fc=4.7,2.9;p=0.0004). Serial transplantation of drl:CMYC marrows resulted in engraftment and disease propagation (7/7;17/18). We used the cellular barcoding GESTALT technique to barcode single cells using CRISPR-CAS9 and injected barcoded embryos with drl:CMYC to induce AEL and perform HSPC and niche clone tracing. HSPC clones were decreased by half (p=0.008) indicative of a clonal expansion of AEL. Barcode and scRNA-Seq of niche ECs revealed a decrease in the number of EC clones (fc= -3.5;p< 0.05) paired with the discovery of a novel AEL venous EC population upregulating 99 genes (fc>1;p< 0.05) suggestive of angiogenesis. AEL marrows had less niche SC clones (fc= -2.1;p< 0.01) and scRNA-Seq of 3,263 SCs revealed an increased fraction of lepr+ mesenchymal SCs (66 vs 24%). We hypothesized that AEL progenitors secrete a signal that can remodel niche clones to promote disease progression. We identified the hormone apelin upregulated in AEL progenitors (fc=2.6;p< 0.0001) and receptors aplnra/aplnrb expressed on >50% of AEL ECs. We tested if apelin alone could remodel the niche by overexpressing apelin in blood cells and found fewer niche clones compared to controls (fc=2.06;p=0.004). EC numbers were unchanged and EC clone size was increased (fc=2.05;p< 0.02) while SC numbers were decreased and SC clone size was increased (fc=-2.2,2.14;p< 0.01). Blood clone analysis revealed more myeloid clones (fc=4.01;p< 0.0001) demonstrating a niche-mediated alteration of hematopoiesis via apelin signaling. Our data reveals that apelin signaling is responsible for AEL-induced clonal and transcriptional niche remodeling to promote disease progression.

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Keywords: Hematopoietic Stem Cell Niche, Acute Erythroid Leukemia, Apelin signaling

TOPIC: KIDNEY

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HUMAN PLURIPOTENT STEM CELL DERIVED KIDNEY ORGANOIDS FOR HIGH THROUGHPUT DISEASE MODELLING IN DRUG DISCOVERY

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Human kidney contains around one million nephrons and more than two dozen different cell types. Reproducing kidney cell types in-vitro is limited and a key challenge for generation of translational datasets. Recent advancements in human induced pluripotent stem cell (hiPSC) differentiation provide an opportunity to use multicellular kidney organoids. We have compared methods of trans-well 'aggrewell' organoids and suspension culture of 'micro-organoids'. This allows comparison of industrial scale and traditional low throughput trans-well organoids. The method involves differentiation of iPSCs to intermediate mesoderm using CHIR and FGF9 and spontaneous aggregation in suspension culture or fixed trans-wells leads to mature kidney organoids, which can be used to study kidney disease in a low to high-throughput manner. We aimed to model human kidney inflammatory and genetic disease in-vitro using kidney organoids, treatment with different insults to reproduce CKD microenvironment (IL-1b, TGFb, Angiotensin-II, protamine sulphate). We compared culture conditions using APEL versus E6 media. We applied bulk and single cell RNA sequencing with immunofluorescence and immunohistology to enable analysis of gene signatures with renal structures within organoids. Kidney organoids recapitulate human kidney cell composition with modest differences in podocyte and tubular fractions between methods. After 24h of stimulation with CKD

stressors, we noted significant upregulation of injury biomarkers including KIM1 and inflammatory cytokines. With a notable increase in IL6, TNF in micro-organoids cultured in E6 media with IL-1b. These observations clearly demonstrate the use of kidney organoids to study renal diseases in-vitro for drug discovery applications with human translatable functional biomarkers. More work is now underway to perform renal functional characterisation of organoids, including cation transport and electrophysiology to translate findings under pathophysiological conditions. These kidney organoids provide a platform for modelling of human kidney diseases related to fibrosis, inflammation, and regeneration. Comparing different organoid methodologies will help select appropriate method for application in drug discovery and target validation for chronic kidney diseases.

Funding Source: AstraZeneca

Keywords: Kidney Organoids, Modelling disease, CKD

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BIASING IPSC-DERIVED KIDNEY ORGANOIDS TOWARDS A DISTAL NEPHRON FATE

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Congenital anomalies of the kidney and urinary tract are identified in ~1% of newborns world-wide and are responsible for 40% of childhood end-stage renal disease (ESRD), where normal kidney function is lost. Infant patients have no therapeutic options beyond dialysis and organ transplantation and have a 93% 1-year mortality rate. Kidney function in the distal nephron is performed by specialized cell populations that control homeostatic regulation of salt, blood pressure, and water. How and where these cells form during development and the mechanisms underpinning their maturation and function are unknown. A challenge facing research on distal nephrogenesis is that kidney organoids fail to form normal distal cell types, thus developing regenerative therapeutics for ESRD patients is impeded. To address this issue, we are delineating how distal cells form in vivo, and replicating this process in organoids. Transcription factors Hnf1b and Pou3f3 drive murine distal nephron cell specification, and their human equivalents are expressed in the distal nephron and are linked to renal birth defects. We show that human and mouse nephrogenesis follows a deeply conserved developmental program. Single cell RNA-seq and single nucleus ATAC-seq data show NPCs differentiate into HNF1B+/POU3F3+/TFAP2A+ distalizing cells via a β-catenin-dependent mechanism. β-catenin and its co-transcription factor bind putative cis-regulatory regions for Hnf1b and Pou3f3 and deeply conserved and accessible DNA regions in human cells have predicted POU and TCF/LEF motifs. Our analyses suggest that a distal HNF1B+/POU3F3+ domain prefigures putative distal precursors along a conserved developmental program. To drive distal cell-fates in organoids, we tuned the dosage, duration, and timing of β-catenin activation using small molecule a(nta)gonists. Modified β-catenin activities altered patterning and distalized nephrons, with genes displaying dosage- and duration-specific activities. Our systematic analyses of dosages/durations of β-catenin signaling across development, combined with tuning other distal-biased pathways, show a dependency on signaling pathways that drive distalization and maturation of distal cell types.

Keywords: kidney organoids, nephron development, distal nephron

TOPIC: LIVER

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PRIMARY AND PLURIPOTENT STEM CELL DERIVED HUMAN MULTICELLULAR HEPATIC ORGANOIDS REVEALS COMPLEX INTRACELLULAR AND INTERCELLULAR INTERACTIONS AND METABOLIC PHENOTYPES

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Nonalcoholic fatty liver disease (NAFLD) currently impacts upwards of 40% of Americans and 25% of adults globally and has rapidly become the most common cause of chronic liver disease in the United States and worldwide. Unfortunately, currently in vitro models and mouse models poorly replicate the complex metabolic interactions seen in human metabolic diseases such as NAFLD. Consequently, the molecular underpinnings of the disease are poorly understood and no clinical therapies are available. We have endeavored to address these gaps with the development of new multicellular models that can recapitulate the complex interactions seen in metabolic disorders. We have developed a scalable multi-well platform that enables the rapid generation of three-dimensional (3D) multicellular organoids composed of primary and stem cell derived human liver parenchymal cell types including hepatocytes, liver sinusoidal endothelial cells (LSEC), stellate cells (HSC), and Kupffer cells. This 3D system can maintain hepatocellular function long-term and can be used to model the interactions between hepatocytes and nonparenchymal cells (LSEC, HSC, and Kupffer cells). NAFLD is associated with chronic hyperglycemia and hyperlipidemia. We generated multicellular organoids and examined how chronic hyperglycemia and hyperlipidemia impacted multicellular organoids. We found that multicellular organoids maintain insulin-sensitive glucose metabolism in contrast to traditional hepatocyte-culture systems. Moreover, we found that chronic hyperglycemia and hyperlipidemia led to impaired insulin sensitivity, increased hepatocyte fat accumulation, hepatocyte injury, and macrophage and stellate cell activation. We evaluated drug compounds currently being used in clinical studies and found that some can mitigate these effects. Our work demonstrates that multicellular organoids composed of primary and stem cell derived human liver parenchymal cell types including hepatocytes, LSEC, stellate cells, and Kupffer cells have stable function and can model complex disease phenotypes in vitro. Leveraging these technologies focused on metabolic phenotypes enabled studies of metabolic injury and can be leveraged in fatty liver drug discovery.

Keywords: disease modeling, liver biology, fatty liver

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TIME RESOLVING PROTEOMIC ANALYSIS OF HEPATIC STELLATE CELL DIFFERENTIATION IDENTIFIES RORA AS A REGULATOR OF CELL COMMITMENT AND FIBROSIS

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Hepatic Stellate Cells (HSCs) are liver cells involved in the maintenance of extracellular matrix homeostasis. Upon injury they activate, acquire myofibroblastic features and become the main fibrogenic cell type. Understanding the trajectory of HSCs differentiation and activation is fundamental to define cell identity in homeostasis and disease and identify potential therapeutic targets. By profiling the differentiation of human iPSCs into differentiated (d) dHSCs, we aim to understand the protein dynamics across differentiation and identify drivers of cell commitment and activation. MS proteomics was performed in four differentiations and primary human HSCs. In vitro models were used to validate the role of RORA in differentiation and activation, using both genetic (iPSC-RORA+/-) and chemical (agonist/antagonist) strategies. In vivo, staggerer mice (RORA-/-) were treated with CCl4 to induce liver fibrosis. Three tissue fibrosis models (liver, heart, and kidney) were used to evaluate the effect of RORA agonist upon injury. RORA expression was evaluated in a cohort of 33 patients with chronic liver disease.Time-resolving proteome analysis of dHSCs differentiation identified three stages of maturation. Comparison of dHSCs with primary human HSCs revealed that RORA is a transcription factor involved in HSC commitment and activation. iPSC-RORA+/- failed to differentiate into dHSCs. The dHSC phenotype was rescued by stimulating differentiating cells with RORA agonist. We also identified RORA to be involved in HSC activation. In vitro, the silencing of RORA in dHSCs promoted activation, whereas the RORA agonist deactivated the cells. In vivo, the staggerer mice showed an exacerbated fibrogenic response. Increasing the activity of RORA using a synthetic agonist in the three models of tissue fibrosis reduced the fibrogenic response. In humans, RORA expression correlated negatively with liver fibrogenic markers in chronic liver diseases. This study shows that understanding cell differentiation protocols may be useful to identify new therapeutic targets. The present study describes the proteomic trajectory of dHSCs differentiation and identifies a dual role of RORA in HSCs commitment and activation, suggesting that promoting RORA activity might be a promising strategy for mitigating fibrosis.

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Keywords: Fibrosis, Cell trajectories, iPSCs differentiation

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SECRETOME DERIVED FROM HUMAN PRE-CONDITIONED MESENCHYMAL STROMAL CELL CONFERS A RENAL AND HEPATOPROTECTIVE EFFECT IN AN ANIMAL MODEL OF SEVERE ACUTE ON CHRONIC LIVER FAILURE.

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Acute on Chronic Liver Failure (ACLF) is a syndrome characterized by the acute deterioration of liver function in patients with a pre-existing compensated or decompensated chronic liver disease. In this condition, the development of acute kidney injury (AKI) is associated with extremely poor survival of the patients. Mesenchymal Stromal Cells (MSCs) therapy represents an emerging approach for the treatment of complex diseases like ACLF. MSCs releases a broad array of immunomodulatory and trophic molecules defined as MSC-secretome. Moreover, the MSC secretion of trophic and anti-inflammatory factors can be enhanced by in vitro pre-conditioning. Our aim was to determine whether the endovenous administration of secretome derived from human

preconditioned-MSC was able to prevent liver failure and AKI in a rat model of severe ACLF. Human adipose derived MSCs were in vitro preconditioned with TNF-α/INFγ. ACLF was induced in rats by the i.p. administration of porcine serum for 11 weeks (chronic liver disease), followed by the administration of D-gal/LPS (acute liver failure). One group received endovenously 200 µl of saline solution (ACLF group), whereas the other was treated with the concentrated MSC-secretome derived from 1x106 MSCs (ACLF-sec group). The administration of MSC-secretome substantially alleviated ACLF, increasing the survival rate (40% in ACLF vs 85% in ACLF-sec group). This result, was correlated with increased hepatocyte proliferation rate (PCNA expression), and decreased hepatocyte apoptosis rate (TUNEL) in the ACLF-secretome group. Furthermore, secretome administration, preserved the liver histological architecture. Both ACLF and ACLF-secretome groups, presented an increased expression of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β, Cinc-1 and MCP-1). However, the ACLF-sec group showed an increase in anti-inflammatory molecules (IL-4, IL-5 and TGFβ1). In the same line, the renal histopathological study of ACLF-group, evidenced tubular injury, increased apoptotic rate and upregulation of Kim-1, HMGB-1 and IL-6 expression. The MSC-secretome administration ameliorated renal tubular lesions, reduced apoptosis rate and downregulated injury marker expression. These data support the potential use of MSCs-secretome in the treatment of multiorgan failure associated to ACLF

Funding Source: Fondecyt 1200308

Keywords: Acute on Chronic Liver disease, Acute Kidney Injury, Mesenchymal stroma cell secretome

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CRISPR EPIGENETIC ENGINEERING OF IMPROVED IPSC-DERIVED HEPATOCYTES

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The liver is unique in its ability to regenerate after external damage from various toxins or genetic defects. However, after prolonged chronic damage, the liver loses the ability to regenerate and becomes irreversibly cirrhotic. Due to the shortage of liver donors for transplantations and the scarcity of primary human hepatocytes for in vitro toxicology studies, there is a growing interest in the in vitro reprogramming of induced pluripotent stem cells (iPSCs) to mature hepatocytes using chemical and genetic methods, termed iPSC-derived hepatocytes (iHeps). However, current methods for producing iHeps yield cells with significant differences in gene expression and epigenetic signatures compared to primary human hepatocytes (PHHs). Further, our generated single-cell multiomic RNA- and ATAC-seq profile of iHeps indicates substantial transcriptomic and epigenetic heterogeneity within the final cell population. These epigenetic differences likely contribute to the reported deficiencies in using iHeps. Epigenetic editing using CRISPR-dCas9 allows the precise dissection and programming of intricate cellular pathways. To address the limitations of current protocols for iHep generation, we used CRISPR activation screens of all transcription factor and epigenetic modifier genes. Fluorescence-activated cell sorting (FACS) for cells that upregulate albumin or alpha-fetoprotein generated a list of guide RNAs (gRNAs) and corresponding gene targets implicated in mature and immature liver phenotypes, respectively. Several independent gRNA hits from the screens target the same gene promoters, providing strongly implicating these transcription factors in iHep differentiation and/or maturity. Validation experiments have confirmed the ability of CRISPR-based activation of screen hits to upregulate the expression of mature liver genes. Future studies will involve studying combinatorial interactions of multiplex gene activation and performing CRISPR activation screens on whole transcriptome readouts using scRNA-seq. Phenotypic validation of the iHeps in vitro and in vivo in mouse models will test the function of the engineered iHeps. This will move the field towards a more accurate and successful protocol for iHep generation that could benefit both research and clinical applications.

Funding Source: NSF Graduate Research Fellowships Program (GRFP) and NIH Impact of Genomic Variation on Function (IGVF)

Keywords: CRISPR Epigenetic Engineering, Single Cell Multiomics, iPSC Differentiation

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HUMAN PLURIPOTENT STEM CELL-DERIVED HEPATOCYTES: PURIFICATION BY METABOLIC SELECTION AND INFECTION BY RISK GROUP 4 VIRUSES

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Ebola viruses are deadly viruses that infect the liver and require biosafety-level-4 (BSL4) containment. Pure human hepatocytes could constitute physiologically-relevant models to study the effects of these viruses. We charted a roadmap for human pluripotent stem cell (hPSC) differentiation into hepatocytes and delineated stepwise gene expression and chromatin changes during liver differentiation. This revealed the production of >80% pure hepatocytes alongside primarily intestinal cells. We developed metabolic selection, a straightforward approach to purify hepatocytes by withholding exogenous nutrients: non-liver cells rapidly died, whereas hepatocytes survived. Purified hPSC-derived hepatocytes transcriptionally and functionally approximated primary adult human hepatocytes, compared to interferon-defective liver cancer cells frequently used in BSL4 virology. Ebola viruses extensively infected and replicated in hPSC-derived hepatocytes. We thus map human liver differentiation, purify human hepatocytes, and use the purified cells to study the cellular effects of Ebola viruses.

Funding Source: The Thomas & Stacey Siebel Foundation, the Stanford Maternal & Child Health Research Institute, Additional Ventures, and the California Institute of Regenerative Medicine funded this study.

Keywords: Pluripotent Stem Cells, Liver Differentiation and Purification, Biosafety-Level-4 Viruses

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IDENTIFICATION OF AN FXR-MODULATED LIVER-INTESTINE HYBRID STATE IN IPSC-DERIVED HEPATOCYTE-LIKE CELLS

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Pluripotent stem cell (PSC)-derived hepatocyte-like cells (HLC) have enormous potential as a replacement for primary hepatocytes in drug screening, toxicology and cell replacement therapy, but their genome-wide expression patterns differ strongly from primary human hepatocytes (PHH). We differentiated human induced pluripotent stem cells (hiPSC) via definitive endoderm to HLC and characterized the cells by single-cell and bulk RNA-seq, with complementary epigenetic analyses. We then compared HLC to PHH and publicly available data on human fetal hepatocytes (FH) ex vivo and performed bioinformatics-guided interventions to improve HLC differentiation via lentiviral transduction of the nuclear receptor FXR and agonist exposure. Single-cell RNA-seq revealed that transcriptomes of individual HLC display a hybrid state, where hepatocyte-associated genes are expressed in concert with genes that are not expressed in PHH – mostly intestinal genes – within the same cell. Bulk-level overrepresentation analysis, as well as regulon analysis at the single-cell level, identified sets of regulatory factors discriminating HLC, FH, and PHH, hinting at a central role for the nuclear receptor FXR in the functional maturation of HLC. Combined FXR expression plus agonist exposure enhanced the expression of hepatocyte-associated genes and increased the ability of bile canalicular secretion as well as lipid droplet formation, thereby increasing HLCs' similarity to PHH. The undesired non-liver gene expression was reproducibly decreased, although only by a moderate degree. DOI: 10.1016/j.jhep.2022.07.009

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Keywords: Hepatocyte-like cells, Hybrid State, FXR

TOPIC: MESENCHYMAL STROMAL CELLS, ADIPOSE, AND CONNECTIVE TISSUE

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HEREDITARY RETINOBLASTOMA IPSC MODEL REVEALS SPLICEOSOME AS A THERAPEUTIC VULNERABILITY OF RB1-MUTANT CANCERS

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The RB1 tumor suppressor has been widely recognized for its role in inhibiting tumor initiation, development, and progression. Patients with hereditary retinoblastoma (RB), caused by autosomal dominant mutations in the RB1 tumor suppressor gene, commonly develop retinoblastoma and non-ocular tumors (e.g.,

osteosarcoma), emphasizing a strong mechanistic link between RB1 loss and tumorigenesis. Here, we generate RB iPSC model to delineate the pathological mechanisms of RB1 mutation in osteosarcomagenesis. The results are backed up by RB1-corrected isogenic control iPSC lines. We demonstrate that the RB iPSC disease model serves as a "disease in a dish" for cancer researchers to study the potential initiation stage of osteosarcomagenesis. By investigating transcriptomes and genome occupancies in RB iPSC-derived osteoblasts, we elucidate the unidentified role of RB1/E2F3a signaling in controlling spliceosome function by regulating spliceosomal gene expression. Patient tumor specimen studies support the clinical relevance of the RB1/E2F3a regulatory network in modulating spliceosomal gene expression. Analyses of splicing events in RB1-deficient cells treated with a spliceosome inhibitor indicate aberrant retained intron (RI) events in tumor-promoting genes. By pathway analyses, these genes with higher rates of RIs in RB1-mutant cells are involved in spliceosome-associated signaling pathways such as NOTCH, TGFβ, α5β3 Integrin, and NF-κB. We further globally dissect the differentiated RNA splicing variants between RB1 wild-type and mutant cells and identify numerous cancer-associated alternative splicing (TRMU, SNX32, LIMS1, SHB) preferentially existed in RB1-mutant osteosarcoma. Pharmacological inhibition of the spliceosome in RB1-mutant cells leads to global intron retention, decreased cell proliferation, and impaired tumorigenesis, suggesting that the spliceosome is an "Achilles' heel" of RB1-mutant osteosarcoma and that cancers harboring RB1 mutation/deletion may be vulnerable to spliceosome inhibition.

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Keywords: hereditary retinoblastoma, spliceosomal genes, osteosarcoma

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QUIESCENCE RECOVERS ADIPOSE TISSUE-DERIVED STROMAL CELLS FROM SENESCENCE-ASSOCIATED SECRETARY PHENOTYPE, LEADING TO ENHANCED ANGIOGENESIS AND DIABETIC WOUND HEALING

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Despite of the therapeutic potential of mesenchymal stem cells (MSCs) through their anti-inflammatory functions, the number of clinical applications available today is still limited. One of the reasons is that MSCs exhibit a senescence-associated secretory phenotype (SASP) in age-related diseases, which results in the attenuation of their anti-inflammatory functions. We previously reported that MSCs become quiescent in gels with a stiffness mimicking that of adipose tissue or bone marrow. In this study, we investigated whether or not introducing quiescence in MSCs will abrogate SASP observed in diabetes, both in vitro and in vivo. Once adipose tissue-derived stromal cells (ADSCs), one type of MSCs used in multiple clinical trials, became quiescent, they initiated to produce soluble factors responsible for their anti-inflammatory functions, even when they are cultured in high glucose media. High glucose-induced attenuated migration and tubular formation of HUVECs, an endothelial cell line, were ameliorated more significantly by conditioned medium from quiescent ADSCs cultured under high glucose than by that from non-quiescent ADSCs cultured under high glucose. Transplants containing quiescent ADSCs enhanced wound healing more than those containing non-quiescent ADSCs in streptozotocin (STZ)-induced diabetic mice, demonstrating a higher efficacy of quiescent ADSCs over non-quiescent ADSCs for diabetic wound healing. Furthermore, ADSCs cultured at a high glucose level in vitro enhanced angiogenesis and wound healing in STZ-induced diabetic mice similar to ADSCs cultured at a normal glucose level in vitro, when they were made quiescent in gels and subcutaneously injected

into the mice. These results suggest that quiescence restores ADSCs from SASP and enhances angiogenesis even under diabetic conditions, which may be crucial for MSC-based cell therapies against diabetic microangiopathy.

Keywords: Quiescence, senescence-associated secretory phenotype, mesenchymal stem cell

TOPIC: MUSCULOSKELETAL

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TRANSGENE-FREE DIRECT CONVERSION OF FIBROBLASTS INTO FUNCTIONAL MUSCLE STEM CELLS UTILIZING SYNTHETIC MRNA AND SMALL MOLECULES

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Transcription factor-based reprogramming provides an attractive approach to produce desired cell types for regenerative medicine purposes. Such cellular conversions are widely dependent on viral vectors to efficiently deliver and express defined factors in target cells. However, use of viral vectors is associated with unfavorable genomic integrations that can trigger deleterious molecular consequences, rendering this method an impediment to clinical applications. To address this limitation, we report on an efficient approach to directly convert mouse fibroblasts into transgene-free induced myogenic progenitor cells (iMPCs) via overexpression of synthetic MyoD-mRNA in concert with an enhanced small molecule cocktail. First, we performed a candidate compound screen and identified two molecules that enhance fibroblast reprogramming into iMPCs by suppression of the JNK and JAK/STAT pathways. Simultaneously, we developed an optimal transfection protocol to transiently overexpress synthetic MyoD-mRNA in fibroblasts. Combining these two techniques enabled robust and rapid reprogramming of fibroblasts into Pax7-positive iMPCs in as little as 10 days. Through various assays including single-cell transcriptomics, we illustrate that nascent transgene-free iMPCs proliferated extensively in vitro, expressed a suite of myogenic stem cell markers, and could differentiate into highly contractile muscle fibers. Furthermore, using global and single-cell transcriptome assays, we delineated gene expression changes associated with JNK and JAK/STAT pathway inhibition during reprogramming and identified in iMPCs a Pax7+ stem cell subpopulation akin to satellite cells. Last, transgene-free iMPCs robustly engrafted skeletal muscles of a Duchenne muscular dystrophy (DMD) mouse model, restoring dystrophin expression in hundreds of myofibers. In summary, we report on an improved and clinically safer approach to convert fibroblasts into myogenic stem cells that can efficiently contribute to muscle regeneration in vivo.

Keywords: muscle stem cells, direct lineage conversion, small molecule cocktail

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NEUROMUSCULAR ORGANOIDS AS A UNIQUE MODEL TO STUDY NEUROMUSCULAR INJURY AND REGENERATION IN VITRO

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Spinal cord motor neurons innervate skeletal muscles and instruct body movement through the formation of functional neuromuscular junctions (NMJs). The skeletal muscles and axons have remarkable capacities for regeneration after injury due to cross-talk between diverse cell types. Damage to muscle fibers and axons triggers an inflammatory response that activates satellite cells to exit quiescent, proliferate, and enter the myogenic differentiation program. In parallel, glial cells guide regenerating axons to their target myofibers and aid them in reforming NMJs. Still, the skeletal muscle loses its regenerative abilities in cases such as severe wounds, myopathies, and degenerative diseases. While significant progress has been made in recent years to engineer skeletal muscle tissue in vitro, no in vitro human injury models have been described which include myofibers, motor neurons and, by extension, functional NMJs. Here we show that our iPSC-derived neuromuscular organoid (NMO) offers a unique and dynamic model for studying neuromuscular development and the mechanisms of neuromuscular injury and regeneration. NMOs are characterized by the simultaneous development of spinal cord neurons and skeletal muscles that self-organize and form functional NMJs. Mechanical injury in NMOs is achieved by severing the muscle fibers and axons. Analysis of NMOs two days post-injury revealed activation and proliferation of satellite cells, indicated by significantly increased numbers of PAX7+ and MYOD1+ cells, marking the initiation of myogenic regeneration. Additionally, injury resulted in a significant reduction in the number and size of NMJs. Strikingly, after two weeks the NMJs recovered to levels comparable to the pre-injury state. Moreover, in response to injury, macrophages (IBA-1+ cells) appeared in the muscle region of the NMOs. Collectively, our data illustrates that iPSC-derived NMOs can be used as a robust model to study neuromuscular injury. This model also opens up the opportunity to investigate the impaired muscle healing mechanisms in various myopathies.

Keywords: Neuromuscular Organoids, Neuromuscular injury, Skeletal Muscle

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423F DRUG DIVERSELY AFFECTS DIFFERENTIATION OF DISTINCT CD90 HUMAN MUSCLE SUBSETS IN MULTIPLEXED CULTURE CONDITIONS VIA GP130

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Chronic muscle injuries, such as massive rotator cuff tears, are associated with progressive muscle wasting, fibrotic scarring, and intramuscular fat accumulation. While progenitor cell subsets are usually studied in culture conditions that drive either myogenic, fibrogenic, or adipogenic differentiation, it is still unknown how combined myo-fibro-adipogenic signals, which are expected to occur in vivo, modulate progenitor differentiation. We therefore evaluated the differentiation potential of subsets of primary human muscle progenitors in multiplexed conditions in the presence or absence of 423F drug, a modulator of gp130 signaling. Retrospectively generated human muscle progenitors were typified as CD90+CD56+ myogenic and CD90-CD56- fibro-adipogenic progenitors (FAP). In addition, we identified a novel CD90+CD56- non-adipogenic progenitor subset. These human muscle subsets exhibited varying degrees of intrinsically regulated differentiation in single and mixed induction cultures. 423F/gp130 modulated muscle progenitor differentiation in a dose-, induction-, and cell subset-dependent manner and resulted in decreased fibro-adipogenesis of CD90-CD56- FAP. Conversely, 423F mediated increased myogenesis of CD56+CD90+ myogenic subset, indicated by increased myotube diameter and number of nuclei per myotube in treated non-induced cultures. 423F treatment eliminated FAP-derived mature adipocytes from mixed adipocytes-FAP cultures but did not affect the growth of non-differentiated FAP in these cultures. Collectively, these data demonstrate that capability of myogenic, fibrogenic, or adipogenic differentiation is largely dependent on the intrinsic features of cultured subsets, and that the degree of lineage differentiation varies when signals are multiplexed. Moreover, our tests performed in primary human muscle cultures indicate that 423F drug could diminish degenerative fibro-adipogenesis of muscle after injury without disrupting myogenesis or augmenting muscle atrophy.

Funding Source: Epstein Research and Discovery Fund.

Keywords: muscle, myo-fibro-adipogenesis, drug

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MECHANORESPONSIVE 3D BIOPRINTED BONE ORGANOIDS FROM HMSC-LADEN HYDROGELS

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Bone undergoes constant finely tuned remodeling processes orchestrated by osteocytes, bone-resorbing osteoclasts, and bone-forming osteoblasts. The activity of these cells is uniquely responsive to mechanical stress. An imbalance in the activity of these cells or an inherent fault in their function gives rise to many diseases. However, the relationship between mechanical loading and unloading and overall gene expression is poorly understood, especially in diseases that impair ambulation. Stem cell-derived organoids are a promising tool to study such molecular processes in health and disease under different conditions. Here, we show that 3D bioprinted stem-cell based osteocyte bone organoids respond to an increase in mechanical loading frequency by increasing mineral density maturation. The osteocyte bone organoids were formed from stem-cell laden graphene oxide hydrogels with mechanical loading using 5 Hz frequency within a pre-culture time of 4 weeks. Subsequently, the effects of mechanical unloading, continued loading with 5 Hz, and with 10 Hz on organoid stiffness, mineral density maturation, bone volume and gene expression were investigated. The organoid stiffness was monitored longitudinally within dynamic compression bioreactors in combination using a mechanical stimulation unit. Our results show that loading with 10 Hz frequency induces a rapid increase in the organoid stiffness, resulting in organoids with 40% higher stiffness compared to mechanical unloading. Time-lapsed micro-computed tomography (micro-CT) shows that the increase in stiffness is due to a higher mineral density increase. To determine whether mechano-regulated pathways have been activated in the bone organoid, we conducted RNA-Seq on a total of 6 organoids. Notably, mechanical loading with 5 and 10 Hz differentially regulated 49 and 173 genes as compared to mechanical unloaded conditions. These results suggest that stem-cell derived bone organoids hold great potential as a tool to investigate the relationship between molecular processes in mechanically loaded or unloaded bone under physiological or pathological conditions, especially when combined with patient-derived cells.

Funding Source: This project was supported by the grant # 2022-487 of the Strategic Focal Area “Personalized Health and Related Technologies (PHRT)” of the ETH Domain (Swiss Federal Institutes of Technology)

Keywords: bone organoid, hMSC-laden hydrogels, mechanobiology

TOPIC: NEURAL

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MULTIPLEXED CELL-BASED ASSAYS FOR EVALUATING THE STRUCTURE AND FUNCTION OF EXCITABLE CELLS

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The flexibility and accessibility of induced pluripotent stem cell technology has allowed complex human biology to be reproduced in vitro at high throughput scales. Indeed, rapid advances in stem cell technology have led to widespread adoption for the development of in vitro models of neuron and cardiomyocyte electrophysiology to be used in screening applications in drug discovery and safety. Furthermore, advanced cell preparations, such as organoids, are under investigation with aims toward establishing mature human phenotypes in vitro. For the development and validation of relevant in vitro neuronal and cardiac models, it is critical to evaluate the structure and function of neuronal synapses and networks, as well as cardiomyocyte viability, electrophysiology, and contractility. The objective of this work is to develop and validate a multiplexed structure-function assay as an efficient approach for evaluating neuronal and cardiomyocyte models in vitro. A planar grid of microelectrodes embedded in the substrate of each well interfaces with cultured cellular networks to continuously monitor both electrophysiological function and structural viability. The electrodes detect the raw electrical activity from the cells to identify changes in function, while structural effects, such as morphological changes and cell viability, are detected as changes in impedance at the cell-electrode interface. Here, we characterized and validated this multiplexed assay using known control compounds that differentially affected cell structure and function. For iPSC-derived neuronal models, all compounds tested (DMSO, glutamate, tributyltin, ionomycin, and Triton X-100) altered functional spiking activity. Glutamate, ionomycin, and tributyltin all produced a dose- and time-dependent decrease in viability, as measured via impedance, with Triton X-100 serving as the positive control for complete loss of membrane integrity. For cardiomyocytes, all compounds (E-4031, nifedipine, isoproterenol, doxorubicin, and blebbistatin) affected aspects of electrical or contractile function, but only doxorubicin decreased cell viability. These results support the continued development and use of human iPSC-derived neuronal and cardiomyocyte assays for high throughput drug discovery and safety assessment.

Keywords: Assay development, Electrophysiology, Viability

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CHEMICALLY DEFINED SUBSTRATES IN A READY-TO-USE FORMAT THAT PROMOTE ADHESION, NEURITE EXTENSION AND LONG-TERM CULTURES FOR MULTIPLE INDUCED PLURIPOTENT-DERIVED NEURAL SUBTYPES

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Induced pluripotent stem cells (iPSCs) and their neural derivatives hold great promise for regenerative medicine, environmental toxin screens, and modeling neurodegenerative diseases. However, successful differentiation of iPSCs and the use of differentiated cells in down-stream workflows often requires applying animal-derived substrates to multiwell plates prior to plating cells. These animal-derived substrates often show batch-to-batch variability in composition that affects cell fate and downstream metrics. Further variability in culture and assay outcomes are introduced through manual coating protocols. Additionally, recent supply shortages in the biomedical sciences have also shown the potential difficulty sourcing animal-derived substrates from vendors. In response, Stem Pharm has developed chemically defined substrates that use norbornene-functionalized polyethylene glycol (PEG) and synthetic peptides offered precoated on ready-to-use standard multiwell plates. The substrates form a 5-8 µm coating on tissue culture polystyrene or cyclic olefin co-polymers and provide a substrate with an elastic modulus of 80-100 kPa as measured by atomic force microscopy. We tested the performance of these plates with commercially available iPSC-derived neurons, astrocytes, and microglia and found that for many neural subtypes, the substrates support sustained long-term cultures better than substrates currently in use. For example, co-cultures of astrocytes and glutamatergic neurons were successfully supported for >70 days. A separate study found iPSC-derived microglia adhesion was sustained over two weeks and cells were morphologically diverse. Microglia response was further interrogated using a combination of lipopolysaccharide (LPS) and interferon gamma (IFN-γ) stimulation with p65 translocation as an endpoint metric. Our results indicated heightened sensitivity to inflammation as measured by higher p65-positive nuclei in Stem Pharm's substrate versus poly-D-lysine. Taken together, our synthetic substrate provides a simple, ready-made foundation to enable robust readouts of neuronal cultures over extended durations in a more physiologically relevant context.

Funding Source: This work has been supported by NIH NINDS SBIR grants 1R43NS102088 and 2R44NS102088.

Keywords: Chemically-defined substrate, iPSC-derived neural cells, multiwell plates

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MODELING NEURAL PROCESSING IN VITRO WITH AGED IPSC NEURONS

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IPSCs have enabled the generation of a wide variety of cellular models for interrogating the fundamental bases of neurological and psychiatric conditions, but the generation of an in vitro model of neural processing has been elusive. Our lab recently described DishBrain, a novel system that integrates human iPSC cortical neurons in a closed-loop in silico environment allowing them to "play" the classic video game Pong. This models what we term synthetic biological intelligence (SBI). In order to determine whether the differences in information processing that occur because of age-related changes can be seen in our system, we paired DishBrain with a novel iPSC line, N2P, which expresses the mutant LMNA protein Progerin after forward programming of NGN2 overexpression leading to an aged neuronal phenotype. In addition, we examined whether pharmacological intervention could be used to rescue, enhance, or modulate the gameplay of this system. Our initial investigations revealed N2P aged neurons were significantly less active in functional measures compared to NGN2 control neurons after 27 days of differentiation. Furthermore, it emerged that our cultures display neural criticality in a variety of fundamental ways, indicating that N2P neurons exist in a differing functional state to control neurons. Unexpectedly, in the DishBrain system, N2P aged neurons significantly outperformed controls during active gameplay across a range of performance metrics, perhaps indicating that rather than displaying a decline in information processing, these cells have inherent advantages in cellular function or networking within the in silico environmental context. To test whether we were able to modulate the system pharmacologically, we treated cultures with carbamazepine, a sodium channel blocker clinically-used as an anticonvulsant, and found that the resulting reduction in spontaneous activity was sufficient to improve NGN2 control neuron gameplay performances, bringing them in line with the N2P aged neurons. This data provides compelling evidence for the use of SBI approaches in cellular models of neural processing and concomitant pharmacological interventions.

Funding Source: Cortical Labs Pty. Ltd. provided reagents and equipment for this study. B.W. and B.J.K. are employees of Cortical Labs Pty. Ltd.

Keywords: Neural Processing In Vitro Model, DishBrain, Aged Neurons

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PATIENT-DERIVED CELLS CARRYING A CACNA1D VOLTAGE-GATED CALCIUM CHANNEL GAIN-OF-FUNCTION MUTATION SHOW DYSREGULATED NEURODEVELOPMENT AND ALTERED NEURONAL FUNCTION

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Voltage-gated calcium channels (VGCCs) are highly expressed in the human brain and are involved in many physiological processes. Recent reports suggest that VGCC are key modulators of early neurodevelopment. VGCC gain-of-function mutations, as observed in the Cav1.3 encoding CACNA1D gene, have been linked to a range of neurological pathologies, including Autism Spectrum Disorders (ASD). One such mutation affects the Cav1.3 L271 residue. Electrophysiological studies in HEK cells overexpressing Cav1.3 L271H indicate that this mutation lowers the voltage dependency of channel activation and inactivation, thereby permitting increased subthreshold inward Ca2+ currents. However, currently no functional studies are available how this mutation affects early neurodevelopment or the physiology of disease-relevant human neurons. Here, we describe the generation of an induced pluripotent stem cell (iPSC)-line, carrying the heterozygous Cav1.3 L271H mutation, through reprogramming of peripheral blood mononuclear cells (PBMC) obtained from a patient diagnosed with a severe neurodevelopmental disorder. This novel iPSC line was used to establish a disease model including multiple stages of neurodevelopment, comprising neural progenitor cells (NPC) and human (midbrain) neurons. Functional analysis of the cells revealed alterations in calcium signaling and electrical activity affecting the resting membrane potential, firing frequency and action potential shapes of the NPCs as well as neurons. Additionally, three-dimensional cell culture systems of early human neurodevelopment revealed structural alterations, indicating a deficit in neural rosette self-organization capacities of the mutated cells. Furthermore, transcriptomic analysis of the patient-derived cell lines highlights the impact of upregulated genes, which have previously been associated to ASD and other neurodevelopmental disorders. Overall, this study will broaden our understanding of the role Cav1.3 channels play during neurodevelopment and how such gain-of-function mutations contribute

to CACNA1D channelopathies, thereby paving the way for novel therapeutic strategies for affected individuals.

Keywords: Voltage-gated calcium channel, iPSC-based disease model, Neurodevelopment and neuronal function

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DEVELOPMENT OF IPSC-DERIVED PRE-CLINICAL DRUG SCREENING ASSAYS FOR KIF1A ASSOCIATED NEUROLOGICAL DISORDER (KAND)

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KIF1A Associated Neurological Disorder (KAND) is a rare and progressive neurodegenerative disorder caused by mutations in the KIF1A gene. Affecting over 500 diagnosed individuals, this disorder has a broad phenotypic presentation, including spastic paraplegia, seizures, hypotonia, optic nerve atrophy, cerebral and cerebellar atrophy, and intellectual disability. Despite the overwhelming need for therapeutics, there are no clinical trials, let alone approved therapies for KAND in which the lack of established and reproducible assays for therapeutic discovery and development is a major barrier. Therefore, we generated iPSC-derived glutamatergic and GABAergic neurons from E253K and P305L KIF1A mutation patients in order to develop pre-clinical assays for KAND drug discovery. Here, we describe phenotypic assays that represent the clinical manifestations of KAND i.e., seizures and developmental delay, that can be used to screen for therapeutics. KIF1A mutations lead to impairments in neurotrophic support of axonal outgrowth, cargo trafficking deficits, as well as altered neuronal electrophysiology in iPSC-derived neurons. These KIF1A mutant phenotypes further our understanding of KAND biology and also represent translatable biomarkers that can be readily adapted to high-throughput screening platforms to identify KAND therapeutics.

Keywords: KIF1A, Seizurogenics assay, Drug-sceen

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IDENTIFYING HISTONE POST-TRANSLATIONAL MODIFICATIONS DRIVING GLOBAL TRANSCRIPTIONAL DYSREGULATION IN DOWN SYNDROME

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Down syndrome (DS), the most common genetic form of intellectual disability (ID) with a prevalence of 1 in 750 live births, is caused by triplication of chromosome 21 (HSA21). Although only the genes on HSA21 are triplicated, trisomy 21 nevertheless results in global transcriptional dysregulation. Gene expression is altered both spatially, depending on the tissue, and temporally, depending on the developmental timepoint. Defining the dynamics of this complex differential gene expression is crucial to understanding the clinical hallmarks of DS. We hypothesize that epigenetic changes, namely histone post-translational modifications (PTMs), drive this dynamic transcriptional dysregulation. To investigate the connection between changes in histone PTMs and differential gene expression, we utilize isogenic iPSCs derived from individuals with DS that are differentiated into multiple neuronal cell types. We have identified altered abundance of specific histone methylation and acetylation marks that are consistent across cell type and individual, including changes that are cell-type specific. To understand how these alterations affect gene expression, we perform Cleavage Under Targets and Tagmentation (CUT&Tag) coupled with RNA-seq to correlate changes in chromatin binding with transcriptional output. This work begins to decode the molecular underpinnings of the global transcriptional dysregulation found in DS and lays the foundation for correlating these molecular changes to distinct cellular phenotypes.

Keywords: Down Syndrome, Epigenetics, Histone modifications

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DISEASE MODELING OF NGLY1 DEFICIENCY WITH PATIENT IPSC-DERIVED BRAIN ORGANOIDS

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N-glycanase 1 deficiency (NGLY1 deficiency) is a rare, autosomal recessive disorder caused by mutations of the N-glycanase 1 gene (NGLY1). The first patient was reported in 2012 and the total number of diagnosed cases is now approximately 100, with more than a dozen patients waiting for a diagnosis. Typical features of NGLY1 deficiency are global developmental delay, hypotonia, abnormal involuntary movements, poor tear production, microcephaly, intractable seizures, EEG abnormalities, and liver disease. There are no approved treatments to date. It is also unknown how exactly NGLY1 mutations cause these symptoms. In this study, we investigated the pathogenesis of NGLY1 deficiency using iPSC-derived cortical organoids (COs) from patients and isogenic controls generated by the CRISPR-Cas9 system to explore effective drugs. Immunohistochemical analysis and transcriptional profiling of organoids on day 26 of differentiation were carried out. There were morphological alternations and some of the most up-regulated differentially-expressed genes (DEGs) included genes that play an important role in GABAergic interneuron of medial ganglionic eminence (MGE). Laser capture microdissection of COs followed by gene expression profiling revealed that NGLY1-deficient COs have a neuronal heterotopia-like phenotype characterized by GABAergic interneurons located in regions that do not follow differentiation conditions of cortical brain organoids. From a disease-related gene network generated by artificial intelligence using curated information and DEGs of COs, it was found that approved drugs targeting dopamine receptor D2 (DRD2) and GABAergic neurons are likely to have therapeutic effects. This study will deepen our understanding of the pathogenesis of NGLY1 deficiency and highlight the utility of patient

iPSC-derived brain organoids for modeling ultra-rare neurodevelopmental disorders in humans.

Keywords: NGLY1 deficiency, Brain organoid, Neurodegenerative disease

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LONGITUDINAL PROGRAMS OF CELLULAR DIVERSIFICATION OF THE HUMAN CEREBRAL CORTEX IN BRAIN ORGANOIDS

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Realizing the full utility of brain organoids to study human cortical development requires understanding whether organoids precisely replicate endogenous cellular and molecular events of the embryonic human developing cortex. Here, we present a comprehensive single-cell transcriptomic, epigenetic, and spatial atlas of human cortical organoid development, comprising over 610,000 cells, from generation of neural progenitors through production of differentiated neuronal and glial subtypes. We show that processes of cellular diversification correlate closely to endogenous ones, irrespective of metabolic state, empowering the use of these data to study human fate specification. We define longitudinal molecular trajectories of cortical cell types during organoid development, identify genes with predicted human-specific roles in lineage establishment, and uncover early transcriptional diversity of human callosal neurons. Based on this latter finding, we are now further leveraging cortical organoids to explore the mechanistic underpinning of callosal projection neuron expansion and diversification in the human cerebral cortex. In sum, our work provides a comprehensive, single-cell molecular map of cortical organoid development, and validates this comprehensive atlas of human corticogenesis in vitro as a resource to prime investigation into the mechanisms of human cortical development.

Keywords: Human cortical development, Human brain organoids, Cellular diversification

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DERIVATION OF POSTERIOR AXIAL ASSEMBLOIDS USING HUMAN STEM CELLS

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During embryonic development, the post-cranial body axis is constructed from a highly proliferative population of axial progenitors (AxPs) located at the caudal tip of the embryo. AxPs simultaneously self-renew and generate neural tube (NT), neural crest (NC), and somatic mesoderm (SM) progenitor cells. We have developed a monolayer protocol that can recreate the AxPs lineage tree from human pluripotent stem cells in vitro. Through modulation of growth factor conditions, we can generate and maintain AxPs that go through colinear Hox activation and subsequently derive pre-somitic mesoderm (PSM), pre-neural crest (NC), ventral neural progenitors (vNT) and dorsal neural progenitors (dNT) of distint A-P levels. We generate organoids from these distinct lineages and assemble these into sophisticated 3D Posterior Axial Assembloids (PAXAs). The PAXAs can be maintained in vitro for months at a time, express mature neural and mesodermal markers and display spontaneous contractions. We anticipate that our protocols and PAXAs will aid in vitro modeling of human development and neuromuscular diseases.

Keywords: Assembloids, Anterior-Posterior programing, Neuromuscular Junction

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CHEMICAL GENETIC DISSECTION OF CANONICAL BAF COMPLEX FUNCTION DURING CEREBRAL CORTEX DEVELOPMENT

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SWI/SNF ATP-dependent nucleosome remodeling complexes (also known as BAF complexes) remodel nucleosomes at cis-regulatory elements to facilitate binding of transcription factors and promote transcription. The canonical BAF complex (cBAF), characterized by incorporation of ARID1 subunits (ARID1A or ARID1B), functions primarily at enhancers to facilitate cell type-specific gene expression programs. Mutations in Arid1a are frequently observed in a variety of human cancers, whereas heterozygous loss-of-function mutations in Arid1b are one of the most common large-effect mutations observed in children with neurodevelopmental disorders. However, the specific functions of ARID1A/cBAF and ARID1B/cBAF complexes in gene regulation during brain development have not been defined and thus the mechanistic underpinnings of neurodevelopmental disorders in individuals with Arid1b mutations remain unclear. To delineate the specific functions of ARID1A and ARID1B/cBAF complexes during embryonic development, we generated inducible degron alleles (Arid1adTAG, Arid1bdTAG) in mouse pluripotent stem cells. The Arid1adTAG allele allows for rapid, inducible, and reversible degradation of ARID1A protein. We performed extensive validation experiments to confirm that dTAG addition to ARID1 proteins does not disrupt their function. Importantly, we demonstrate that constitutive degradation of ARID1AdTAG phenocopies a null mutation in a spontaneous differentiation assay. Using a novel protocol developed in our lab, we generated cerebral cortical organoids from Arid1adTAG, Arid1bdTAG EpiSC lines. Arid1b+/dTAG cortical organoids are akin to an inducible model of Arid1b haploinsufficiency, and can be used to examine the impact of stage-specific Arid1b loss-of-function as well as to perform rescue experiments. With these new models, we are charting the direct/proximal functions of ARID1A/cBAF and ARID1B/cBAF complexes in gene regulation during cerebral cortex development using scRNA-seq. Taken together, our data provide a proof-of-concept that degron alleles can be used in neural organoids to characterize mechanisms of gene regulation during brain development, with important implications for the design of new cellular models of neurodevelopmental disorders.

Keywords: neurodevelopmental disorders, brain organoids, transcriptional regulation

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UNDERSTANDING PARKINSON'S DISEASE PATHOGENESIS THROUGH REGIONALLY SPECIFIC ASTROCYTES

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Mutations and/or deletions in LRRK2, SNCA and PRKN genes result in a high probability of developing Parkinson's disease (PD), suggesting a critical role for these genes. While the loss of pigmented dopamine neurons in the substantia nigra is an established pathology seen in these genetic backgrounds, the potential role of astrocytes is unknown. Astrocytes are seen in the areas of neurodegeneration and sometimes pathological profiles, which was previously considered a downstream response to neuronal death, however, recent evidence suggests these cells may also actively participate in disease progression. To understand this role, we will use induced pluripotent stem cells from patients with mutations or deletions in the LRRK2, PRKN and SNCA genes, as well as unaffected controls, and differentiate them into regionally specified astrocytes. By creating astrocytes with regional identity i.e., cortex or ventral midbrain, we aim to more specifically model astrocyte-associated pathological phenotypes associated with different brain regions. i.e., are ventral midbrain astrocytes more/less affected by certain PD mutations when compared to cortical astrocytes? We will explore differences in gene and protein expression and will also co-culture astrocytes with healthy neurons in vitro. To further extend on this, these patient-derived astrocytes will be studied in the environment of a living brain through xenotransplantation which will provide a unique paradigm to reveal temporal, spatial and cellular components of non-cell intrinsic PD pathobiology.

Funding Source: Aligning science across Parkinson's (ASAP); The Michael J. Fox Foundation for Parkinson's Research.

Keywords: Regionally specific astrocytes, Stem cell derived Astrocytes, LRRK2, SNCA, PRKN

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MODELING THE HUMAN NEUROMUSCULAR JUNCTION IN VITRO

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Neuromuscular junctions (NMJ) are affected early in amyotrophic lateral sclerosis (ALS). An in vitro model that replicates the NMJ and its destruction in ALS would further our understanding of this early event in the disease and may aid in finding disease modifiers. To generate a reproducible in vitro system of the NMJ, as well as modelling its early destruction in ALS, we differentiate human pluripotent stem cells (PSCs) into spinal motor neurons (MN) and skeletal muscle from a healthy donor and isogenic lines with genome engineered ALS-causing mutations. Maturation of human iPSC-derived myofibers is assessed by myofiber striation with immunostaining of TITIN, a protein of the contractile apparatus. Preliminary work to validate the generation of functional NMJs was done using human primary myoblast-derived myotubes expressing the fluorescent calcium indicator MHCK7::GCaMP cultured with PSC-derived MNs. Functional connectivity was detected by an increase in fluorescence intensity produced by the calcium indicator of the myotubes after glutamate stimulation of MNs. Interactions between MN processes and AChR clusters on myotubes were detected by immunostaining for TUBB3, SV2A, and BTX, indicating formation of NMJ-like structure. Research in progress in this project includes RNA-sequencing to analyze the transcriptomic changes derived from MN-myofiber interaction in ALS, and thus, studying the role of each cell type in the disease.

Keywords: neuromuscular junction, spinal motor neurons, amyotrophic lateral sclerosis

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INVESTIGATING THE ROLE OF NUCLEOLIN IN NEURAL REPAIR POST-STROKE

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Stroke occurs in two ways; ischemic stroke where an artery is blocked, or hemorrhagic stroke which is a burst of a blood vessel. Specifically in ischemic stroke, brain repair is limited. The process called axonal sprouting is a process which encourages brain recovery post stroke injury. Axonal sprouting takes place from the motor cortex to the premotor cortex. Stroke is the leading cause of adult disability, affecting around 800,000 individuals each year. Current therapy options for individuals are limited and difficult to find. Therefore, we look to investigate potential therapies for which the brain can recover after injury. My project is concerned with investigating the role of nucleolin in neural repair post-stroke. Behavioral tests, pasta matrix and gid walk analysis were conducted on the cohorts in order to investigate the effects of nucleolin inhibition in functional recovery. Cohorts were euthanized at 30 days following brain mapping analysis. Results showed stroke cohorts did in fact regain motor function after treatment. Behavioral tests, pasta matrix and grid walk, showed virus and stroke cohorts improved motor function compared to control and stroke cohorts.

Funding Source: Mike Fainzilber, Ph.D. Department's of Biomolecular Sciences and Molecular Neuroscience Weizmann Institute of Science S. Thomas Carmichael, M.D., Ph.D. Department. of Neurology UCLA

Keywords: mice, vmouse stroke model, neurology

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IN VITRO GENERATION OF PSEUDOUNIPOLAR RODENT AND HUMAN IPSC-DERIVED SENSORY NEURONS

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While multiple protocols describe the generation of human sensory neurons (SNs) from iPSCs, proper recapitulation of specific

hallmarks of SN morphology and physiology remains challenging. A unique feature of SNs is their specialized pseudounipolar morphology. This property is a crucial prerequisite for SN function and signal transduction from the periphery to the CNS and has not been convincingly shown in human in vitro models so far. Recent data indicate that cultured rodent neurons depend on the presence of dorsal root ganglia (DRG) glial cells to transition into a pseudounipolar morphology. These pseudounipolarized rat DRG neurons recapitulate the formation of a thin and a large diameter axon, resembling the central and peripheral axon development respectively. Live imaging of DRG explants from Thy1-EB3-GFP mice revealed an asymmetric microtubule dynamic in DRG neurons, with the central axon displaying a more dynamic cytoskeleton. The asymmetric cytoskeletal dynamics were recapitulated in in vitro pseudounipolarized rat DRG neurons. We became interested in the question whether these findings could be translated to human iPSC-derived SNs. To address this question we used froward programmed SNs generated by overexpression of the transcription factors NGN1, BRN3A and ISLET1 ('NBI') in iPSCs. After 7 days of continuous transgene expression we observed robust formation of SNs expressing PRPH, NTRK1, TRPV1, NAV1.7 and NAV1.8. IPSC-derived SNs are electrophysiologically functional and show TTX-resistant currents, a hallmark of nociceptive sensory neurons. While their expression profile and electrophysiological properties recapitulate a human SN phenotype, a mature pseudounipolar morphology was not observed. However, upon a 3-week co-culture of 'NBI' SNs with non-neuronal cells from rat DRG more than 20% of the human SNs transitioned into a pseudounipolar morphology, while the number of multipolar neurons was significantly reduced. Our data indicates that an in vivo-like environment containing non-neuronal DRG cells triggers the development of an pseudounipolar morphology in forward programmed human SNs. We expect these cells to provide a more authentic tool for studying human SN development, in vitro disease modeling and drug discovery.

Keywords: iPSC-derived sensory neurons, Dorsal Root Ganglion, Pseudounipolar Neurons

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IMPACT OF CELLULAR METABOLISM ON CORTICOGENESIS: AN OBSERVATION FROM HUMAN CORTICAL ORGANOID CULTURE

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3D-cultured human induced pluripotent stem cell-derived cortical organoids can mimic broad features of developing human cortex. However, even with the best protocol developed so far, many fundamental aspects of human corticogenesis have not been fully recapitulated in organoid culture yet. This has been partially due to aberrantly accelerated radial glia differentiation in an overall non-ideal in vitro environment. Here, our preliminary study has shown that, after being cultured in hypothermal conditions to slow down metabolism, the cortical organoids can have significantly enlarged size and more importantly, a remarkably increased neuronal production, particularly the upper-layer cortical neurons, which led to improved cortical lamination. Such phenotype can be attributed to an enlarged proliferating progenitor pool and a prolonged neurogenic time window. Further investigation has suggested boosted mitochondrial activity as one of the underlying mechanisms. Our study revealed an unexpected relationship between cellular metabolism and corticogenesis, and strongly suggests that metabolic manipulation holds great promise as a generalizable strategy to modulate in vitro model of neurodevelopment.

Funding Source: the Simons Center for the Social Brain Postdoctoral Fellowship at MIT

Keywords: brain organoid, corticogenesis, metabolism

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OPTICAL ALPHA SYNUCLEIN AGGREGATION INDUCTION SYSTEM-BASED COMPOUND SCREENING PLATFORM FOR PARKINSONS DISEASE

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Parkinson's disease (PD) is a devastating age-related neurodegenerative disease characterized by the loss of midbrain dopaminergic (mDA) neurons with pathological aggregation of alpha-synuclein (a-syn). Human induced pluripotent stem cell (hiPSC) technology makes it possible to acquire mDA neurons of PD patients. However, it is difficult to model late-onset human disease including PD because the reprogramming of somatic cells to iPSCs resets their pathological state. In this study, we developed an optogenetics a-syn aggregation induction system (OASIS) that uses optogenetic proteins to allow light-induced control of protein interactions. The OASIS can optically aggregate ±-syn in a light-dependent manner and an exceptionally short temporal window and leads to phosphorylation of ±-syn aggregates, induces cellular toxicity in PD hiPSC-derived mDA neurons and midbrain organoids (MOs). In addition, we developed OASIS-based compound screening platform in human neuronal cells and identified two candidate small molecules that considerably decrease the pathogenic ±-syn characters and significantly rescue the neurotoxicity in PD hiPSC-derived mDA neurons, MOs. Moreover, we validated the efficacy of one small molecule, named compound#1, on ±-syn preformed fibril (PFF)-induced PD-like symptom in in vivo mouse model by a wide range of pathological, behavioral, and biochemical studies as well as on mouse primary neurons, PD hiPSC-derived mDA neurons, suggesting the molecule could be a potent candidate for alleviating PD symptoms. We also found that compound#1 activated autophagic flux by inhibiting the PI3K-PDK1/AKT/mTOR signaling pathway to induce clearance of ±-syn aggregates. Our OASIS provides a new hiPSC-based disease modeling and drug screening platform for PD.

Keywords: Parkinson's disease, human iPSCs, alpha synuclein aggregation

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CREATION OF A MODEL FOR FRIEDREICH'S ATAXIA BY INDUCING PATIENT-DERIVED IPSC CELLS TO RETINAL GANGLION CELLS

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Friedreich's Ataxia (FRDA) is autosomal recessive disease caused by GAA trinucleotide repeat expansion in frataxin (FXN) gene intron 1. FRDA affects multiple organs, such as central and peripheral nervous system, heart. 30% of the FRDA patients reported visual ophthalmic manifestations, including optic neuropathy and retinitis pigmentosa-like syndrome. Optical Coherence Tomography (OCT) also showed decreased ganglion cell complex (GCC) and mean peripapillary retinal nerve fiber layer (RNFL) thickness. Since mouse model cannot recapitulate the retinal ganglion cell (RGC) loss phenotype, in this study, we used FRDA patient derived iPSC and iPSC derived RGCs as guide RNA screening platform and disease model. The aim of the therapy is to increase FXN protein expression level by 2-fold as indicated by FARA. We developed a chemical defined RGC differentiation protocol, using AggreWell to make embryoid body (EB) for the first 4 days of differentiation with dual Smad inhibition, then expand retinal neuronal progenitor cells (RPC) in monolayer for another 14 days, specify the PRCs to retinal ganglion progenitors (RGP), and mature RGPs to Brn3b(+)/CD90(+) RGCs. Flow cytometry analysis (FCA) and ELISA assays showed 2.4±0.41-fold increase of FXN protein in patient iPSC lines with the current gene therapy strategy. Matured RGCs robustly express RGC markers, such as Brn3a, Brn3b, TuJ, RBPMS, etc. FRDA patient iPSC showed impaired neurite growth, decreased mitochondria membrane potential and increased apoptosis, which recapitulated phenotype of FRDA pathology. In summary, FRDA patient iPSC and iPSC derived RGCs exhibit a spectrum of molecular and cellular defects of FRDA which can be corrected by the current gene editing strategy. This study underscores the application of patient derived iPSC and iPSC derived, relevant cell type in gene therapy discovery and preclinical stage when there is no feasible animal model.

Keywords: Friedreich's Ataxia, inducible pluripotent stem cell (iPSC), retinal ganglion cells (RGC)

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MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF HUMAN THALAMOCORTICAL AND CORTICOTHALAMIC INTERACTIONS IN IPS CELL-DERIVED ASSEMBLOIDS

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Brain organoids and assembloids are useful techniques for understanding the human brain development and pathogenesis of neuropsychiatric disorders. Because thalamocortical neural circuits are pivotal for higher brain functions and neurodevelopmental disorders such as autism spectrum disorders, reconstructing and characterizing human thalamocortical interactions in vitro is an essential approaches to human neurobiology. Despite the generation of human thalamocortical assembloids, it remains unknown how much the assembloids mimic thalamocortical interactions of the brain. Here, we report cell type-specific circuit organization, molecular mechanisms of axonal projections, and structural and functional maturation in human thalamocortical assembloids using the CRISPR-Cas9 system, rabies monosynaptic tracing, enhancers, tissue clearing, and two-photon imaging. We directed the differentiation of human induced pluripotent stem cells to thalamic organoids and cortical organoids each of which expressed brain region-specific genes. We subsequently fused the thalamic and cortical organoids to generate thalamocortical assembloids. Labeling the organoids with fluorescent proteins visualized reciprocal projections in the assembloids. These axonal projections were steered by guidance molecules. Trans-synaptic tracing using rabies viral vectors showed synaptic connections between the thalamic organoid and cortical organoid in the assembloids. Moreover, corticothalamic neurons and pyramidal tract neurons in the cortical region labeled with adeno-associated viruses carrying cell type-specific enhancers expressed layer-specific genes and projected to the thalamic region in the assembloids. These excitatory neurons were more mature in the assembloids than in the cortical organoids alone. Furthermore, two-photon imaging revealed mature spontaneous activity in cortical neurons of the assembloids. These results demonstrate that thalamocortical assembloids faithfully recapitulate thalamocortical interactions in the developing human brain. The assembloid we produced here will facilitate understanding human brain development and developing drugs for neurodevelopmental disorders.

Keywords: Neural organoid, Neural assembloid, Viral vector

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INVESTIGATING THE ROLE OF NEUROINFLAMMATION IN AMYOTROPHIC LATERAL SCLEROSIS USING HUMAN IPSC DERIVED MICROGLIA

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Amyotrophic lateral sclerosis is a multifactorial neurodegenerative disease characterised by the irreversible loss of upper and lower motor neurons. Accumulating evidence suggests that non-cell autonomous mechanisms are involved in ALS pathology and motor neuron degeneration itself is accompanied by an inflammatory response. Microglia activation is evident in post-mortem brain tissues and neuroimaging data from patients with ALS. However, the mechanisms of microglia-mediated neurotoxicity and loss of homeostatic functions remain elusive. Therefore, delineating microglia phenotypes in ALS is now imperative. To address this, we derived induced pluripotent stem cell-microglia from patients with ALS caused by mutations on TDP-43 and C9ORF72 genes. ALS-microglia exhibited a significant impairment in homeostatic functions such as phagocytosis and migration, upregulated release of inflammatory cytokines and reactive morphologies consistent with a neuroinflammatory phenotype. RNA sequencing revealed that these mutations promote a transition to Interferon Response Microglia (IRM) accompanied by differentially expressed or spliced genes involved in apoptosis, endocytosis, formation of the inflammasome and cytoskeleton organisation. Finally, ALS-microglia recapitulated the pathological hallmark of TDP-43 cytoplasmic localisation and phosphorylated-TDP-43-positive inclusions. A deeper understanding of the role of microglia in the cellular level of ALS is expected to unveil microglia heterogeneity and impact on motor neuron survival, leading towards the development of mechanistically rationalised therapies.

Funding Source: Wellcome Trust

Keywords: Microglia, human iPSCs, Amyotrophic Lateral Sclerosis

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INVESTIGATING ENVIRONMENTAL NANOPLASTIC TOXIN IN PROMOTING THE ONSET OR AGGRAVATION OF NEURODEGENERATIVE DISEASE

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Incidence of neurodegeneration has increased over the past decades, but this is not entirely explained by longer lifespans. We noted that this coincides with the increased use of single-use plastics from the 1970s. Various studies have reported increase oxidative stress following accumulation of nanoplastic in the brain tissue. Increase oxidative stress may affect many cellular processes leading to apoptosis and neuroinflammation, a hallmark of neurodegenerative disease. In this study, we investigated if polystyrene nanoplastics (PS-NPs) can result in an onset or exacerbation of neurodegenerative disorders. Our current work reveals that 50nm PS-NPs can be efficiently taken by human neurons and iPSC-derived motor neurons (MNs). PS-NPs exposure reduces survival, neuronal health (neurite length, soma size) and mitochondrial function. Importantly, Pull-down assay revealed that PS-NPs can bind to TDP43 and result in hyperphosphorylation of TDP43, which is a protein implicated in both ALS (motor neurons disease) and frontal temporal dementia (FTD). Finally, we found that concurrent treatment of activated carbon and PS-NPs reduces bioaccumulation of PS-NPs in spinal motor neurons. Therefore, our study demonstrated that environmental nanoplastic pollution can bind to key proteins involved in neurodegeneration. Its exposure has demonstrated to reduce neuronal health and survival, which can be ameliorated by treatment with activated carbon.

Keywords: NEURODEGENERATIVE DISEASE, NANOPLASTIC, Neurotoxic hazard

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COMPARISON OF DIFFERENTIATION PROTOCOLS FOR PATIENT-DERIVED IPSC ASTROCYTES TO MODEL ALS DEGENERATION

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Astrocytes have been recently recognized as key players in the pathogenesis of neurodegenerative diseases, such as Amyotrophic lateral sclerosis (ALS). Previously, astrocytes were thought to only have structural support roles in the brain, but have since been proven to have immune functions, maintain homeostasis, and induce synapse formation. All those functions have been shown to be dysregulated in ALS. Often, rodent astrocytes are utilized to study neurodegenerative diseases. While these models are helpful, human astrocytes have many morphological and molecular differences from rodents, which may not present the whole picture when studying ALS. Human-derived induced pluripotent stem cells (iPSCs) are a new tool that can help us to model disease mutations and their functional consequences more accurately. Currently, there is no consensus on the gold standard astrocyte differentiation protocol. Here we seek to identify a robust and reproducible protocol to generate ALS and control as-

trocytes. To this end, we characterized and compared two popular astrocyte differentiation protocols using cells from patients who have the ALS C9orf72 mutation and a wild-type cell line. We will investigate the transcriptomic, protein level, and functional changes between the different protocols as well as directly compare changes between the C9 and WT lines. To assess astrocyte functionality, we will evaluate the phagocytic ability and glutamate uptake. Our work will identify a suitable protocol resulting in mature astrocytes that can be used for ALS drug screening in mono- and tri-culture assays. Future research will involve integrating the iPSC-derived astrocytes into tri-cultures that may lead to discovering astrocyte-specific pathways in ALS.

Keywords: astrocytes, amyotrophic lateral scleross, differentiation

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DISTINCT PHENOTYPES IN 2D AND 3D IPSC-DERIVED NEURONAL MODELS FOR CEP290 ASSOCIATED-CILIOPATHIES

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Ciliopathies are a group of human Mendelian disorders caused by dysfunction of primary cilia (PC), small ubiquitous sensory organelles protruding from the surface of most cells, required for signal transduction. Ciliopathy patients show various symptomes, with frequent central nervous system (CNS) involvement. Joubert Syndrome (JBTS) is a representative ciliopathy and a neurodevelopmental disorder. It is characterized by a highly specific mid-hindbrain malformation whose underlying pathomechanism remains unclear. Moreover, the presence of non-structural CNS defects such as seizures or intellectual disability implies a role for PC in neuronal function beyond transmission of developmental signaling pathways. To understand the JBTS-specific CNS malformation, we are generating iPSC-derived in vitro models for JBTS. After using CRISPR editing to knockout the JBTS-associated gene CEP290 in human iPSCs, we apply 2D and 3D protocols to generate neural stem cells, cortical neurons and cerebral organoids. CEP290 mutant iPSCs show a higher propensity of differentiating into FOXG1-expressing NSCs in a 2D protocol than the isogenic parental line. This difference can be corrected by inhibiting Wnt signaling during the differentiation. We further characterized the CEP290 interactome in NSCs, identifying an interaction with APC and β-catenin, both involved in Wnt signaling. Together, these findings suggest a link between cilia/CEP290 and Wnt signaling in human NSCs. Current experiments follow up on this finding. Preliminary data indicate that loss of CEP290 does not preclude generation of cerebral organoids, but might affect organoid patterning. Interestingly, loss of this ciliary gene does not alter cilia number and length in 2D neuronal cultures but leads to strikingly aberrant ciliary morphologies in a subset of cells in 3D cerebral organoids. Applying different differentiation protocols to the same mutant iPSC lines may identify cell-type specific functions for ciliary genes, where different neuronal cells suffer distinct consequences from their loss of function. It becomes apparent that modeling a complex genetic disease requires various models to elucidate underlying pathomechanisms.

Keywords: Joubert Syndrome, Organoids, cortical NSCs

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ELEVATED LEVELS OF FMRP-TARGET MAP1B IMPAIR NEURONAL DEVELOPMENT AND SOCIAL BEHAVIORS VIA AUTOPHAGY PATHWAY

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Fragile X messenger ribonucleoprotein 1 protein (FMRP) binds many mRNA targets in the brain. However, the contribution of these targets to fragile X syndrome (FXS) and related autism spectrum disorder (ASD) remain unclear. Here, we show that FMRP deficiency leads to elevated microtubule-associated protein 1B (MAP1B) in developing human and nonhuman primate cortical neurons. Targeted MAP1B gene activation in healthy human neurons or MAP1B gene triplication in ASD patient-derived neurons inhibit morphological and physiological maturation. Activation of Map1b in mouse prefrontal cortex excitatory neurons impairs social behaviors. We show that elevated MAP1B sequesters components of autophagy and reduces autophagosome formation. Both MAP1B knockdown and autophagy activation rescue deficits of both ASD and FXS patients' neurons and FMRP-deficient neurons in ex vivo human brain tissue. Our study demonstrates conserved FMRP regulation of MAP1B in primate neurons and establishes a causal link between MAP1B elevation and deficits of FXS and ASD.

Keywords: MAP1B elevation, FXS and ASD pathogenesis, Autophagy activity

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MYELOMENINGOCELE HUMAN AMNIOTIC FLUID STEM CELLS-DERIVED SPINAL CORD ORGANOIDS HAVE A UNIQUE GENE EXPRESSION PROFILE AND EXHIBIT ELECTRIC ACTIVITY

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Myelomeningocele (MMC) is a severe form of spina bifida resulting from failed fusion of the caudal region of the neural tube during embryonic development. This study sought to characterize functional dorsal spinal cord organoids (SCOs) generated from human amniotic fluid-derived induced pluripotent stem cells (iPSCs) to in-

vestigate the mechanisms of disease in MMC. After IRB approval, second trimester human amniotic fluid samples (n=3) from healthy (control) and MMC fetuses were reprogrammed into iPSCs and subsequently differentiated into 3D SCOs based on a modified Takahashi protocol. SCOs were extensively characterized prior to functional analysis by microelectrode arrays (MEA, Maxwell Biosystems) and single cell RNA sequencing (scRNAseq). SCOs were composed of multiple neural cell types, including neuroectoderm (SOX1, PAX6), roof plate (Lmx1a), dorsal (Olig3, PAX7), and ventral (Olig2) spinal cord progenitor phenotypes by d15. Dorsal morphogens induced the formation of dorsal interneurons (Brn3a, Lbx1), whereas motoneurons (Lhx3/Islet2), oligodendrocyte progenitors (NG2), and astrocytes (GFAP) were also present. MMC cell lines had significantly fewer neuroectodermal cells than controls at d9 ($p< 0.05$), and progenitors/neurons were more restricted to the dorsal spinal cord at d24. Both MMC and control SCOs showed spontaneous electric activity with a median firing rate of 0.37 Hz and a median spike amplitude equal to 28.72 µV, indicating continuous neuronal activity. Network assay showed numerous burst peaks with a mean of 1158 spikes per burst and a burst duration of 1.42 seconds, suggesting connection between functional neurons. Preliminary scRNAseq data confirms the presence of the different neural cells and reveals that control SCO cells clustered separately from MMC SCO cells, suggesting a unique phenotype. Taken together, this study demonstrates that human dorsal SCOs with functional electrical activity can be generated from MMC fetuses, denoting a patient-specific in vitro platform to study spinal cord development and disease pathogenesis, and a potential autologous source for spinal cord regeneration.

Keywords: Spinal Cord Organoids, Neural Tube Defect, Amniotic Fluid Stem Cells

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NAD+ PRECURSOR RESCUES RETINAL DEGENERATION IN MICE AND HUMAN DISEASE MODEL OF NMNAT1 RELATED INHERITED RETINAL DISEASE

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A bi-allelic mutation in Nicotinamide mononucleotide adenylyltransferase 1(NMNAT1) causes Leber congenital amaurosis (LCA), an early-onset inherited retinal disease, in which there have been no treatment. We established a retinal organoid model using human induced pluripotent stem cells (hiPSCs) derived from a patient with the NMNAT1 c.C709T (p.R238C) mutation, the most prevalent variant of NMNAT1 related LCA in Korea. In addition, a mice model having Nmnat1 c.712C>T (p.R238C) mutation, which is the homologous mutation to human NMNAT1 c.C709T variant was constructed using CRISPR/Cas9. In both models, the relevant disease phenotypes of early-onset retinal degeneration of LCA were confirmed. The retinal organoids of NMNAT1 c.C709T LCA patients-iPSC and NMNAT1 Knock-out ESC showed neural retinal thinning and impaired RPE development, which were rescued in isogenic controls by gene editing. In Nmnat1 mouse model, early-onset retinal degeneration and visual impairment were confirmed. When Nicotinamide adenine dinucleotide (NAD+) precursors were treated in the both, in vivo and in vitro models, the disease phenotypes were partially rescued. We have shown that this result a precursor of NAD+ could be used as a therapeutic agent for NMNAT1-related inherited retinal disease.

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Keywords: Retinal organoid, Leber congenital amaurosis, NMNAT1

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SINGLE CELL CRISPR SCREENS IN IPSC DERIVED IOGLUTAMATERGIC NEURONS

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Functional genomic screening using CRISPR/Cas9 has revolutionised biology and made it possible to casually link gene function to disease phenotypes. However, outside the field of oncology, the lack of relevant tissue types available for screening remains a limiting factor. This is particularly challenging in the study of neurodegenerative diseases where primary tissue is inaccessible. Human induced pluripotent stem cell (hiPSC) derived models offer an opportunity to bypass this problem. These models provide a physiologically relevant system to advance our understanding of neurodegeneration and accelerate drug discovery programs. bit.bio discovery has developed a CRISPR perturbation screening platform to query human biology within the context of hiPSC derived ioCells™. Our opti-ox technology allows controllable expression of inducible transcription factors in hiPSCs, resulting in reliable and scalable generation of various human cell types for drug discovery. Here, we utilise our CROP-Seq workflow in ioGlutamatergic neurons to perturb genes known to be associated with various neurodegenerative diseases. We provide proof-of-concept that functional genomic screening can be performed at both iPSC stage and following forward programming into ioGlutamatergic neurons. Our data highlights the importance of timing when it comes to the identification of essential genes. While certain genes are essential at iPSC stage, they are clearly non-essential if perturbed in differentiated ioCells™. Importantly, a single-cell transcriptomic readout uncovered several examples of disease-linked genes whose knockout had transcriptomic phenotypes. Our results highlight the power of unbiased genetic screens in differentiated cells and provides a platform for systematic interrogation of normal and diseased cell states for disease modelling and target discovery.

Keywords: CRISPR, neurons, functional genomics

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MODELLING SELECTIVE SEROTONIN REUPTAKE INHIBITOR METABOLIC EFFECTS IN NEURODEVELOPMENT AND AUTISM USING HUMAN INDUCED PLURIPOTENT STEM CELLS

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Selective serotonin reuptake inhibitors (SSRIs) are among the most frequently used drugs for depression and anxiety disorders. While their use in pregnancy is considered relatively safe, in-utero exposure to SSRIs has been associated with a higher risk of neurodevelopmental disorders (NDDs), including autism spectrum disorder (ASD). Prenatal exposure in animals is known to affect multiple processes of early brain development and leads to behavioural changes. Only a few studies have used human induced pluripotent stem cell (iPSC) models to understand their impact during neurodevelopment. Studies that do exist, do not compare the molecular effects of SSRIs in routine clinical use. Here, we investigate four commonly prescribed SSRIs (fluoxetine, citalopram, sertraline, and paroxetine) using human iPSC lines in the context of ASD. We aim to identify metabolomic changes following short- and long-term exposures to each SSRI. We further test if a machine learning based prediction model could identify SSRI specific metabolomic signatures of ASD. For the study, iPSC lines from a neurotypical male, neurotypical female and two males with known genetic ASD variants were used. The iPSC-derived neuroepithelial stem cells were differentiated towards neuronal lineage with parallel SSRI exposures and sampled at day 5 and 28. Direct infusion electrospray ionisation mass spectrometry based untargeted metabolomics was performed on cell lysates, with a stratified analytical approach. Preliminary results show significant (adj. $p < 0.05$) metabolite changes across the SSRIs and exposure period. Including, paroxetine significantly increased lysophospholipid levels at both day 5 and 28, while this was only observed at day 5 for fluoxetine. Paroxetine significantly decreased plasmanylcholines at day 5 and 28. Sertraline significantly decreased amino acids and plasmanylcholines, while lysophospholipids were significantly elevated at both time points. We are now validating our findings. Several of the reported metabolites can be classified as phospholipids and amino acids, which are known to be crucial for typical neurogenesis and brain development. An understanding of the specific metabolic signatures for SSRIs in NDDs can help decide their best clinical use-case and safeguard the developing foetus and pregnancy.

Keywords: induced pluripotent stem cells, selective serotonin reuptake inhibitors, metabolomics

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INVESTIGATING HUMAN-SPECIFIC ASTROCYTE INDUCED SYNAPTOGENESIS

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Astrocytes are crucial neural cells in regulating brain development via synapse formation. This function of astrocytes is evolutionarily conserved and analogous across most species. Several studies have highlighted divergent structure, function, and gene regulation between mouse astrocytes and human astrocytes; however, how mouse and human astrocytes molecularly differ in their ability to induce synapse formation and function is not understood. Here we use induced pluripotent stem cell (iPSC) technology to generate human neurons and astrocytes and in vitro co-culture systems using novel combinations of human and rodent cells to identify contact-dependent and secreted signals human astrocytes utilize for their enhanced synaptogenic functions. We aim to determine differences in human and mouse astrocyte-neuron interactions and investigate human-specific factors that induce synapse formation. The results of these studies will provide insight on species-specific functions of astrocytes which may help accurately modeling and studying human diseases.

Keywords: astrocyte, syntaptogenesis, induced pluripotent stem cell

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COMPARISON OF A TRANSGENIC MOUSE MODEL WITH PARKINSONS DISEASE DERIVED BRAIN ORGANOIDS

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Human iPSC derived brain organoids are increasingly being used as 3D models of neurodegenerative disorders. However, this new technology still has limitations, such as the lack of immune and vascular cells. Therefore, it is essential to verify the manifestation of relevant pathological aspects in 3D models e.g. by comparing data obtained from transgenic animals with human organoids that share the disease causing mutation. Here, we examined the effect of chronic stress in two models overexpressing human a-synuclein (a-syn): a transgenic mouse model (h-a-synL62) and brain organoids derived from iPSCs of a Parkinson's disease (PD) patient. We found molecular alterations such as glucocorticoid receptor deficiency and increased a-syn protein levels in the forebrain of mice that underwent daily restraint for 6 weeks and in healthy forebrain organoids after treatment with the synthetic glucocorticoid Dexamethasone (Dex) for 2 weeks. Interestingly, endogenous and synthetic glucocorticoid exposure reduced the level of accumulated a-syn in h-a-synL62 mice and in PD patients

derived brain organoids. Importantly, this reduction of pathological a-syn levels by dex normalized functional network activity in PD patient-derived neurons to the level of control neurons, measured in a multi-electrode array. In summary, we established and validated a human 3D culture model that can be used to study particular molecular and functional alterations associated with neurodegenerative diseases, offering replacement of research animals.

Keywords: brain organoid, stress, synuclein

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REFINING THE DIFFERENTIATION HIERARCHY OF POSTNATAL HUMAN BRAIN PROGENITORS AND THEIR ROLE IN MTOR-DRIVEN EPILEPSY

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Our prior work demonstrated strategies for purifying neural stem cell (NSC) populations and downstream progenitors from primary human fetal brain, and uncovered an intermediate glial progenitor capable of generating both astrocytes and oligodendrocyte progenitor cells (OPCs). However, the stem/progenitor hierarchy governing neural and glial differentiation in the postnatal human brain remains far less understood, in part because this aspect of human biology differs substantially from rodents, where adult NSCs are relatively well-characterized. Improved knowledge of the identity, fate potential, and lineal origin of durable progenitor subpopulations of the human adult brain is crucial to unlocking mechanisms of brain tissue regeneration as well as early events in malignant transformation. Using index sorting and single-cell RNA sequencing (scRNAseq), we have uncovered new strategies to identify and prospectively enrich for distinct progenitor subpopulations from fresh human brain tissue resected from epilepsy surgeries. These include a bipotent glial progenitor that gives rise to both astrocytes and oligodendrocytes in vitro. Preliminary data suggest the in vitro self-renewal of this population is enhanced in tuberous sclerosis (TSC), a genetic condition involving hyperactivity of the mammalian target of rapamycin (mTOR) pathway. Cortical tissue is severely disrupted in TSC by large benign growths, and some patients eventually develop subependymal giant cell astrocytoma (SEGA). Bipotent glial progenitors from TSC tissue may therefore represent a premalignant state, leading to a hypothesis that increased self-renewal could be relevant to the disease trajectory through clonal competition mechanisms favoring progenitors that acquire additional oncogenic mutations. This model is being tested in vivo using fetal human NSCs transplanted intracranially in immunodeficient mice. Human NSCs are genome-edited to introduce clinically relevant mTOR pathway mutations, then co-transplanted with wild-type NSCs. Fluorescent reporters are used to separately track the expansion of mutant cells relative to control. With this model we aim to define and therapeutically disrupt intercellular competition promoting pathological expansion of mutant cells in a live animal model of human epilepsy.

Keywords: Adult Human Glial Progenitor, Tuberous Sclerosis, Clonal Progenitor Expansion

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LOSS OF CTNND2 (DELTA-CATENIN) AFFECTS NEURALIZATION AT THE EARLY STAGE OF NEUROGENESIS

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Loss of CTNND2 has been identified in human patients with various neurodevelopmental disorders such as autism, intellectual disability, learning difficulties and reading disabilities. The encoded protein, delta-catenin, is a brain-specific component of the cadherin-catenin cell adhesion complex. How loss of functional CTNND2 affects neurodevelopment remains, however, unclear. To study disease-relevant cell types, we took advantage of induced pluripotent stem cell (iPSC) technology and have developed an advanced in vitro neural cell model to study the molecular mechanisms caused by the CTNND2 mutation. We have established iPSC lines from three individuals carrying pathogenic variants that leads to the heterozygous loss of CTNND2, and three healthy control individuals. To model disease, we then directed the iPSCs into long-term neuroepithelial stem cells (NESCs). Our results show a clear phenotype at the early stage of neuralization with the patient-derived iPSCs displaying an abnormal formation of neural rosette structures and a failure in establishing stable neural stem cells. Preliminary transcriptome data analysis from different time points of neural induction reveals deregulation of the WNT signaling pathway. When we removed the WNT signaling activator (CHIR: the GSK3 inhibitor) from the neural induction process, we observed a partial phenotype rescue of the patient cells. Altogether, our work increases the understanding of how loss of CTNND2 impacts the early stages of neurogenesis and neuronal maturation.

Keywords: Disease modelling, neurodevelopmental disorder, delta-catenin, CTNND2, neuralization, neuroepithelial stem cells, neural rosette formation, WNT signalling, early neurogenesis

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IMPAIRMENT OF NEURONAL NETWORK ACTIVITY IN IPSC-DERIVED NEURONS CARRYING THE CHROMOSOME 16P11.2 DUPLICATION IS RESCUED BY CO-CULTURE WITH NON-CARRIER ASTROCYTES

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Copy number variants (CNVs) in the 16p11.2 region are associated with a wide spectrum of neuropsychiatric and neurodevelopmental disorders, but the mechanisms by which deletion and duplication (dup) within this genomic segment lead to neuropsychiatric disorders are poorly understood. Astrocytes are the most abundant glial cell type in the human central nervous system and play crucial roles in regulating neurophysiology, and alterations in neuron-glia interactions are implicated in a variety of neuropsychiatric disorders. Preliminary studies from this lab showed that astrocytes derived from 16p11.2dup neural progenitor cells appeared less mature compared with those from non-carriers. Here, we further explore the effects of the16p11.2dup on neurons and astrocytes by using high-density microelectrode arrays to study time-dependent interactions between human induced pluripotent stem cell (iPSC)-derived cortical neurons and astrocytes. We compared network activity and dynamics in 6 co-culture conditions for 7 weeks: 16p11.2dup neurons (4 samples) with (1) 16p11.2dup astrocytes (2) non-carrier astrocytes or (3) no astrocytes; non-carrier neurons (6 samples) with (4) 16p11.2dup astrocytes (5) non-carrier astrocytes or (6) no exogenous astrocytes. When compared with non-carriers, 16p11.2dup neurons have decreased spontaneous network burst activity. No astrocyte shows no significant difference from non-carrier astrocyte co-culture with iPSC-derived cortical neurons. Moreover, non-carrier neurons co-cultured with 16p11.2dup astrocytes show impaired network activities with a reduced number of spikes per burst, burst peak fire rate, burst duration, and the inter-burst interval that begins early and recovers after 6 weeks. Notably, co-culture of non-carrier astrocytes with 16p11.2dup neurons improves network activity. Overall, our results demonstrate impairment in neuronal network activity in 16p11.2dup iPSC-derived cortical neurons that is partially rescued by co-culture with non-carrier astrocytes. These findings add to the growing evidence for the important role of astrocytes in the pathophysiology of neuropsychiatric disorders.

Keywords: MEA, Cortical Neurons, Astrocytes

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REDUCED APICAL CONSTRICTION IS A SHARED PATHOGENIC MECHANISM FOR VALPROIC ACID EXPOSURE AND SHROOM3 KNOCKOUT IN A BRAIN ORGANOID MODEL OF NEURAL TUBE DEFECTS

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Neural tube defects (NTDs) including anencephaly and spina bifida are common major malformations of fetal development resulting from incomplete closure of the neural tube. These conditions lead to either universal death (anencephaly) or life-long severe complications (spina bifida). Despite hundreds of genetic mouse models having neural tube defect phenotypes, the genetics of human neural tube defects is poorly understood. Furthermore, pharmaceuticals thought to be safe (e.g. antiseizure medications) have been found clinically to increase the risk of NTDs when administered during pregnancy. Using our self-organizing single rosette spheroid (SOSRS) brain organoid system, we have developed a high-throughput image analysis pipeline for evaluating SOSRS structure. Small molecule inhibition of apical constriction proteins rho-kinase (ROCK) and non-muscle myosin dramatically increased cell apical surface areas and overall size of the lumen. Similarly, treatment with valproic acid (VPA), a known cause of neural tube defects, increases this same metric in a dose-responsive manner. In fact, the normalized lumen size was the most instructive feature for VPA-treated SOSRS by random forest predictive modeling. We generated an iPSC line with the well-known anencephaly gene SHROOM3 knocked out by biallelic CRISPR indel formation. Compared with the isogenic control, the SHROOM3-KO SOSRS had expansion of the lumen as measured by the radial distribution of apical markers ZO1 or f-actin (phalloidin). While ZO1 was still entirely apically localized, SHROOM3-KO SOSRS had f-actin staining on the basal surface, indicating loss of SHROOM3 results in impaired polarization. Additionally, both VPA treatment and SHROOM3-KO resulted in enlarged apical surface area, and, thus, impairment of apical constriction is a shared mechanism for these two well-known causes of NTDs. Our model system abrogates the long time (7 days vs. months), low N (100-1000's vs. 10-100's), and high heterogeneity associated with prior structural analysis in brain organoid systems. Future studies will use this system to screen chemical libraries and suspected NTD genetic variants.

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Keywords: Neuroteratogen, Anencephaly, Induced pluripotent stem cells

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GLOBAL TRANSCRIPTOMIC EFFECTS OF ATXN2 KNOCKOUT IN HUMAN IPSC-DERIVED MOTOR NEURON MODELS OF TDP-43 PROTEINOPATHY

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Transactive Response DNA Binding Protein 43 (TDP-43) is a ubiquitously expressed nucleic acid transport and processing factor that is essential for normal cellular development and function. Disruptions in TDP-43 function, particularly in the form of re-localization and aggregation in the cytoplasm, have been associated with the development of several neurodegenerative diseases, including Amyotrophic Lateral Sclerosis (ALS). Approximately 97% of ALS patients will develop hyper-phosphorylated, hyper-ubiquitinated inclusions of TDP-43 in spinal cord motor neurons. Previous studies in mouse models of TDP-43 overexpression, wherein mice develop ALS-like symptoms and TDP-43 cytoplasmic aggregates in spinal cord motor neurons similar to humans, have shown that Ataxin-2 (ATXN2) knockout was sufficient to extend life span, mitigate the severity of symptoms, and reduce the burden of TDP-43 cytoplasmic aggregates in mice overexpressing TDP-43. However, the mechanism by which ATXN2 knockout confers this therapeutic potential has yet to be fully explained in a human system. To deeply explore the mechanisms potentially contributing to this effect, we performed bulk RNA sequencing on isogenic iPSC-derived human motor neurons engineered to overexpress TDP-43 or the cytoplasmically restricted TDP-43ΔNLS variant in the context of ATXN2 CRISPR knockout. By identifying and validating the transcriptomic changes between conditions, we will discover which human-specific cellular processes may be disrupted by TDP-43 dysregulation and how ATXN2 knockout may directly or indirectly play a role in allowing the motor neurons to maintain normal health and function in conditions that otherwise lead to neurodegeneration. Furthermore, examining what processes that are disrupted by TDP-43 dysregulation, but are not rescued by ATXN2 knockout will shed light on non-ATXN2 mediated mechanisms for preventing motor neuron degeneration could be worth exploring in the future. We believe that this unbiased evaluation of how ATXN2 knockdown affects the transcriptomic landscape of human motor neurons is an important building block in the process of discovering and developing new therapies for ALS and other diseases where TDP-43 proteinopathy plays an important role.

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Keywords: Neurodegeneration, In-Vitro Modeling, Amyotrophic Lateral Sclerosis

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HUMAN IPSC-DERIVED DOPAMINE NEURONS ON ORGAN-CHIP TO MODEL PARKINSON DISEASE

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Parkinson disease (PD), the second most common neurodegenerative disease, is a complex disorder in which nigral dopaminergic neurons are progressively lost, resulting in a reduction of striatal dopamine levels and aggregation of intracellular proteins leading to motor and non-motor symptoms over time. The molecular and cellular changes leading to dopaminergic neurons loss are complex and likely involve the interplay of different cell types. No current therapeutics effectively stops dopaminergic neuron loss. The diagnosis of PD usually occurs after physical manifestations start, at which point there can be up to 80% dopaminergic neuron loss. While conventional “2D” cell culture models show promise for identifying disease mechanisms, they lack the cellular complexity and developmental maturity to capture complex changes underlying PD. Here, we compare the differentiation of human iPSCs into dopaminergic neurons using 2D culture and the 3D organ-on-chip system. In addition, we differentiated PD patient-derived iPSCs into dopaminergic neuron to understand whether the organ on-chip system can be used to recapitulate PD and find early biomarkers. Discovering novel early biomarkers for PD could permit diagnosis and therapeutic intervention at a time when dopaminergic neurons are still present. Control and patient derived-dopaminergic neurons were differentiated using our published protocol and seeded either in glass coverslip or chips. The microengineered chip has two channels that permit independent culture of cell types separated by a porous membrane. While glass coverslips were fed every other day, chips were connected to constant laminar flow. Cells were collected and analyzed after 4 weeks in culture. An LDH assay demonstrated no signs of neuronal toxicity. ICC and single nuclei RNAseq showed that the 3D chip platform optimizes the generation of human iPSC-derived dopaminergic neurons compared to 2D cultures, with an increased proportion of neurons and more homogeneous culture. Analyzing PD patient-derived dopaminergic neurons showed cells had both previously identified phenotypes and new phenotypes relevant to PD. Ultimately, the organ-chip platform permits co-culture of multiple cell types in separate chambers, for instance to model the brain-blood barrier to test therapeutics.

Funding Source: NIH5UG3NS105703-02

Keywords: Parkinson Disease, Organ on chip, iPSCs

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CASC3 SUPPRESSION MITIGATES NEURODEGENERATION IN DIVERSE FORMS OF ALS/FTD

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Amyotrophic lateral sclerosis (ALS) is a complex neurodegenerative disease characterized by the progressive loss of the upper and lower motor neurons of the brain and spinal cord respectively. ALS affects approximately 30,000 people within the United States and is ultimately fatal, with a life expectancy of 2-5 years following diagnosis. The disease remains incurable and while significant effort has gone into understanding the genetic etiology of ALS, more than 90% of ALS patients have unidentified genetic driver(s) making their pathology challenging to study and cure. However, the nuclear clearance and cytoplasmic aggregation of the RNA binding protein TDP-43 is a hallmark feature observed in 97% of patients regardless of genotype. TDP-43 is a primarily nuclear RNA binding protein that can shuttle between the nucleus and cytoplasm to perform an array of cellular functions and it's mislocalization can induce both loss- and gain-of-function consequences that drive neurodegeneration. To this end, utilizing a 3D-spheroid culture system of induced neurons (iNs) derived from a patient carrying a causal TARDBP mutation (I383V TDP-43), we performed an unbiased genome-wide CRISPR-interference (CRISPRi) phenotypic screen to rescue neuron survival. Our results identified a component of the exon junction complex (EJC), CASC3, to potently increase neuronal survival in vitro. Validation studies using individual CASC3 sgRNAs rescue survival, the hallmark TDP-43 cytoplasmic mislocalization, and improve TDP-43 functional activity to the level of healthy control 3D-iNs. Further, we observe that antisense oligonucleotide (ASO) mediated suppression of CASC3 significantly rescues C9ORF72 and sporadic ALS 3D-iN survival while also improving cryptic-exon repression and TDP-43 compartment localization. Our results demonstrate the potential of CASC3 suppression to prevent highly conserved mechanisms of TDP-43 neurotoxicity. Thus, illuminating the suppression of CASC3 as a potential broadly acting, effective therapeutic approach for diverse forms of ALS.

Keywords: Neurodegeneration, CRISPR Screen, ALS

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CONSTRUCTION OF DUAL-FLUORESCENT REPORTER STEM CELL LINES TO STUDY HUMAN CORTICAL MOTOR NEURON DEVELOPMENT WITH 3D ORGANOID CULTURE

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Cortical motor neurons, long projection neurons located at the deep layer of cerebral cortex, convey signals to muscles and control the movement of muscles. Degeneration of cortical motor neurons underlies debilitating motor neuron diseases including hereditary spastic paraplegias and amyotrophic lateral sclerosis. Mechanisms underlying the development and degeneration of cortical motor neurons in human cortex remain largely unknown, which requires a good model to study these neurons in vitro and in vivo. FEZF2 expression is detected in early forebrain progenitors and in their postmitotic progeny in deep cortical layers, while CTIP2 plays critical roles during neuron-differentiation and axonal extension in deep layer projection neurons of the cerebral cortex. To better understand and study the development of cortical motor neurons, we replaced stop codons of FEZF2 and CTIP2, two key transcriptional factors of cortical motor neurons, with green and red fluorescent genes zsGreen and tdTomato, respectively. After generating FEZF2-zsGreen and CTIP2-tdTomato dual-fluorescent human embryonic stem cell (hESC) lines with CRISPR/Cas9 gene editing tools, the successful integration of these fluorescent reporters was examined and confirmed. To validate the reporter lines, we differentiated these reporter lines into cortical projections neurons. We then examined the expression of FEZF2 and CTIP2, and observed the co-labelling of these markers with the reporters. Moreover, both zsGreen and tdTomato fluorescent were able to recapitulate expression state of FEZF2 and CTIP2 at both the mRNA and protein levels. Finally, the double reporter cell lines were used for 3-dimensional (3D) brain organoid culture, allowing the maturation of the labelled cortical motor neurons. This study demonstrates the construction of FEZF2-zsGreen/CTIP2-tdTomato hESC cell lines using CRISPR/Cas9-mediated gene editing, and these double reporter lines are successfully applied in 3D organoid culture system, providing an innovative approach to study the development and degeneration of human cortical motor neurons.

Funding Source: National Institutes of Health

Keywords: Forebrain motor neuron, fluorescent reporter cell, neural development

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INVESTING DNA DAMAGE AND REPAIR PATHWAYS IN RETT SYNDROME MODELS

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Rett syndrome, the second leading cause of ID in young girls, is associated with mutations in the gene MECP2 resulting in the loss of functional MECP2 protein. However, it's unclear why loss of function for this methylated DNA binding protein causes neuronal dysfunction. Utilizing skin fibroblasts from Rett syndrome patients, hiPSCs were specialized into neurons with and without the MECP2 mutation. Our lab has previously found that a key molecular effect of the loss of MECP2 is DNA damage, but whether the DNA damage is due to increased DNA breaks or defective DNA repair remains unknown. Immunofluorescent staining has shown that different types of DNA damage are significantly higher in mutant cultures compared to control at different stages of differentiation. Furthermore, in COMET assays performed on mutant and WT neurons, the same effect is observed. To better understand the dynamics of DNA damage repair systems, we are exploiting reporter plasmids designed to readout the activity of DNA repair pathways (NHEJ or HR). By transfecting these tools and taking advantage of DNA damage agents, we aim to understand whether

DNA repair is defective in the absence of MECP2, and what types of DNA repair are key to genomic stability in neurons.

Keywords: Rett Syndrome, Stem Cells, DNA Damage

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INVESTIGATION OF NEUROPHYSIOLOGICAL PHENOTYPE IN SEVERE COMBINED IMMUNODEFICIENCY SYNDROME CAUSED BY MUTATIONS IN ADA

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Neurological impairments such as motor and learning difficulties have been reported in patients with ADA-SCID, Severe Combined Immuno-Deficiency caused by a mutation in the ADA gene. Adenosine deaminase (ADA) converts adenosine to inosine in the recycling pathway of ATP and other purine analogs, and a lack of ADA causes a buildup of adenosine and its substrates. While the metabolic effects of this disruption are well studied in immune cells, little is known about the effects in brain cells. In an ADA-/- mouse model, the build-up of adenosine was found to overstimulate adenosine receptors of subtype 2a (ADORA2a), causing their downregulation in the brain. We hypothesized that developing human-derived brain cells which lack ADA would also have a downregulation of ADORA2a and possibly ADORA1, the other most widely expressed adenosine receptor in the brain. To test this hypothesis, we made human-derived induced pluripotent stem cells (iPSCs) by reprogramming fibroblasts from 2 patients and their matched parent controls. By inducing these iPSCs towards a neural fate, we examined the protein levels of ADORA2a and ADORA1 in cortical neural precursor cells (NPCs). Our preliminary findings show that forebrain cortical NPCs of ADA-SCID patients have reduced levels of ADORA2a and ADORA1 compared to their matched controls. Additionally, we are developing a CRISPR-Cas9 ADA knockout line from an isogenic control to confirm these findings. The regulation of adenosine during development and in the adult brain is essential, and our research aims to understand how this is disrupted by the lack of ADA. Our future directions are to develop human-derived astrocytes and neurons from the NPC lines to further understand these effects. With increased survival of SCID patients after treatment of immunodeficiency due to new and better therapies, it is becoming important to better understand the mechanism of potential neurological defects caused by ADA mutations.

Keywords: reprogramming, genetic, purines

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INVESTIGATING THE OCCURRENCE OF NEUROINFLAMMATION IN HUMAN STEM CELL MODEL OF SPG3AINVESTIGATING THE OCCURRENCE OF NEUROINFLAMMATION IN HUMAN STEM CELL MODEL OF SPG3A

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Hereditary Spastic Paraplegia (HSP) is a group of rare neurodegenerative disorders which are characterized by axonal degeneration of cortical motor neurons, leading to muscle weakness and spasticity. The most common early-onset form of HSP, Spastic paraplegia 3A (SPG3A), is caused by mutations in the Atlastin-1 (ATL1) gene. Astrocytes are non-neuronal central nervous system (CNS) cells that could cause inflammation in response to external stimuli. Though changes in the Toll-like receptors (TLRs) and increased production of pro-inflammatory cytokines were observed in neurodegenerative diseases, the role of TLRs present on astrocytes in HSP remains elusive. Here, we examined the link between astroglia TLRs and pathogenesis of SPG3A and we hypothesized that TLR stimulation-mediated inflammation is involved in disease phenotypes in the ATL1 mutation cells. We differentiated the ATL1 mutant hESCs to astrocytes and characterized the differentiated cells using astrocyte specific markers S100β and GFAP. We compared the inflammatory genes expression and cytokines secretion levels after stimulation with ligands in control and ATL1 mutated astrocytes. Gene expression levels revealed the activation of transcription of TLRs in response to stimulus and the further recruitment of NF-κB pathway. Moreover, a significant increase in the levels of inflammatory cytokines was observed via ELISA and proteome profiling. In addition, variation in cytokine secretion levels over the period of 7 days was observed using profiling. These data indicate the occurrence of neuroinflammation and suggest its role in prognosis of the disease. It is essential to study further if anti-inflammatory drugs such as Nonsteroidal anti-inflammatory drugs can be used as a potent agent to mitigate the effects of cytokines to rescue SPG3A neurons from axonal degeneration.

Funding Source: Blazer Foundation, NIH and Department of Biomedical Sciences at UIC College of Medicine, Rockford

Keywords: Neuroinflammation, SPG3A, Glial Cells

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DEFINING THE HUMAN OTIC PROGENITOR NICHE

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Inner ear development involves the assembly of cells from a variety of different embryological origins. There is an unmet need

for developing a human inner ear model in vitro to mimic both the complex structure and the function of the inner ear outside of the body. This project builds on our previous discovery of a 3D culture system that uses hPSCs to generate inner ear (otic) organoids. The current protocol contains three stages. In Stage-I, we induce inner ear progenitors by modulating TGF, BMP, and FGF signaling. In Stage II, the premature otic organoids (otic vesicles) appear after WNT activation. In Stage III, the organoids mature into multi-chambered cysts containing sensory and non-sensory epithelium surrounded by mesenchyme. Our first goal is to generate a better-defined protocol to generate human inner ear organoids. We have identified a founding population of otic placode/vesicle-like epithelial cells, which are defined by the expression of TBX2, PAX2/8, SOX2, and OC90. However, the off-target cell population, such as epidermis and mesenchymal cells still took a large proportion of the single-cell RNA-Seq UMAP clusters. Thus, there is a pressing need to increase the yield of the otic cell population during the early stage of differentiation. Here, we demonstrate how the IODA can be leveraged to focus efforts to modulate otic induction. We identified early Wnt activation might increase the production of posterior placode. Based on the knowledge from existing literature and our single-cell Seq data, we have screened a series of treatments to stimulate the expansion of inner ear organoids. The mature inner ear vesicles usually reside inside the solid aggregate, giving rise to technical difficulties in imaging and closely monitoring the developmental process. Thus, our next goal is to construct new reporter cell lines and novel platforms to visualize the dynamics of emerging inner ear organoids. Here, We highlighted a novel SOX2-GFP/OC90-RFP dual reporter line for monitoring otic induction and organoid growth. We will demonstrate the result of enriching human otic epithelial progenitor cells using MACS-based approaches. Looking ahead, we hope that a better-defined model of the human inner ear can be integral to pre-clinical studies for therapies to treat congenital deafness and balance-related disorders.

Funding Source: NIDCD grant R01 DC017461 and DOD grant RH200050.

Keywords: Human inner ear, Organoid, Development

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USING HUMAN IPSC MODELS TO DECIPHER THE ROLES OF MICROGLIAL TREM2-TYROBP/DAP12 SIGNALING IN LATE-ONSET ALZHEIMER'S DISEASE

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Microglia, the primary immune cells of the brain, are now recognized as central players in the neuroinflammatory response contributing to Alzheimer's disease (AD) and other neurodegenerative disorders. Genetic studies have highlighted that loss-of-function mutations in the microglial cell surface receptor TREM2 exert pleiotropic effects on microglial function and accelerate disease progression, and that TYROBP/DAP12, a cytoplasmic adaptor for TREM2 and other receptors, is a driver of sporadic late-onset AD. Little is known, however, about the role of TYROBP in AD and its interdependence with TREM2. To investigate the impact of these genes on AD-related microglial functions, we have generated an isogenic panel of CRISPR/Cas9-edited human iPSC lines lacking TREM2 or TYROBP, as well as lines edited to delete a key phosphorylation residue in TYROBP involved in downstream signal transduction. Both TREM2- and TYROBP-knockout iPSC-microglia show significantly compromised viability during differentiation, and immunohistochemical analysis identifies aberrant cellular morphology and protein expression patterns in variant microglia, including discontinuous IBA1 distribution. RNA-seq revealed that TREM2- and TYROBP-knockout microglial progenitors exhibit decreased expression of key homeostatic markers and upregulation of MHC class II genes compared to isogenic controls, while TYROBP-phosphorylation mutants exhibit upregulation of genes associated with activated microglial states, such as GPNMB. Using our established protocols for integrating microglia into oligo-cortical organoids containing excitatory and inhibitory neurons, astrocytes and oligodendrocyte lineage cells, we are evaluating how variant microglia respond to AD-related insults within these complex systems, including exposure to amyloid-beta aggregates and/or human Tau fibrils. These studies are working towards a fuller understanding of the functional roles of microglia in AD, with the ultimate goal of identifying viable targets for more informed therapeutic manipulation of disease-relevant microglial signaling pathways.

Keywords: Alzheimer's disease, iPSC modeling, Microglia

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ALZHEIMER'S DISEASE-LIKE PHENOTYPES INDUCED BY SARS-COV-2 INFECTION THROUGH ACTIVATION OF GILA IN CORTICAL-BLOOD VESSEL ASSEMBLOIDS

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A correlation between COVID-19 and Alzheimer's disease (AD) has been proposed recently. Although the number of case reports on neuroinflammation in COVID-19 patients has increased, studies of SARS-CoV-2 neurotrophic pathology using brain organoids have restricted recapitulation of those phenotypes due to insufficiency of immune cells and absence of vasculature. Cerebral pericytes and endothelial cells, the major components of blood-brain barrier, express viral entry receptors for SARS-CoV-2 and response to systemic inflammation including direct cell death. To overcome these limitations, we developed cortical-blood vessel assembloids by fusing cortical organoid with blood vessel organoid to provide vasculature to brain organoids and obtained the characteristics of increased expression of microglia and astrocytes in brain organoids. Furthermore, we observed AD pathologies, including β-amyloid plaques, which were affected by the inflammatory response from SARS-CoV-2 infection. These findings provide an advanced platform to investigate human neurotrophic

diseases, including COVID-19, and suggest that neuroinflammation caused by viral infection facilitates AD pathology.

Funding Source: This study was supported by the Korean Fund for Regenerative Medicine (KFRM) grant (No. 22A0101L1-11) and partially supported by the Research Institute for Veterinary Science, Seoul National University.

Keywords: Assembloids, Blood vessel organoids, Cortical organoids

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HOXD1 INHIBITS TUMOR PROGRESSION BY PROMOTING GLIOBLASTOMA STEM CELL DIFFERENTIATION

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Glioblastoma, the most aggressive brain cancer, has no effective treatment. Cancer stem cells (CSC), distinctive of these types of tumors, are a promising therapeutic target since they are associated with tumor emergence, progression, invasion, resistance to treatment, and poor clinical prognosis. HOX genes encode a broad family of transcription factors that, in addition to playing a central role during embryonic development, have been associated with the development and progression of different types of cancer, including glioblastoma. The alteration of the expression profile of the HOX genes suggests their role as tumor modulators, being able to play a suppressive role in tumors or have an oncogenic role that may be associated with the acquisition of a CSC phenotype. We hypothesized that the activation of tumor suppressor HOX gene expression could induce CSC differentiation, thereby preventing glioblastoma progression, a matter not addressed far. We performed different bioinformatics approaches based on transcriptomic and clinical data from public databases of developing brains and cancer. The different analyzes suggest that HOXD1 is a potential tumor suppressor gene in glioblastoma associated with a differentiated phenotype. Our in vitro analyses, associated with the differentiation and stemness assessment in different glioblastoma cell lines, are consistent with the bioinformatics results and suggest that the increase of HOXD1 expression leads to the differentiation of CSC in glioblastoma. We are currently working on the in vivo validation of HOXD1 as a tumor suppressor gene in glioblastoma. Our results will contribute to a better understanding of the mechanisms underlying the initiation and progression of glioblastoma and will allow the description of new biomarkers associated with prognosis.

Funding Source: ANID National Doctorate Scholarship 21181605 Millennium Institute Center for Genome Regulation ICN2021_044

Keywords: cancer stem cells, glioblastoma, HOX genes

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HUMAN STRIATAL-MIDBRAIN ASSEMBLOIDS FOR MODELING OF ALPHA-SYNUCLEIN PATHOGENESIS

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Alpha-synuclein aggregation in the form of the Lewy bodies and the dysfunction of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc) is a factor in Parkinson's disease (PD). Most PD models are based on non-human experiments, which have limitations in mimicking human neural tissue and finding human-specific mechanism of pathophysiology. However, recent advances in human pluripotent stem cell (hPSC)-derived three-dimensional in vitro organoid technology for the neural tissue have improved the study of brain development and neurological disorders. In this study, we generated human striatal-midbrain assembloids (hSMAs), which are assembled human striatal organoid (hSO) and human midbrain organoid (hMO) generated from hPSCs to recapitulate neuronal projections and neural activity. The results demonstrated that both neural organoids contains a variety of cell types including multiple types of neurons, astrocytes, oligodendrocytes, and progenitors and showed transcriptional profiles that resemble in vivo prenatal tissue. Additionally, our study further demonstrated that hSMAs with flourescently labeled hSO and hMO, were confirmed formation of reciprocal nigrostriatal and striatonigral projections through various imaging techniques and showed higher electrophysiological activity compared to hSO and hMO individual neural organoids. Next, we assembled the two regionalized neural organoids incorporating SNCA overexpression (SNCA O/E) which mimic SNCA triplicate patients, and they showed fewer axonal projections and less network burst frequency and firing rate compared to the wild-type hSMAs. Importantly, we observed alpha-synuclein antidromic propagation from striatum to midbrain region in SNCA O/E hSMA, leading to the Lewy body-like inclusions. Our current study suggest that hSMAs can be utilized for modeling synucleinopathies, allowing us to elucidate the alpha-synuclein propagation mechanism and eventual vulnerability of DA neurons.

Funding Source: This work was supported by Okinawa Institute of Science and Technology Graduate University from Japan; Medical Research Center (NRF-2019R1A5A2026045) of National Research Foundation of Korea at Ajou University School of Medicine.

Keywords: human striatal-midbrain assembloid (hSMA), nigrostriatal projection, alpha-synuclein aggregation

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KCNQ2-RELATED GENOTYPE SPECIFIC NETWORK CHARACTERISTICS IDENTIFIED BY HIGH DENSITY MEA RECORDINGS IN IPSC-DERIVED EXCITATORY NEURONS

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KCNQ2 encodes for a voltage-gated potassium channel subunit (Kv7.2), that forms Kv7 channels which regulate the resting membrane potential and dampen repetitive neuronal firing. Human pathogenic variants in KCNQ2 are responsible for a spectrum of neurodevelopmental and epileptic disorders; KCNQ2 Loss-of-Function variants cause Self-limited Neonatal Epilepsy (KCNQ2-SLNE), Dominant Negative (DN) variants cause Developmental Delay with neonatal seizures (KCNQ2-DN), and Gain-of-Function (GOF) variants cause DD with or without later onset seizures (KCNQ2-GOF). No therapies exist that improve the DD of KCNQ2-related disorders. To address this problem, we developed patient-derived human induced pluripotent stem cell (hiPSC) lines from 2 KCNQ2-SLNE, 2 KCNQ2-GOF, 3 KCNQ2-DN patients and 2 controls. hiPSC lines were differentiated towards excitatory cortical neurons using NGN overexpression. Electrophysiological characterization was performed using high density Multi Electrode Arrays (MEA) from day in vitro (DIV) 12 until DIV49, and patch clamp was performed around DIV21. KCNQ2-DN neuronal cultures showed an increased firing rate compared to the control group, and more proment differences in the synchronous bursting behaviour were observed from DIV35 onwards, such as an increase in burst frequency and a decreased inter spike interval outside of the bursts. We further comfirmed this hyperexcitability using patch clamp in one of the KCNQ2-DN lines and its respective isogenic control. In comparison to DN lines, KCNQ2-GOF neuronal cultures showed a consistent delay in the generation of synchronous network bursts compared to the control group. Additionally, a general decrease in netwerk activity and a decrease in the spikes per bursts and burst frequency was observed. Chronically knocking down KCNQ2 expression using ASO technology in the KCNQ2-GOF neuronal cultures rescued the KCNQ2-GOF phenotype, whereas the effect of an aspecific Kv7 channel blocker, XE991, led to a partial restoration of the network activity. In conclusion, using high density MEA technology we are able to extract specific KCNQ2 variant alterations in network activity over time. Using this network fingerprint we validated a new potential precision therapy using ASO technology that rescued the in vitro KCNQ2-GOF phenotype.

Funding Source: SW received funding from FWO (1861419N and G041821N), KCNQ2 Cure, KCNQ2 e.v., EJP-RD (TreatKCNQ), and the Queen Elisabeth Medical Foundation. ND receives support from FWO-SB (1S59221N)

Keywords: KCNQ2, High density micro electrode array, ASO

TOPIC: PANCREAS

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BETA CELL REGENERATION UPON EFFEVTIVEMONONUCLEAR CELLS (E-MNCS) AS A POSSIBLE ALTERNATIVE TO INSULIN THERAPY

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Sjögren syndrome (SS) and type 1 diabetics (T1DM) are autoimmune diseases characterized by salivary gland (SG) and pancreatic islet destruction leading to loss of secretory function. Recently, we developed an effective culture method that enhances the anti-inflammatory and immunomodulatory phenotypes of peripheral blood mononuclear cells (PBMNCs), and the resulting effective-mononuclear cells (E-MNCs) which contained an enriched population of M2 macrophages could be obtained. Then, we demonstrated that E-MNC transplantation improved the function of radiation-injured SGs due to immunomodulatory and regenerative actions. The aim of this study is to investigate the efficacy of E-MNC therapy against the onset of T1DM, as well as SS, in non-obese diabetic (NOD) mice. The anti-inflammatory characteristics of E-MNCs were firstly analyzed using a co-culture system with CD3/CD28-stimulated (T-cell activated) PBMNCs. Then, E-MNCs were transplanted to SGs and pancreases of NOD mice via local (at 9 weeks of age) and systemic routes (1 time injection per week for 3 consecutive weeks from 9 weeks of age). Subsequently, secretory function of saliva and insulin, histological and gene expression (associated with T-cell infiltration) analyses of harvested tissues were performed. E-MNCs, which exhibited induction of CD11b/CD206-positive M2-like macrophages, inhibited inflammatory gene expressions in T-cell activated PBMNCs. E-MNCs administered via both local and systemic routes suppressed the infiltration of CD4-positive T-cells up to 4 weeks post-transplantation and restored the saliva secretion up to 8 weeks. Similarly, E-MNCs delivered to pancreas after systemic injection led the mice to have lower blood glucose levels at 8 weeks post-administration. At that time point, the insulitis score on lymphocyte infiltration was significantly improved in pancreatic islets after E-MNC treatment, and insulin-producing beta cells were also found in severely damaged islets, classified as grade 4 on that score. These results suggest E-MNCs have therapeutic effects for the regeneration of beta cells, as wells as SG epithelial cells. This study demonstrated E-MNC therapy partially prevents the development of SS- and T1DM-like diseases in mice.

Keywords: Cell therapy, Type 1 diabetics (T1DM), Beta cell regeneration

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TOBACCO CARCINOGENS FACILITATE PANCREATIC CANCER DEVELOPMENT VIA INCREASED NRF2 PATHWAY ACTIVITY

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Pancreatic ductal adenocarcinoma (PDAC) has a 5-year survival rate of 10%. It is one of the deadliest cancers and is expected to become the second leading cause of cancer death by 2025. Tobacco is one of the main risk factors for PDAC. It is estimated that tobacco users make up 30% of pancreatic cancer cases. Besides their role in mutagenesis, tobacco products induce oxidative stress, thereby promoting PDAC initiation and progression. However, the effector pathways activated following tobacco carcinogen exposure remain unclear. Therefore, the impact of tobacco-induced changes in oxidative stress needs to be studied. This project aims to address the impact of carcinogens in pancreatic cancer etiology. We hypothesize that the elevated reactive oxygen species level upon chronic tobacco exposure impacts redox gene pathways, and thereby increases the transformation potential in the pancreas to contribute to disease initiation and progression, specifically involving the key redox regulator Nrf2-Keap1 pathway. Nrf2 is a transcription factor that translocates into the nucleus and initiates transcription of cytoprotective genes upon elevated reactive oxygen species. In this project, we will investigate redox pathways using two major subclasses of carcinogens, cadmium (Cd) and benzo(a)pyrene (BaP) in premalignant and normal mouse pancreatic ductal organoid cultures. Chronic exposure to tobacco products was simulated by dosing organoids with Cadmium and BaP over multiple passages. We interpret the impact on the redox pathway via levels of Nrf2 and the downstream target genes, including Nqo1, Gstp1, and GCLC1. We find via RNA-seq that in premalignant organoid cultures Nrf2 and its downstream targets are downregulated after chronic carcinogen exposure.Collectively, this project contributes to the understanding of carcinogen exposure and the effects downstream of reactive oxygen species elevation in PDA pathogenesis. This project will elucidate a deeper connection between redox metabolism and pancreatic cancer by which PDAC can be induced, and can provide insights to early detection of smoking-induced PDAC.

Keywords: pancreatic ductal adenocarcinoma, reactive oxygen species, RNA-seq

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THE PATH TOWARD ENGINEERING HYPOXIA-RESISTANT STEM-CELL DERIVED ISLETS

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Type 1 diabetes (T1D) is a disease that results from loss of insulin-producing beta (β) cells or β cell function. Transplantation of primary cadaveric islets with healthy β cells has been proposed as a long term solution to T1D, but patients that receive islet transplantations return to exogenous insulin dependence within a matter of years due to a gradual loss of function of transplanted islets. The most recognized contributor of islet transplant death and dysfunction is hypoxia, as isolated, oxygen-hungry islets are transplanted into a low-oxygen microenvironment. Although hypoxia has been identified as the major cause of islet transplant dysfunction, the field still lacks a mechanistic understanding of the effect of hypoxia on islets as well as a high resolution analysis of transcriptome changes in response to hypoxia that could elucidate a mechanistic understanding. Additionally, it has not been shown how hypoxia impacts survival and function of stem cell-derived islets (sc-islets). To address these gaps, we performed static glucose stimulated insulin secretion (sGSIS) on primary human and sc-islets that were subjected to 1% O2 to observe signs of dysfunction after hypoxic culture; primary islets had a markedly lower stimulation index after 48 hours in 1% O2, while sc-islets had a low stimulation index after 6, 24, and 48 hours in 1% O2. We also performed single cell RNA sequencing on these same islet samples, along with normoxic controls. Thus far, we have determined differentially expressed genes and differentially regulated pathways that are specific to the hypoxic response in primary islets or sc-islets, as well as some that are shared between the two. Together, our findings will serve as a resource for the field to use to determine the cell-type specific mechanisms that are differentially regulated under hypoxic conditions.

Keywords: Islet, Hypoxia, Sequencing

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COXSACKIEVIRUS B INFECTION INVOKES UNIQUE CELL-TYPE RESPONSES IN A HETEROGENOUS PANCREATIC ORGANOID MODEL

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Coxsackievirus B (CVB) infection has long been considered an environmental factor precipitating Type 1 diabetes (T1D), an autoimmune disease marked by loss of insulin-producing beta cells within pancreatic islets. Previous studies have shown CVB infection negatively impacts islet function and viability. However, there are no reports on how virus infection individually affects the multiple cell types present in human islets. Therefore, we hypothesized the various islet cell populations have unique transcriptional responses to CVB infection. To test our hypothesis, we first performed single-cell RNA sequencing on human islets treated with either CVB or Poly(I:C), a viral mimic, for 24 and 48 hours. This approached resulted in a list of candidate genes upregulated only in CVB infected endocrine cells. Genetically modified human stem cell-derived islets (SC-islets) were then generated to elucidate the role of these genes in cellular response to CVB infection and replication. SC-islets are a powerful asset because, unlike primary islets derived from patient donors, they can be generated in large supply and are genetically identical, which allows for more robust

analyses. We also find a time-dependent increase in mitochondrial genes in both CVB and Poly(I:C) groups. Functional assays confirmed a decrease in mitochondrial function in CVB and Poly(I:C) treated islets with the former having a more drastic effect. Our transcriptomic data also indicate beta cells have a unique temporal immune response compared to other islet cell types. Additionally, beta, alpha, and ductal cells have the strongest immune responses whilst delta cells are virtually transcriptionally unaffected despite having similar virality levels when compared to other cell types. Together, these findings demonstrate a cell-specific transcriptional, temporal, and functional response to CVB infection and provide new insights into the relationship between CVB infection and T1D.

Keywords: SC-islets, Coxsackievirus, scRNA-seq

TOPIC: NO TISSUE SPECIFICITY

829

INTRAVITAL IMAGING AND SINGLE CELL TRANSCRIPTOMIC ANALYSIS FOR ENGRAFTMENT OF MESENCHYMAL STEM CELLS IN AN ANIMAL MODEL OF INTERSTITIAL CYSTITIS/BLADDER PAIN SYNDROME

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Mesenchymal stem cell (MSC) therapy is a promising treatment for various intractable disorders including interstitial cystitis/bladder pain syndrome (IC/BPS). However, an analysis of fundamental characteristics driving in vivo behaviors of transplanted cells has not been performed, causing debates about rational use and efficacy of MSC therapy. Here, we implemented two-photon intravital imaging and single cell transcriptome analysis to evaluate the in vivo behaviors of engrafted multipotent MSCs (M-MSCs) derived from human embryonic stem cells (hESCs) in an acute IC/BPS animal model. Two-photon imaging analysis was performed to visualize the dynamic association between engrafted M-MSCs and bladder vasculature within live animals until 28 days after transplantation, demonstrating the progressive integration of transplanted M-MSCs into a perivascular-like structure. Single cell transcriptome analysis was performed in highly purified engrafted cells after a dual MACS–FACS sorting procedure and revealed expression changes in various pathways relating to pericyte cell adhesion and cellular stress. Particularly, FOS and cyclin dependent kinase-1 (CDK1) played a key role in modulating the migration, engraftment, and anti-inflammatory functions of M-MSCs, which determined their in vivo therapeutic potency. Collectively, this approach provides an overview of engrafted M-MSC behavior in vivo, which will advance our understanding of MSC therapeutic applications, efficacy, and safety.

Keywords: Single cell analysis, Mesenchymal stem cell, Intravital imaging, Interstitial cystitis/bladder pain syndrome

831

HIGH EXPRESSION OF CD58 AND ALDH1A3 PREDICTS A POOR PROGNOSIS IN BASAL-LIKE BREAST CANCER

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CD58 is an immune adhesion molecule on the cellular surface. It was previously found that a high expression of CD58 predicted a poor prognosis of patients with lower-grade gliomas. Therefore, the aim of this paper was to investigate the assocation between CD58 and breast cancer. In the present study, CD58 gene expression downloaded from cBioPortal was compared in the different subtypes of breast cancer. Clinical prognosis was examined using Kaplan-Meier analysis and multivariable Cox regression analysis. The association between CD58 expression and immune cell infiltration was estimated using the TIMER 2.0 web platform.

Finally, the tumour sphere formation of aldehyde dehydrogenase 1 (ALDH1)high basal-like breast cancer cells in which CD58 was knocked down using siRNA was measured. The results revealed that CD58 mRNA was mainly enriched in claudin-low and basal-like subtypes. The high expression of CD58 predicted a good prognosis in of patients with luminal A and luminal B breast cancer. This good prognosis prediction may be due to the association of immune cell infiltration with CD58. Notably, patients with luminal A breast cancer with a high expression of CD58 in association with ALDH1A3 exhibited a good prognosis; however, this did not apply to patients with basal-like breast cancer. The in vitro experiments revealed that the knockdown of CD58 inhibited the tumour sphere formation ability of ALDH1high basal-like cancer cells. Overall, the present study demonstrates that CD58 may function as a potential prognosis biomarker and therapeutic target in ALDH-positive basal-like cancer stem cells.

Keywords: CD58, cancer stem cell, ALDH1

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ANEUPLOID EMBRYONIC STEM CELLS DRIVE TERATOMA METASTASIS

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Aneuploidy, the unbalanced numerical alterations of whole or partial chromosomes, is one of the hallmarks of cancer. High levels of aneuploidy are typically associated with metastasis and poor outcomes in cancer patients. However, the causality of aneuploidy in cancer metastasis remains obscure. We have established several trisomic mouse embryonic stem cell (ESC) lines carrying an extra copy of chromosome 6, 8, 11 or 15 respectively. To more systemically investigating the functional roles of aneuploidy in cancer metastasis, we labeled the isogenic diploid and aneuploid mouse ESC lines with enhanced green fluorescence protein (EGFP)/Luciferase dual reporters. We found that teratomas derived from aneuploid murine ESCs, but not from isogenic diploid ESCs, disseminated to multiple organs, for which no additional copy number variations were required. Meanwhile, no cancer driver gene mutations were detected in any metastases. Aneuploid circulating teratoma cells were successfully isolated from peripheral bloods and showed high migration and organ colonization capacity. Single-cell RNA sequencing of aneuploid primary teratomas and metastatic samples identified a unique cell population with high stemness that was absent in diploid ESCs-derived teratomas. Aneuploid cells exhibited impaired proteasome activity and overactivated endoplasmic reticulum (ER) stress during differentiation, thereby diminishing the degradation of proteins produced from extra chromosomes in the ESC state and causing differentiation deficiencies. Importantly, both proteasome activator Oleuropein and ER stress inhibitor 4-PBA can effectively inhibit aneuploid teratoma metastasis. Our results provide insights into the causal relationship between aneuploidy and tumorigenesis.

Funding Source: The National Science Fund for Distinguished Young Scholars (T2125002), and the National Natural Science Foundation of China (31970813, 92042303 and 32170771),etc.

Keywords: aneuploidy, metastasis, proteasome dysfunctional & ER stress.

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MODELING TUBEROUS SCLEROSIS COMPLEX WITH 3-D HUMAN CORTICAL ORGANOIDS

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Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder caused by mutations in either TSC1 or TSC2, presenting with systemic growth of benign tumors. In addition to brain lesions, neurologic impairment causes the greatest morbidity in TSC patients. In particular, approximately 50% of TSC patients are affected with autism spectrum disorders (ASDs). Studies from animal or human stem cell models with TSC1/2 knockout suggest that hyper-activation of mTORC1 signaling due to TSC1/2 deficiency may lead to aberrant neurodevelopment. However, how the causal variants of TSC1/2 genes identified in TSC patients affect human cortical development and how they contribute to the neurological manifestations in TSC remain largely unexplored. To address these questions, here we generated 3-D cortical organoids from induced pluripotent stem cells (iPSCs) derived from TSC patients carrying TSC2 mutations as well as healthy controls. To investigate how TSC2 mutations affect the molecular trajectories during cortical development, we generated transcriptome profiles across several developmental stages of TSC and control organoids. Gene regulatory network analyses at four stages (day 35, 56, 98, and 126, respectively) revealed significantly up-regulated gene pathways involving nervous system development, neurogenesis, and chemical synaptic transmission during long-term organoid development compared to controls. We observed multiple genes involved in neurodevelopment, synapse and glial cells, including SYN, SYT1, GRIN1, GRM3, KCNK9, NNAT, and S100B, were upregulated during organoid development. Moreover, the differentially expressed genes in TSC organoids are significantly enriched among genes associated with ASD, intellectual disability, and epilepsy. To further understand the specificity and complexity of the impact of TSC2 mutations on human brain development at the single cell level, we performed single cell RNA-seq (scRNA-seq) on these cortical organoids. The scRNA-seq and time trajectory analyses suggest that the TSC2 mutations lead to alternative and differential developmental progression. Collectively, our study illustrated that disease-associated TSC2 mutations may impair neurodevelopment by the perturbation of gene regulatory networks during early cortical development.

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R21MH123711), and Department of Defense grant (No. W81XWH1910353 to ZW).

Keywords: Tuberous sclerosis complex, TSC2 mutations, Cortical organoids

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ADVANCING NK CELL IMMUNOTHERAPIES FOR OVARIAN CANCER THROUGH A PATIENT-SPECIFIC ORGANOID CO-CULTURE SYSTEM

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Ovarian cancer is the fifth leading cause of cancer deaths in females, with a 5-year survival rate below 30% for most patients. Yet the standard of care remains virtually unchanged after decades. While immunotherapy has revolutionized the treatment of blood cancers and saved countless lives, the benefits have yet to be realized for solid tumors like ovarian. Ovarian cancer brings the unique challenges of frequent relapses, drug resistance, and high variability among tumors. Tumor-derived organoids have emerged as a revolutionary patient-specific cancer model with unlimited expansion capacity and the ability to recapitulate patient drug responses, offering remarkable potential for precision oncology research. At the NYSCF Research Institute, we have implemented tumor organoid technology for ovarian cancer, using fresh tumor resections from the NCI-supported Cooperative Human Tissue Network (CHTN) and our clinical collaborators at Memorial Sloan Kettering Cancer Center (MSK) and the Englander Institute for Precision Medicine at Weill Cornell Medicine (EIPM). We are building a large biobank of patient-specific organoid models as a collaborative research resource. The ability of organoid models to capture tumor biology and heterogeneity, including in responses to immunotherapies, offers the opportunity to accelerate novel treatments and combinations. Their scalability allows us to assess efficacy across patient populations, including ethnic minorities who have been underrepresented throughout the research pipeline and have systematically worse outcomes. To advance immunotherapies for ovarian cancer, we are leveraging NYSCF's human induced pluripotent stem cell (iPSC) technology platform to test the emerging promise of natural killer (NK) cells as an immunotherapy for solid cancers. Our co-culture system of iPSC-derived NKs and patient organoids already shows preliminary evidence of NK killing activity; we are now using it to evaluate how genetically and clinically diverse patients will respond, and whether additional targeted therapies enhance NK killing of certain tumors. This study will accelerate the development of NK cell therapies for ovarian cancer and the design of tailored combination therapies that improve patient outcomes.

Funding Source: This work was supported by the New York Stem Cell Foundation Research Institute (NYSCF) and by the NCI R21CA240219. Tissue samples were provided by the NCI Cooperative Human Tissue Network (CHTN) RRID SCR_004446.

Keywords: Tumor organoids, Natural Killer cells, coculture

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HIGH-THROUGHPUT EDITING OF GENOMIC DELETIONS IN HUMAN INDUCED PLURIPOTENT STEM CELLS

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Our CRISPR-based deletion strategy has proven to be a highly efficient method for generating gene knockout alleles in human induced pluripotent stem cells (iPSCs). The preferred outcome is either deletion of the entire coding region, or removal of a 'critical' exon resulting in a frameshift following mRNA splicing. To achieve this, we specifically targeted the region of interest using flanking CRISPR/Cas9 guide RNAs and utilized a bridging donor to enhance the likelihood of homozygous deletions. This was achieved under conditions optimized for homology-directed repair (HDR). Another key aspect of our bridging donor method is the use of barcoding to identify homozygous clones. This provides us with a level of confidence in our results and allowed us to screen clones efficiently by Sanger sequencing. We were able to demonstrate the effectiveness of this approach by efficiently recovering homozygous clones with deletions up to 64kb in size. In conclusion, our deletion strategy provides a scalable, high-throughput method for generating definitive null alleles in human iPSCs. This will be of significant benefit for downstream loss of function analyses.

Keywords: CRISPR, Knockout, Homology-directed repair

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THE DEVELOPMENT AND USE OF STEM CELL-BASED TOOLS TO SUPPLEMENT OR EVEN REPLACE ANIMAL TESTING IN DRUG DEVELOPMENT: ETHICAL AND REGULATORY CONSIDERATIONS

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Ever since the early 1960's, FDA has required that sponsors demonstrate safety (toxicity) and efficacy of their investigational agents in animals prior to testing them in humans. If FDA deems that, based on the animal data, it would be safe for the agent to be administer to human beings, the FDA may let the clinical trial move forward. However, experience over the last few decades has shown that many animal models can be poor predictors of safety in humans: often, products have shown unexpected serious adverse effects in humans that were not seen in the animals. Uncertainties and disappointments regarding the predictive capacity of animal models, together with pressure from animal rights advocates, has led Congress to pass and President Biden to sign an amendment to the Federal Food, Drug, & Cosmetic Act's ("FD&C Act"). Removing the requirement for animal testing, the revised Act now refers to the requirement for "nonclinical tests," which may include data from "a test conducted in vitro, in silico, or in chemico, or a nonhuman in vivo test." Of interest to the stem cell research community is the potential of human stem cell-derived in vitro systems to supplement, if not replace, animal testing in drug development. Human pluripotent stem cell-derived systems such as organoids and microphysiological systems (MPS), including "organ-on-a-chip" technology, provide promising tools for drug screening and for making predictions of a potential drug's toxicity, efficacy, and biochemical mechanism of action. It is still an open question as to whether these systems can provide sufficient data to replace animal testing altogether. Herein is a discussion of ethical and regulatory considerations regarding the use and validation of stem cell-derived in vitro models in drug development, including thoughts on the qualities necessary for these systems to convincingly and safely mitigate and even obviate the need for testing in animal models.

Keywords: stem cell-derived in vitro systems, drug development, regulatory requirements

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USING STEM CELLS TO UNDERSTAND THE EFFECTS OF VAPING ON HUMAN PRENATAL DEVELOPMENT

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While the electronic cigarette (EC) market has grown rapidly during the last decade, very little work has been done to understand how vaping by pregnant affects prenatal development. EC fluids contain many chemicals including synthetic coolants. This study addresses WS-23, a synthetic coolant that is added to 4th generation ECs, often at concentrations as high as 60 mg/mL. This study examined the hypothesis that WS-23 activates TRPM8 channels on hESCs, thereby altering normal development. hESCs are similar to the epiblast stage of post-implantation embryos, contain numerous TRPM8 channels. Activation of the TRMP8 channels in hESCs by WS-23 was studied by measuring calcium influx using Fluor-8 in conjunction with a Synergy multiplate reader. The presence of WS-23 activated the TRPM8 channels by increasing the calcium influx. The highest calcium influx was observed at 58 nM WS-23. The cytotoxicity of WS-23 was evaluated using the MTT assay. The IC50 (330 µg/mL) and NOAEL (4.5 µg/mL) were determined from concentration-response curves. The IC50 was used to determine the effective concentration of the TRPM8 inhibitor (TCI-2014). hESCs were treated with various concentrations of TRPM8 inhibitor in the presence of WS-23. Using the MTT assay, the effect of WS-23 was reversed at 0.01 nM of TRPM8 inhibitor. Live cell imaging experiments were conducted in a Nikon BioStation to visualize the effect of WS-23 on colony growth and morphology. CL Quant and StemCellQC softwares were used to create videos and extract features (e. g., Colony Area and Number of Protrusions). hESCs were treated with three concentrations of WS-23 (26 to 2600 nM). The colony size decreased in a concentration-dependent manner. Additionally, 260 and 2600 nM concentrations of WS-23 limited the number of protrusions. WS-23 affected adhesion by creating large gaps between hESCs . Inhibition of TRPM8 channels with its antagonist reversed the effect of WS-23 on colony area and the size of gaps. For an exposure of 140 puffs/day, the estimated concentration of WS-23 circulating in the blood of an 85 kg pregnant would be around 14 nM. Therefore, sufficient WS-23 would reach and affect the embryo in a pregnant who vapes ECs with high WS-23 concentrations. These effects could increase the chances of small birth weight babies and other birth defects.

Funding Source: CIRM Bridges: EDUC2-12695

Keywords: hESCs, WS-23 Synthetic coolant in ECs, Human Prenatal Development

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NOVEL 3D CULTURE SUBSTRATE FOR ORGANOIDS CONTAINING COLLAGEN, LAMININ-E8, AND HYALURONAN

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We evaluated gels composed of (1) collagen, (2) laminin 511E8 fragments containing interaction sites with cell surface integrin receptors, and (3) hyaluronic acid as three dimensional cell culture substrates capable of inducing tissue formation that mimics in vivo conditions. The efficacy was examined using liver organoid formation, cancer organoid formation, and cranial nerve organoid formation as indices. When primary human hepatocytes were cultured in gel, this gel, hereafter referred to as MM511 gel, showed superior effects on proliferation and hepatic parenchymal differentiation compared to Matrigel culture. Primary hepatocytes proliferated well in type I collagen gel, but most cells were induced to differentiate into bile ducts. On the other hand, in MM511 gel, despite using the same concentration of type I collagen, in addition to some bile duct differentiation, many cells

formed spheroids with positive hepatic parenchymal markers. The above induction of hepatic parenchymal spheroid formation in MM511 gel culture was also notable in MM511 gel culture mixed with pepsin-solubilized type IV collagen. For developing mouse brain neuron culture, dispersed cells formed spheroids in MM511 gels with additional type V collagen and in MM511 gels with additional hyaluronic acid and proteoglycans. An induction of astrocyte differentiation was observed in addition to neuronal differentiation. In contrast to cultures in Matrigel, numerous large sphere formations positive for astrocytic cell markers were observed in MM511 gel cultures. Patient-derived colorectal cancer cells in MM511 gel culture showed good proliferation and formation of large colorectal cancer spheroids. In MM511 gel cultures of patient-derived colorectal cancer cells, rapid proliferation and formation of large colorectal cancer spheroids were observed. The spheroids contained cells positive for metastatic markers that were not observed in cultures in Matrigel. Cross-sectional observation of the spheroid structure in frozen sections revealed a two-layered structure with an outer cell population and an inner cell population. The interior of the spheroid was rich in hyaluronic acid, while the exterior of the spheroid was rich in type I collagen. MM511 gel provides a diverse extracellular environment to induce various differentiation.

Keywords: collagen, hyaluronic acid, hydrogel

TOPIC: EPITHELIAL INCLUDES SKIN GUT LUNG

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DRUG SCREENING BY PATIENT DERVIED QUANTIFIABLE ORGANOIDS

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Organoids, stem cell-derived organ-mimicking multicellular systems, have recently been emphasized as an important preclinical model for various studies, including stem cell biology, disease modeling, the development of potential therapeutics, and drug screening for personalized therapies. Necessarily, a large-scale culture of organoids is a prerequisite for their industrial and clinical applications. Current organoid culture systems relying on embedding single cells or clusters in bulk extracellular matrix (ECM) hydrogels (e.g., Matrigel™) have limitations for large-scale production and precise observation in terms of morphology, size non-uniformity, and physiological relevancy in multicellular interaction. Here, we demonstrate an advanced hydrogel-based, fluidic organoid called “quantifiable organoids” and a scalable culture platform using colorectal cancer organoids. Quantifiable organoids generated from a novel assembly-type (ready-to-assemble) 96-well-based quantifiable culture platform equipped with strips of U-shaped 9 microwells are not only functionally compatible with organoids generated from conventional 24 well Matrigel platforms, but they are also significantly improved in terms of consistency, uniformity, and reproducibility. Importantly, quantifiable organoids induce distinct transcriptomic changes compared to conventional single cell-based organoids, resulting in the presence of more primitive cancer stem cells, lesser differentiation, and differential drug sensitivity. Taken together, the newly introduced quantifiable culture type may reprogram the transcriptional pattern that affects the functional behavior of organoids, resulting in more precise and reliable drug screening results.

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Keywords: Colon cancer organoids, quantifiable drug screening, a novel organoid-screening-platform

TRACK: MODELING DEVELOPMENT AND DISEASE (MDD)

Session 3: Even

2:15 PM – 3:00 PM

TOPIC: CARDIAC

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GENERATION OF A HIPSC-DERIVED 3D MICROTISSUE MODEL FOR THE STUDY OF THE CARDIAC PHENOTYPES IN MARFAN SYNDROME

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Marfan Syndrome (MFS) is an autosomal dominant disorder of the connective tissue caused by mutations in the FBN1 gene encoding fibrillin-1, a major structural component of microfibrils and, thus, of the elastic fiber. Clinical manifestations of MFS affect skeletal , ocular and cardiovascular systems. Our group has produced isogenic hiPSCs with null and dominant-negative mutations in the FBN1 gene to develop in vitro models of the disease. In particular, we are interested in understanding the molecular mechanisms involved in dilated cardiomyopathy, present in 20-25% of MFS patients. Although hiPSCs can be efficiently differentiated into cardiomyocytes in 2D cultures, these do not express FBN1 hampering their use in modeling the disease in vitro. Use of 3D cardiac microtissues (MTs) composed of hiPSC-derived cardiac fibroblasts, cardiomyocytes and endothelial cells might prove valuable models for the study of heart phenotypes of MFS, since fibroblasts express FBN1, they enable higher maturation state of hiPSC-derived cardiomyocytes, and MTs better represent the organ. We have effectively differentiated all four hiPSC lines used in the study into the three cell types of interest. Cells were characterized by flow cytometry and immunocytochemistry assays for cTNNT (cardiomyocytes), CD31 and CD144 (endothelial cells), DDR2 and COL1A1 (cardiac fibroblasts). We generated MTs by co-culturing the three cell types, and characterized them by immunofluorescence with markers for CD31, cTNNT1 and COL1A1, in order to assess if all cell types were present in the model as well as their organization level. The MTs with different mutations in the FBN1 gene will be deeply phenotyped in order to understand

if they can model dilated cardiomyopathy and other cardiac phenotypes in MFS.

Funding Source: Funding for this research has been provided by CAPES.

Keywords: Marfan Syndrome, Dilated Cardiomyopathy,Cardiac Microtissues.

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EXPRESSION PATTERNS OF THREE-DIMENSIONAL TISSUE USING TURNER SYNDROME IPSCS

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Turner Syndrome is a syndrome caused by non-disjunction of the X chromosome in females. 45,XO, 46,X,i(X), 46,X,r(X) caused by complete or partial loss of one X chromosome, or mosaicism, including various karyotypes. Metabolism, cardiovascular, ovarian, and skeletal abnormalities occur mainly, resulting in symptoms of heart malformation, obesity, diabetes, and infertility. In particular, about 20-70% of Turner syndrome patients have heart disease, and about 20% show congenital anomalies, which are known to be the most common cause of death in Turner syndrome patients. In previous studies, there is insufficient evidence for the identification of the expression mechanism and the genes related to the symptoms of Turner syndrome. Therefore, we tried to find the relationship between the disease and the heart and blood vessels using induced pluripotent stem cells derived from patients with Turner syndrome. Cardiac and vascular organoids were formed by referring to previously established protocols. We confirmed the expression of genes specific to the heart and blood vessels through the formed organoids. As a result of analyzing the single-cell RNA sequencing data, overlapping populations were also identified, but clearly distinct clusters were also found. This paper not only presents a basis for screening genes related to heart and blood vessel diseases, the cause of death in Turner syndrome but also contributes to the creation and treatment of Turner syndrome disease models in the future.

Keywords: Turner syndrome, X monosomy, X chromosome inactivation

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EPICARDIOID SINGLE-CELL GENOMICS UNCOVER PRINCIPLES OF HUMAN EPICARDIUM BIOLOGY IN HEART DEVELOPMENT AND DISEASE

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Cardiovascular disease is the main cause of adult mortality worldwide. It ultimately leads to heart failure associated with a reduction of the heart mass and function. As the renewal potential of the adult heart is minimal, there is a need for approaches to promote cardiac regenerative capabilities. The epicardium, the outermost heart layer, is essential during cardiac development, homeostasis, and repair. During embryonic development, the epicardium is the source of several cardiac cell types, including the majority of fibroblasts and vascular smooth muscle cells, and provides signals critical to the development and proliferation of the myocardium. In pathological settings, some of these programs are re-activated in the adult epicardium, making it a promising target for therapy. However, the mechanisms underlying these processes in humans are unclear. Here, we generated human pluripotent stem cell-derived heart organoids showing self-organization of the epicardium and myocardium (called epicardioids). The epicardial layer of epicardioids promotes unprecedented morphological, molecular, and functional self-patterning of the myocardium that recapitulates features of the ventricular wall. We used scRNA-Seq and scATAC-Seq to reveal key transcriptional programs guiding cellular segregation and fate decisions along the epicardial lineage tree, a long-standing pursuit in the field. Finally, we demonstrated that epicardioids offer an advanced system to investigate functional cross-talks between cardiac cell types and study the multicellular pathogenesis of hypertrophy and fibrotic ventricular remodeling. Overall, epicardioids provide a powerful platform for investigating the role of the epicardium during cardiac injury and developing prospective strategies to aid revascularization and heart regeneration. As such, they offer a wide range of applications in cardiovascular medicine and drug discovery.

Keywords: organoids, heart, epicardium

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NANOPLASTICS EXPOSURE IMPAIRS FUNCTION OF HUMAN STEM-CELL DERIVED CARDIOMYOCYTES

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Plastic has become an ubiquitous environmental pollutant and nanoplastics (NPs) that are within the size range of 1 to 1000nm could form upon weathering. Considering its sheer size which contributes to greater ease of cellular internalisation, NPs are speculated to be more hazardous than their larger counterparts. Despite the growing concern, most studies focused the effects of NPs on the brain, gastrointestinal tract and the lungs, while a lack of studies on the human heart was noted. Moreover, current NPs studies were mainly conducted on animal models, which may lack physiological relevance to humans. To address that, we utilised human embryonic stem cells-derived (hESCs) cardiomyocytes (CMs) to investigate the effects related to the uptake and accumulation of NPs in human heart. Size-dependent uptake was first investigated and cellular uptake of 50nm nanoplastics was found to be significantly higher than 500nm particles. Over a time course experiment, the dose-dependent accumulation of 50nm NPs was established, which coincided with an upregulation of both oxidative stress and endoplasmic reticulum stress. Alongside that, an elevated cleaved-caspase 3 expression for NPs-treated cells was noted, suggesting that NPs exposure could induce apoptosis in CMs. Corresponding to these cellular effects, functional assessment of calcium transient dynamics demonstrated that NPs treatment in CMs triggered an arrhythmic phenotype that constituted greater variability in the duration of beat-to-beat and repolarisation. Collectively, our study revealed that NPs have

an adverse effect on the human heart and future studies could provide mechanistic insights to the cardiac risks associated with the ever-increasing nanoplastic pollution.

Keywords: Nanoplastics, Cardiac, Environmental Pollution

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HIGH THROUGHPUT SCREENING IDENTIFIES NEW COMPOUNDS TO ENHANCE HUMAN IPSC-DERIVED CARDIOMYOCYTES MATURATION

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The maturation level of cardiomyocytes derived from hiPSCs (human induced pluripotent stem cells) is still far from their in vivo mature counterparts. This fact limits the fully functional applicability of hiPSC-derived cardiomyocytes (hiPSC-CMs) in treating cardiac injury affected by a massive loss of myocardium. Mature cardiomyocytes are mitotic-refractory cells in a quiescent state, suggesting that a complete withdrawal from the cell cycle is a trigger toward cardiac maturation in postnatal stages. Fluorescent Ubiquitination based Cell Cycle Indicator (FUCCI)-based method showed that hiPSC-CMs are proliferative like those fetal-like cells before maturation, highlighting proliferation activity as a barrier in maturation. Previous studies showed that supplementing hiPSCM-CMs with fatty acids, hormones, and small compounds, enhanced the maturation of some properties, yet retained residual proliferation, a roadblock toward cardiac maturation. Here, we aim to identify compounds promoting cardiac maturation through a complete cell cycle withdrawal. By combining FUCCI-expressing iPSC-cardiomyocytes and our maturation method, we have performed high throughput screening (HTS), identified several compounds that halt proliferation and improved the maturation of hiPSC-CMs. Enhanced structural maturation is being monitored by increased levels of isoform 3 of the cardiac troponin I (TNNI3) and decreased fetal isoform (TNNI1), an essential developmental switch during cardiac maturation. Our results showed that these compounds could significantly enhance levels of TNNI3, suggesting that other properties of cardiac maturation also improved. We are currently optimizing the different combinatory on the compounds and evaluating molecular and electrophysiological properties of our cardiac cells. Optimizing the combination and usage of these compounds will guarantee pharmacological safety on a safer delivery toward clinical applications of mature hiPSC-CMs.

Funding Source: This work was supported by JST SPRING, Grant Number JPMJSP2110.

Keywords: Cardiomyocyte, Cell cycle, High throughput screening

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APPLICATION OF INDUCED PLURIPOTENT STEM CELLS TO STUDY NOVEL EMERIN MUTATION IN PATIENT WITH DILATED CARDIOMYOPATHY

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Emerin (EMD) is a member of the nuclear lamina-associated protein family and is highly expressed in both skeletal and cardiac muscle cells. EMD gene is located on X chromosome and mutations in EMD gene are responsible for an inherited degenerative myopathy - Emery-Dreifuss muscular dystrophy. Most of the diagnosed patients develop heart failure or abnormalities in heart rhythm such as atrial fibrillation, bradycardia, syncope or sudden cardiac death. Searching for genetic cause of cardiac failure of male patient, we have found a deletion in EMD gene leading to stop codon cancellation and subsequently to formation of 10kDa longer protein product. The transcription of mutated EMD protein was confirmed by cDNA analysis, but immunochemistry labeling did not detect any EMD protein in the patient's heart tissue. To elucidate functional impact of this novel mutation, we have differentiated cardiomyocytes from patient-specific induced pluripotent stem cells. Mutant EMD protein was also not detected in patient-derived cardiomyocytes, and abnormalities in the nuclear morphology were observed. We further characterized production and localization of emerin binding partners and other nuclear proteins including LMNA, LMNB1, BAF and LAP2. To determine the cause of undetectable EMD protein with preserved mRNA transcription, iPSC-derived cardiomyocytes were treated with proteasome inhibitor. Upon this treatment, we detected increased expression of mutant EMD protein, suggesting rapid proteasome degradation of the mutant protein form. Our results illustrate that iPSC are a valuable tool for generation of patient-specific models allowing to improve insight into the effects of genetic variants on disease pathogenesis.

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Keywords: cardiomyocytes, emerin, nuclear proteins

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DEVELOPMENT OF A NOVEL MICRO-ENGINEERED HEART TISSUE PLATFORM ON CHIP WITH MULTICELLULAR BIOMIMICRY

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Cardiovascular diseases (CVD) are the leading cause of death globally and there is currently no cure available. The efficient generation of human pluripotent stem cell derived cardiomyocytes (hPSC-CMs) enabled their use for disease modeling, drug discovery and regenerative medicine in CVD. Moreover, in order to enhance their maturity in vitro, several three dimensional (3D) in vitro systems, such as Engineered Heart Tissues (EHTs) have been developed and proven to be efficient in improving CM maturation. Nevertheless, conventional EHT models require high cell numbers, which makes them expensive, and lack flow, a key element in heart function. Additionally, recapitulation of the complex CM and non-CM interactions as they occur in vivo is key to further enhance CM maturation. Heart-on-chip (HoC) systems allow for precise control of flow and facilitate dynamic adjustments of the required microenvironment. Nevertheless, current microfluidic HoC do not feature flexible pillar-based EHTs that allow for precise assessment of contractile forces and do not exhibit the self-organization of the cardiac tissue as occurs in vivo. Moreover, their arduous use, complex fabrication and limited robustness hamper their use. Therefore, in this study we designed a novel and user-friendly HoC platform featuring four micro-EHTs (µEHTs) consisting of 50,000 cells each. We show that the addition of flow improves contractile performance of the µEHTs. Importantly, we demonstrate for the first time that culture of cardiomyocytes (CMs), endothelial cells (ECs), smooth muscle cells (SMCs) and fibroblasts (FBs) results in self-assembled µEHTs, where ECs form a wrapping layer around the tissue, better mimicking the in vivo-like CM-EC interface while preserving direct cell-cell contact. We found that this combination of cell types enhances cardiac performance by improving contractile force, post-rest potentiation, maintenance of high frequency pacing and conduction velocity. Additionally, delayed drug responses are observed compared to µEHTs with only CM:FBs, suggesting a possible role of the EC layer in separating the CMs from the drug exposure. Thus, we believe that this innovative HoC model will help understanding heart biology in vitro and facilitate accurate disease modeling and cardiac toxicity screening.

Keywords: Engineered Heart Tissues (EHTs), Heart-on-Chip (HoC), In vitro advanced cardiac modeling

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ALLELE-SPECIFIC TARGETING OF BAG3 USING CRISPR/CAS9 IN MFM6 PATIENT-DERIVED IPSCS

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Myofibrillar myopathy-6 (MFM6) is a devastating muscle disorders caused by a genetic mutation in the BAG3 gene. As its genetic defect has been known, correction or inactivation of BAG3 mutation represents a rational therapeutic strategy for MFM6. Research on animal models involving MFM6 have been performed, however, studies on patient-derived iPSC cells have not yet been conducted due to the rarity of the disease. Here, we explored allele-specific CRISPR/Cas9 strategies of selectively inactivating the mutant BAG3 allele in patient-derived iPSC lines. MFM6 patient-derived iPSCs showed capacity of self-renewal and differentiation into endodermal, mesodermal, and ectodermal cells, indicating that the BAG3 mutation does not affect the characteristics of pluripotent cells. Moreover, patient iPSC lines hemizygous for BAG3 due to allele-specific CRISPR/Cas9 could differentiate into all three germ layers and cardiomyocytes, suggesting that one copy of normal BAG3 is sufficient for differentiation. Our data demonstrating the feasibility of allele-specific CRISPR/Cas9 genome editing strategies for the BAG3 mutation represents novel therapeutic approaches for MFM6. In addition, our panel of patient-derived iPSCs and CRISPR/Cas9-targeted lines provide powerful tools for in vitro disease modeling and developing novel therapeutic modalities for MFM6.

Keywords: Allele-specific CRISPR/Cas9 in genetic mutation, Myofibrillar myopathy-6 (MFM6) patient-derived iPSC, BAG3 mutation

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GENERATION OF SPLANCHNIC MESODERM-DERIVED CARDIAC ORGANOID FROM HUMAN PLURIPOTENT STEM CELLS BASED ON THE ACTUAL DEVELOPMENTAL PROCESS

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The heart is representative mesoderm-derived organ that plays pivotal roles in circulatory system by pumping-out a lot of blood containing oxygen and nutrients. Hence, the cardiac damage caused by chemotherapy drugs or cardiovascular diseases is critical enough to decide the crossroads of life and death. Cardiac organoid has been widely investigated as a platform to study drug

evaluation and cardiac pathology as it recapitulates the complex three-dimensional microenvironment of the heart. While most researches have actively studied heart cell differentiation efficiency and patterning, the understanding of how to differentiate cardiac cells based on the actual developmental process remains yet to be disclosed. Here, we introduced the developmentally more sophisticated and self-organized cardiac organoid from human pluripotent stem cells (PSCs). First, we PSCs were sequentially differentiated PSCs into the primitive streak (PS) and lateral plate mesoderm (LPM) stages by modulating TGF, BMP, WNT signal pathways. Then, the LPM cells were further differentiated into a splanchnic mesoderm (SPLM) cells under regulation of BMP signals in a spherical state. After verifying the representative markers for SPLM, we embedded the SPLM spheres into the matrigel dome to induce the self-organization under the B medium supplemented with C and D. About 48 hours from embedding, the SPLM spheres spontaneously assembled in the center, and pulsating began to be observed. Correspondingly, the increased expression of early cardiac markers were also validated by qRT-PCR and immunostaining data. Although self-organized cardiac organoids were observed as above, a more advanced method for the differentiation and patterning of region specific cardiac cells such as cardiomyocyte within the left ventricle still remains to be discovered. Collectively, we differentiated cardiac cells step-by-step from PSCs using a developmentally more sophisticated method, and these cardiac cells not only self-organized but also dynamically heart-beat with increased cardiac markers.

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Keywords: Pluripotent stem cell, Caridac organoid, Development

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MECHANICAL INSIGHTS INTO HYPERTROPHIC CARDIOMYOPATHY ASSOCIATED TNNT2 VARIANTS R278C USING HIPSC-CMS

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Hypertrophic cardiomyopathy (HCM) is the most common heritable cardiovascular disease and is the leading cause of sudden cardiac arrest (SCA) in youth and young adults. HCM primarily results from variants of genes encoding for sarcomeric contractile proteins. Variants in troponin T are of clinical interest because they are less likely to be associated with cardiac hypertrophy and more likely to be associated with SCA. In this study, a heterozygous R278C+/− TNNT2 variant was introduced into human induced pluripotent stem cells (hiPSCs) using CRISPR/Cas9 and subsequently differentiated into ventricular cardiomyocytes (hiPSC-CMs). Then a functional analysis was conducted on R278C+/− and their isogenic control (WT) hiPSC-CMs to investigate the mechanism by which R278C+/− affect contractility. To measure the kinetics of contraction of the WT and R278C+/− hiPSC-CM 2D monolayers, SarcTrack, a Matlab algorithm was used. Since immature hiPSC-CMs fail to recapitulate the physiological complexity of intact cardiac system, a physiologically relevant substrate was generated using polydimethylsiloxane (PDMS). Finally, WT and R278C+/- hiPSC-CMs were treated with 0.3 μM and 1 μM of Mavacamten, an allosteric myosin ATPase inhibitor, while their mechanical properties were measured using SarcTrack. The results of contractility analysis showed a significant increase in the sarcomere shortening and contractile kinetics of R278C+/- compared to WT hiPSC-CMs when they paced at 1Hz and 2Hz. Moreover, a significant decrease in the sarcomere shortening and relaxation kinetics of R278C+/− hiPSC-CM were observed upon Mavacamten treatment. To further investigate the underlying mechanism of increased contraction in R278C+/- variant, the phosphorylation status of cardiac-myosin binding protein C (cMyBP-C) and myosin light chain (MYL2) were studied using Mass spectrometry (MS). A reduction in the super relaxed state myosin due to the cMyBP-C and MYL2 phosphorylation may be regarded as a possible mechanism for increased contractility in R278C+/- hiPSC-CMs. However, further studies are required to assess the underlying mechanisms of increased contraction in TNNT2 variants. Studying the alterations in cardiomyocytes' function set the stage for identifying new therapeutic targets for this clinically challenging disease.

Keywords: Hypertrophic cardiomyopathy (HCM), hiPSC-CMs, Contractile kinetics

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MITOTHERAPY RESCUES MELAS-ASSOCIATED DEFECTS OBSERVED IN CARDIOMYOCYTES AND ENDOTHELIAL CELLS

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Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS), is a prevalent maternally inherited mitochondria disorder, resulting from a m.3243A>G mutation in the MT-TL1 gene, which codes for tRNA Leu(UUR). This condition is associated with a myriad of clinical syndromes, with cardiac abnormalities being present in one-third of MELAS patients, including altered vasculature and stroke-like episodes. The mutation causes complex I deficiency and leads to organ dysfunction through defects in the electron transport chain and increased reactive oxygen species production. Therefore, maintaining healthy mitochondria is crucial to counter the negative effects of pathogenic mtDNA mutations and restore normal tissue functions. To model MELAS at an in vitro level, induced pluripotent stem cells derived from MELAS patients harboring >80% of m.3243A>G mutant mtDNA were used to differentiate into disease cardiomyocytes and endothelial cells. Results demonstrated that MELAS derived-endothelial cells displayed vascular functional defects such as impaired wound healing and tube formation capabilities, which results in angiopathy and reduced blood flow to the microvessels in several organs. These processes eventually lead to the manifestation of stroke-like episodes. Given the heart being an energetically demanding organ, disruptions in oxidative phosphorylation process can lead to structural damage and impaired function in the heart, which progressive accumulation results in cardiac conditions such as arrhythmia and hypertrophy. In line with previous clinical observations, our study found that MELAS cardiomyocytes display both hypertrophic and arrhythmic phenotypes. In this study, mitotherapy, the transplantation of healthy mitochondria into diseased cells, demonstrated to ameliorate

pathologies manifested in diseased cardiomyocytes and endothelial cells through the restoration of redox and cellular bioenergetics, leading to improvements in cellular functions. Our findings suggest that mitotherapy may be a promising treatment option for mitochondrial related diseases where conventional therapies such as pharmacological treatment and dietary supplementation offer little or no long-term benefits.

Keywords: mitotherapy, mitochondria-genetic disease, cardiac

TOPIC: EPITHELIAL INCLUDES SKIN GUT LUNG

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IN VITRO EXPANDED STROMAL CELLS RECONSTITUTE A LONG-LIVED PHENOCOPY OF THE NATIVE HUMAN THYMUS EX VIVO AND IN A NOVEL HUMANISED MOUSE MODEL

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The thymus is a primary lympho-epithelial organ where the T cell-mediated immune function develops. Alterations of thymus development or function result in severe immunodeficiency and autoimmunity. Despite its crucial role in immune regulation, the cellular and molecular mechanisms that regulate the generation and maintenance of the human thymus remain largely unknown. Therefore, there has been long-standing interest in developing models to study thymus generation and the potential to manipulate it clinically. Here, we developed the conditions for isolation of human thymic stomal cells capable of extensive in vitro expansion and characterized by an epithelial-mesenchymal phenotype, an unique, cell-intrinsic feature of thymus epithelial stroma that is stably maintained over many passages in culture. Moreover, we accomplished the full reconstitution of a human organ (not an organoid) long-term in vivo thanks to the cooperation of thymic epithelial, interstitial cells and haematopoietic stem cells (HSC) supported within a natural extra-cellular matrix (ECM) obtained by a novel perfusion-decellularization approach. Crucial to demonstrating the functional competence of thymic stroma upon in vitro expansion was its capacity to attract circulating human HSC, support T cell development and repopulate the periphery of athymic NSG mice. Such a whole human system opens the possibility of addressing many immunological questions including the development and functional maturation of conventional and unconventional human T cells; positive and negative selection; and the establishment of tolerance. These findings support the feasibility of a multidisciplinary approach to rebuild a functional human organ and establish a basis for studying the crosstalk between stroma, ECM and thymocytes, thus offering practical prospects for treating congenital and acquired immunological disorders.

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Keywords: human thymus, whole-organ regeneration, natural extracellular matrix

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THE ROLE OF RFX6 IN INTESTINAL PATTERNING AND FUNCTION

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The small intestine is patterned in an anterior-to-posterior manner by a gradient of growth factors such as WNT and FGF pathways. iPSC-derived human intestinal organoids (HIOs) have been used to generate intestinal tissue and to show manipulation of these signaling factors can be used to control regional patterning. Although, the signaling factors needed to pattern the tissue are relatively known, the specific transcription factors contributing to this regionalization remain to be identified. Here, we focus on transcription factor RFX6. Human RFX6 has been associated with Mitchell-Riley Syndrome which is characterized by intestinal growth retardation, atresia of the duodenum and permanent neonatal diabetes. At Cincinnati Children's Hospital, we were able to generate an iPSC line from a patient with a compound heterozygous mutation in RFX6. We generated Intestinal organoids and were able to replicate the phenotypes seen in the patient's biopsy. The organoids allow for further examining of the molecular functions of RFX6 and the effects of its loss and showed abnormal patterning of the duodenum. Rfx6-mutant duodenal HIOs exhibited ileal characteristics suggesting abnormal patterning and loss of duodenal function. To further understand the role of Rfx6 we aimed to rescue the phenotypes via 3 different approaches. First, one allele from our mutant line was corrected using CRISPR to test whether one WT allele was sufficient. We also attempted to rescue the phenotypes with downstream target Pdx1 and finally, by re-introducing endogenous levels of Rfx6. With this project, we aim to further understand the role and function of RFX6 in the development, patterning and function of the small intestine. Specifically, we want to identify possible downstream targets of RFX6 and understand their role in intestinal function and enteroendo-

crine cell lineage differentiation. Additionally, this project allows us to test our organoid model in a more disease-focused context which in combination with CRISPR could advance the field of regenerative medicine by correcting genetic mutations and generating healthy tissue from the corrected cell line that could be introduced back to the patient.

Keywords: Intestinal patterning, intestinal organoids, duodenal identity

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ESTABLISHMENT OF HUMAN IPSC-DERIVED WNT-ACTIVATING SKIN ORGANOID ENAVLED ATOPIC DERMATITS MIMIC MODEL BY INFECTION OF STAPHYLOCOCCUS AUREUS AND ITS PROTECTIVE EFFECTS BY CUTIVACTERIUM ACNES

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A recently developed human PSC-derived skin organoid model has opened up new avenues for studying skin development, diseases, and regeneration. The current model has limitations since the generated organoids are enclosed, circular aggregates with an inside-out morphology with unintended off-target development of cartilage. Here, we first demonstrated that Wnt signaling activation resulted in larger organoids without off-target cartilage. We optimized further using an air-liquid interface (ALI) culture method to recapitulate structural features representative of human skin tissue. Finally, we used the ALI-skin organoid platform to model atopic dermatitis by Staphylococcus aureus (SA) colonization and infection. SA infection led to a disrupted skin barrier and increased production of epidermal- and dermal-derived inflammatory cytokines. Additionally, we found that pre-treatment with Cutibacterium acnes had a protective effect on SA-infected organoids. Thus, this ALI-skin organoid platform may be a useful tool for modeling human skin diseases and evaluating the efficacy of novel therapeutics.

Funding Source: This study was partially supported by the Research Institute for Veterinary Science, Seoul National University Korean Fund for Regenerative Medicine(KFRM) grant (No. 22A0101L1-11)

Keywords: Human Pluripotent Stem cell, Skin organoid, Atopic dermatitis

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COMPARATIVE STUDY OF COMMERCIALLY AVAILABLE MATRICES FOR PSC MAINTENANCE AND INTESTINAL ORGANOID GENERATION

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Extracellular matrix (ECM) plays a critical role in cell behavior and development. Organoids generated from human induced pluripotent stem cells (iPSCs) came to the forefront of research in the last years. However, one of the biggest setbacks of using iPSCs is that classical cell culture material lacks the physiological cues to differentiate iPSCs. Incorporating commercially available ECM into stem cell culture provides physical and chemical cues beneficial for cell maintenance. Commercially available products derived from animal sources, such as Matrigel® and Cultrex™, are composed of ECM proteins and growth factors that support cell maintenance. Since the ECM holds tissue-specific properties that can modulate cell fate, an increasing demand for xeno-free matrices drove the development of products such as VitroGel™. While commercially available matrices are widely used in iPSC and organoid work, the equivalency of these matrices has not been evaluated yet. Here, we have performed a comparative study of iPSC maintenance and intestinal organoids (IO) generation in four different commercial matrices: Matrigel®, Geltrex®, Cultrex™, and VitroGel™. We found that the reported round shape of iPSC colonies cultured in Vitronectin is affected by culturing these on alternative matrices such as Matrigel®, Geltrex®, and Cultrex™. Despite the colonies' morphologic changes, minimal spontaneous differentiation was observed as of over 85% of the cells expressing the stem cell marker SSEA-4. VitroGel™ led to the formation of 3D clumps with over 65% of the cells expressing SSEA-4. While there is reduced viability after seeding iPSC on VitroGel™, increasing the concentration of growth factors (mTeSR Plus™ supplement) in the hydrogel solution improved iPSC viability and SSEA-4 expression. While the different matrices led to minimal variations in the IO maintenance, differentiation of Geltrex®-maintained iPSC resulted in a lower number of spheroid releases during Mid-/Hindgut stage. Altogether our results suggest that variations in the composition of different matrices might affect stages of IO differentiation. This study contributes to the stem cell and organoid fields by raising awareness about the differences in commercially available matrices and providing a guide for matrix optimization during iPSC and IO work.

Keywords: Intestinal Organoids, iPSC, Extracellular Matrix

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DEVELOPMENT OF NOVEL HIPSC-DERIVED TRIPLE CO-CULTURE MODEL TO INVESTIGATE SARS-COV-2 INFECTIVITY IN THE LUNG

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COVID-19, caused by SARS-Cov-2, continues to be a fatal disease resulting in respiratory problems. SARS-CoV-2 is known to impact the vasculature and immune cells in the lung. Most human model systems utilize only the lung epithelial population to understand viral infectivity. To understand the mechanisms of lung injury in humans, develop novel strategies, and therapeutics to reduce infection, we require a model system containing lung epithelial cells interacting with endothelial and immune cells. Cell responses after acute infection with SARS-CoV-2 will allow a better un-

derstanding of the viral life cycle, cells being targeted, and cell communication. We propose to create and study a physiologically relevant assay representing human lung tissue and study cell signaling before and after infection with SARS-CoV-2. This system will include iPSC derived epithelial cells, endothelial cells and macrophages to model a biologically relevant lung injury. We have successfully differentiated lung organoids, endothelial cells and macrophages. Lung organoids were differentiated in a stepwise fashion through the endodermal lineage of embryogenesis into lung progenitor cells. After 6 weeks, 3D proximal lung organoids were dissociated into transwells and airlifted to induce an airway cell phenotype. Lung organoids expressed the proteins e-cadherin, ZO-1 and EPCAM. Endothelial cells were differentiated over 7 days. iPSCs were exposed to the hematopoietic signaling factors BMP4, CHIR, Forskolin and VEGF. Endothelial cells expressed CD31 and were sorted to create a pure population. Macrophages were derived from iPSCs over 2 weeks via embryonic bodies, hematopoietic progenitors, and myeloid cells using a combination of growth factors including BMP4, VEGF, IL-3, and M-CSF. The macrophages express CD45 and we are currently working on differentiating them into alveolar macrophages expressing CD11c. We are preparing to co-culture lung epithelial cells and macrophages on the apical side of the transwell and the endothelial cells on the basolateral side. IL-1β, TNF-α , IL-10, and TGFβ will be analyzed before and after infection with SARS-CoV-2. These findings will enable the understanding of SARS-Cov-2 in a human lung and the signaling mechanisms involving endothelial cells and macrophages.

Keywords: co-culture, infectivity, cell signaling

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HUMAN LUNG STEM CELL MODELS FOR SCREENING ENVIRONMENTAL EFFECTS ON THE AIRWAY

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The adult stem cells of the airways play a critical role in repair and regeneration by responding to injury with an initial self-renewal response followed by differentiation. Aberrant repair can lead to excessive self-renewal and/or altered differentiation which can lead to lung diseases with poor mucociliary clearance, premalignant lesions or fibrosis. Previous research on environmental effects on the human airways has mainly utilized immortalized lung cell lines or lung cancer cell lines, which are not the most representative model of human airways. We are utilizing two human adult stem cell models to examine the effects of environmental exposures such as smoking, vaping and viral infection, on the airways. We cultured ABSCs in an air-liquid interface (ALI) system to develop a fully differentiated human mucociliary epithelium. We developed an exposure chamber controlled by a microprocessor to expose the surface of the ALI cultures to environmental pollutants. We then compared the effects of cigarette smoke (CS) and vaping on the ALI airway. CS exposure resulted in ABSC proliferation, increased mucus production and loss of ciliated cells. In contrast, vaping resulted in less ABSC proliferation and loss of cilia, but increased mucus production. We also exposed the ALI cultures to SARS-CoV-2 viral infection. Exposure to CS increased the amount of viral infection and, similar to human patients, remdesivir and paxlovid showed efficacy against SARS-CoV-2 infection, whereas hydroxychloroquine did not. To generate the distal airway to examine the effects of environmental exposures on stem cells, we grew human alveolar type II (AT II) stem cells as spheroids. Similar to the ALI model, when the cells are exposed to cigarette smoke extract (CSE) the AT II cells show increased proliferation but lost AT II cell markers and underwent dedifferentiation. Similar to our ABSC ALI cultures, CSE increased SARS-CoV-2 infection by five-fold in AT II cells. Both stem cell airway models can be cultured in a 96 well format for high throughput drug screening for antivirals or for toxicity testing. As it is challenging to study environmental exposure on the airway, these stem cell models can elucidate these effects and help to discover new drug treatments for airway repair and/or antiviral therapeutics.

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Keywords: Airway Stem Cell Organoids, Airway Stem Cell Injury Response, Airway Stem Cells Environmental Exposures

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REVEALING THE UNEXPECTED ROLE OF TMPRSS2 IN SARS-COV-2 INFECTION AND TRANSMISSION USING HPSC-DERIVED LUNG ORGANOIDS

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To enter a host cell, SARS-CoV-2 uses its spike protein to bind to the ACE2 cellular receptor and then is primed by the type II transmembrane serine protease TMPRSS2. However, the exact role and mechanism of TMPRSS2 in this process remains unclear. Our study revealed that TMPRSS2 is expressed in intracellular and extracellular vesicles (EVs). In addition, EVs containing TMPRSS2 and ACE2 enhanced SARS-CoV-2 pseudovirus transduction rate in a dose-dependent manner. Furthermore, we identified unexpected cell populations with TMPRSS2 protein on the cell surface that do not express TMPRSS2 mRNA. These findings suggest potential mechanisms by which TMPRSS2 participates in SARS-CoV-2 infection/transmission, particularly to cell types that do not express this specific protease. Our findings may facilitate the development of therapeutic strategies that target TMPRSS2 which could help treat COVID-19 and other deadly respiratory viral diseases.

Keywords: SARS-CoV-2, COVID-19, hPSC-derived lung organoids, TMPRSS2, ACE2, extracellular vesicle, infectivity, human lung, respiratory infectious disease

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NOVEL ROLE OF BPIFA1 IN MAINTAINING AIRWAY EPITHELIUM HOMEOSTASIS IN PREMATURE HUMAN LUNG DEVELOPMENT

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The human airway epithelium protects the host from environmental insults, however, infants born prematurely have underdeveloped lungs unable to aptly defend themselves. At birth, the transition from hypoxia (in-utero) to normoxia (postnatal) initiates the secretion of homeostatic proteins that line the airway surface to protect the lung. But soon after birth, premature neonates are at extreme risk of acquiring severe respiratory syncytial virus (RSV) infections because the mechanisms of airway defense have yet to be established. The airway surface layer (ASL), lines the surface of the lung epithelium and is critical in maintaining airway homeostasis in neonates. BPIFA1 is the airway surfactant that maintains ASL balance to prevent disease. However, while BPIFA1 is known to support lung homeostasis and prevent pathogen induced airway disease in mice or in human adults, no research has investigated the value of BPIFA1 in a developmentally compromised human airway. Therefore, we wish to assess: the correlation between epithelium maturity and BPIFA1 expression, the influence of oxygen tension on BPIFA1 expression, and if immature epithelium lacking BPIFA1 has worsened RSV infections. First, temporal and localized expression of BPIFA1 was assessed in vitro using human stem cell derived airway epithelial cultures; BPIFA1 expression was found to correlate with the maturation of goblet epithelial cells, validated with primary human fetal lung tissue samples. Next, influence of oxygen tension on BPIFA1 was investigated by culturing airway epithelial cells in 5% or 21% oxygen; BPIFA1 expression was found to increase with increase in oxygen tensions. Lastly, antiviral response of epithelium by BPIFA1 expression evaluated; BPIFA1 was found to be upregulated after RSV infection. These findings investigate if premature birth potentially stunts the production of homeostatic protein BPIFA1 to explore the therapeutic potential of supplementing BPIFA1 in the premature infant population.

Funding Source: CIRM Training grant EDUC-4 12813

Keywords: Lung Development, Lung Differentiations, Airway Surfactant

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AN ORGANOID CRISPRI SCREEN REVEALED THAT SOX9 MAINTAINS HUMAN FETAL LUNG TIP PROGENITOR STATE BY ENHANCING WNT AND RTK SIGNALING

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The fine balance between self-renewal and differentiation in human fetal lung epithelial progenitors drives human lung epithelial branching morphogenesis and cell fate determinations. Details of the molecular networks regulating human lung progenitor self-renewal and differentiation remain unknown. Mouse models have been the primary model to study lung development. However, given the differences in sizes, cellular compositions and gene expressions, mouse lung cannot fully recapitulate human lung development. We have established a human fetal lung organoid culture system to facilitate human lung development studies. Advancing this platform, we have established targeted gene integration, CRISPR interference and CRISPR activation, and NanoBlade to facilitate efficient gene targeting, gene up-regulation and down-regulation, and genetic knockout, empowering lung organoids for gene function studies. Leveraging these tools, we performed the first CRISPRi screen in primary human lung organoids to identify transcription factors controlling progenitor self-renewal. We showed that a pair of SOX proteins display distinct functions in progenitor self-renewal regulation. SOX2, although is expressed in human fetal lung tip progenitor cells doesn't involve in progenitor cell self-renewal. However, another SOX protein, SOX9, promotes proliferation of lung progenitors and inhibits precocious airway differentiation. Moreover, we used Targeted DamID to identify SOX9 direct regulatory factors and discovered that SOX9 is at the intersection of amplifying WNT and RTK signaling to stabilize the progenitor cell state. The proof-of-principle CRISPRi screen and Targeted DamID tools establish a new strategy for using primary human organoids to elucidate detailed functional mechanisms underlying normal development and disease.

Keywords: Human lung development, Organoids, CRISPRi and CRISPRa

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POINT MUTATION IN P63 LEADS TO STEM CELL FAILURE THAT CAN BE RESCUED BY A SMALL MOLECULAR WEIGHT COMPOUND

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Adult stem cell (SC) failure may be caused by both environmental and genetic insults. The impact of disease-causing SC mutations on tissue integrity and cellular dynamics remains largely unknown, mainly due to the lack of reliable in-vivo models. Here, we utilized the cornea that serves as a robust model for monitoring SC dynamics and failure in live animals. SCs that constantly renew the corneal epithelium are located at the peripheral zone known as the limbus, and their self-renewal is highly controlled by the transcription factor P63. We report that single L514F point mutation in P63 leads to loss of corneal transparency and blindness in human patients, a clinical condition known as limbal SC deficiency (LSCD). We established a conditional mouse model carrying the L514F mutation in limbal/corneal epithelial cell lineage and discovered that it recapitulates the LSCD phenotype found in patients carrying the same mutation. Quantitative lineage-tracing revealed hyper SC proliferation coupled with extensive SC loss, leading to SC exhaustion and failure to maintain tissue homeostasis. Moreover, RNA-sequencing revealed loss of corneal identity, abnormal cell adhesion and imbalanced expression of pro-angiogenic factors. Intriguingly, treatment with a small molecular weight compound, PRIMA-1MET, substantially prevented the development of LSCD and alleviated its aggravation following injury. In conclusion, this study demonstrates that L514F mutation disrupts SC function, dynamics and tissue integrity in both mice and human, leading to loss of corneal transparency and potential blindness. Furthermore, PRIMA-1MET showed substantial therapeutic potential in vivo and may serve as a future therapy for human patients.

Keywords: Stem cell failure and rescue, P63 mutation, Mutated stem cell dynamics

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DYNAMIC NETWORK-GUIDED CRISPRI SCREEN REVEALS CTCF LOOP-CONSTRAINED ENHANCER FUNCTION IN CELL STATE TRANSITIONS

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Enhancers play key roles in gene regulation. However, comprehensive enhancer discovery is challenging because most enhancers, especially those affected in complex diseases, have weak effects on gene expression. Through gene regulatory network modeling, we identified that dynamic cell state transitions, a critical missing component in prevalent enhancer discovery strategies, can be utilized to greatly improve the cells' sensitivity to enhancer perturbation. Guided by the modeling results, we performed a mid-transition CRISPRi-based enhancer screen utilizing human embryonic stem cell definitive endoderm differentiation as a dynamic transition system. The screen discovered a comprehensive set of enhancers (4 to 9 per locus) for each of the core lineage-specifying transcription factors (TFs), including many enhancers with weak to moderate effects. Integrating the screening results with enhancer activity measurements (ATAC-seq, H3K-27ac ChIP-seq) and three-dimensional enhancer-promoter interaction information (CTCF looping, Hi-C), we were able to develop a CTCF loop-constrained Interaction Activity (CIA) model that can better predict functional enhancers compared to models that rely on Hi-C-based enhancer-promoter contact frequency. Together, our dynamic network-guided enhancer screen and the CIA enhancer prediction model provide generalizable strategies for sensitive and more comprehensive enhancer discovery in both normal and pathological cell state transitions.

Funding Source: National Institutes of Health grant U01HG012051 National Institutes of Health grant U01DK128852

Keywords: Enhancer discovery, Gene regulatory network, Enhancer prediction

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EXPERIMENTAL PLATFORMS FOR MALE DIFFERENTIATION OF HUMAN IPSC DERIVED GERM CELLS

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The indifferent gonad provides cues to primordial germ cells (PGC) in order to initiate male or female development. Male fate depends on the presence of Sertoli cells (SC). The cacsacde initiated by SRY expression leads to Sertoli cell progenitors. Via aggregation and outgrowth, these cells form seminiferous cords which provide the necessary compartment for livelong spermatogenesis. PGCs enclosed in the cords arrest mitotically and become gonocytes and spermatogonia in the later stage of testicular oganogenesis. Here we describe three different organoid platforms which mimic the cellular environment during transition from bipotential gonad to the testis. A critical prerequisite is cord structure formation by somatic cells. Human induced pluripotent stem cells (iPSC) derived PGCs can be added to explore somatic germ cell cross-talk and testicular niche interaction. In our system the primary cells from immature rats and human testis (transgender) form cord like structures in the presence of the appropriate extracellular matrix and culture conditions. As a third track, we can create a bipotential gonadal environment from human iPSC using cytokine cocktails and stepwise cell selection strategies. Our systems are standardized and reproducable. SOX9 is applied as Sertoli cell marker and SMA as a marker for peritubular myoid cells. Cord like structures usually appear at 7 days of culture and can be maintained for an extended culture period of another 10 days. PGCs are characterized on protein level by the three markers OCT4, SOX17 and AP2gamma. When added on Day 1 to the platforms PGCLCs can be maintained for up to 14 days. Therefore, all three systems can be used as feeders to maintain germ cell like status. hPGCLCs populate the organoids and grow in specific patterns detected by immunofluorescence. Interestingly, also hiPSC populate and respond to the somatic environment of the organoids and few cells undergo fast transition into PGCLCs (AP2gamma, OCT4, SOX17) in just 7 days. Our platforms provide strategies for exploring early germ line development and PGC interaction with male somatic environments. We foresee that this

system is useful for toxicological testing and drug screening on human germ cells. Long term cultures may provide options for generating and expanding human male germ cells from pluripotent cells.

Funding Source: Deutsche Forschungsgemeinschaft, (CRU 326)

Keywords: testicular organoids, germ cells, development, ipsc, toxicological screening, spermatogenesis, sertoli cells, differentiation, male germ line, testicular organoids, germ cells, development, ipsc, toxicological screening, spermatogenesis, sertoli cells, differentiation, male germ line, organoid, testis, male germ line

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GENERATING SINGLE CELL-DERIVED HUMAN BLASTOIDS IN 3D BIOREACTOR CULTURE

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Blastoid is a useful model of the blastocyst derived from naïve pluripotent stem cells to study early human development. Human blastocyst is formed about five days after fertilization with a fluid-filled cavity and contains different lineages including the epiblast, trophectoderm, and primitive endoderm. Current methods can make blastoids within 4 day starting with cell aggregates, while nature embryos develop from a single cell. Here, we use a stirred-tank bioreactor to form blastoids in seven days starting from single cells. By collecting cells at different timepoints, we show that the cells grow from single cells to two-cell stage, four cell-stage, eight-cell stage, morula-like structures, and finally blastoids. These bioreactor-blastoids have a diameter of about 150um-250um and comprise around 200 cells, which are comparable to the human blastocyst. Thus, we propose that the bioreactor-blastoid can be used to study human implantation and early development in a faithful and ethical manner.

Keywords: blastoid, early development, bioreactor

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JADE1 AND THE HBO1 COMPLEX ARE SPATIAL-SELECTIVE COFACTORS OF OCT4

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Oct4 is a master regulator of pluripotent, undifferentiated gene expression states. The interaction partners of Oct4 are incompletely defined. Like other POU domain proteins, Oct4 is capable of binding to DNA in different configurations, including for example simple octamer, PORE (palindromic octamer-related element) and MORE (more palindromic octamer-related element). However, the effects of these different configurations on transcription output are unknown. Here, we show that Oct4 interacts with common and unique proteins when bound to DNA in different configurations. One of these proteins, Jade1, more strongly associates with Oct4 when bound to MORE DNA sequences that bind Oct4 dimers. We show that multiple Jade1 isoforms interact with Oct4 in solution. We are performing ChIP-seq and CUT&RUN-seq with HBO1 antibodies, which is in the same complex with jade1, to test preferential HBO1 and Jade1 binding to MORE sites in vivo. We are also using purified recombinant Oct4 and a Jade1/HBO1-containing complex to test for direct binding and test the effect of Oct4 DNA binding on histone acetyltransferase activity.

Keywords: Pou5f1/Oct4, Jade1/HBO1, spatial-selective binding

TOPIC: HEMATOPOIETIC, IMMUNE, AND ENDOTHELIAL

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GENOME OF ENTEROVIRUS 71 CROSSES IN VITRO MODEL OF THE HUMAN BLOOD-BRAIN BARRIER

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Central nervous system (CNS) viral infections often bypass or disrupt the restrictive character of the human blood-brain barrier (BBB). Enterovirus 71 (EV-A71) infections are highly associated to the development of neurological complications, such as brain stem encephalitis due to the risk of reaching peripheric nervous, and migrate into the CNS via axonal retrograde transport. However, the invasion of EV-A71 into the CNS through the BBB is still under investigation. To evaluate the direct interaction between the EV-A71 and the human BBB, we reproduced a cellular model by using brain-like endothelial cells (BLECs) derived from hematopoietic progenitors CD34+ isolated from umbilical cord blood. BLECs achieve BBB phenotype when co-cultured with pericytes in semi-permeable membranes known as transwells. The BBB model was infected via apical side, where BLECs were cultured. The infection was performed by using three EV-A71 strains genetically distinct at multiplicity of infection (MOI) = 1 (equivalent to one virion per cell). The immunofluorescence analysis showed active viral replication in a limited number of BLECs. These cells, even under monoculture revealed low sensibility to EV-A71 infection. The evaluation of cell viability showed high levels of living cell indicator, the GF-AFC until 48 hours post infection (h.p.i). The EV-A71 infection did not affect the protein expression of tight junction Zonula occludens 1 (ZO-1) neither altered the endothelial permeability, the permeability coefficient of the BBB model remained

near 0.5 x 10-3 cm.min-1, similar to uninfected condition. The titration of the cell supernatants showed that the infection of BLECs at apical compartment does not cause a leakage of infectious particles into the basal compartment. Nevertheless, RT-qPCR analysis revealed the presence of EV-A71 genome in both apical and basal supernatants. Our study concludes that EV-A71 does not present an imminent risk of directly disrupts the human BBB, but during this infectious context the BBB may release the EV-A71 genome at the brain parenchyma. Our study highlights that investigate the implication of EV-A71 genome in the brain parenchyma may help to better understand the EV-A71 neuropathogenesis.

Funding Source: This work was founded by Marie Skłodowska-Curie Actions - Innovative Training Networks (MSCA-ITN)

Keywords: Blood-brain barrier (BBB), Viral infections, Neuropathogenesis

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ANALYSIS OF TUMOR MICROENVIRONMENT IN MOUSE ESCS -> FLK-1 KO CHIMERIC MICE GENERATED BY BLASTOCYST COMPLEMENTATION

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Humanized animal models are important for both basic research and drug development in oncology field. Although several kinds of humanized mice have been developed by the transplantation of human cells to immunodeficient mice, there are several hurdles to overcome including incomplete reconstitution and limited cellular lineages. Especially, vascular endothelial cells and tissue-resident macrophages of embryonic origin are difficult to be reconstituted in the current humanized animal models, though these cells are key players in tumor microenvironment (TME). One possible solution for this is to generate the humanized animal models by blastocyst complementation. To explore the possibility, we performed blastocyst complementation with ICR Flk-1 (Vegfr2) knockout embryos as a host and Azami-Green (AG)-positive C57/BL6 (B6) mouse-derived embryonic stem cells (ESCs) as a donor. By flow cytometry and immunofluorescent analysis, we observed that vascular endothelial cells, hematopoietic cells, and tissue-resident macrophages including microglia, were derived from the injected ESCs (AG+). To further examine the capability of using this model for immuno-oncology studies, we first confirmed the engraftment of MC38, B6-derived tumor cells, into the chimeras, and analyzed TME of the engrafted tumors by using mass cytometry. The analyses showed that not only tumor infiltrating lymphocytes (TILs) and tumor-associated macrophages (TAMs), but also majority of cancer-associated fibroblasts (CAFs) were derived from donor cells, and that TILs (AG+) worked correctly as in B6 mice. In addition, angiogenesis by donor cell-derived vascular endothelium occurred successfully in the tumors. These data suggest that humanized animals created by blastocyst complementation using Flk-1 KO as a host would be useful models for immuno-oncology research and drug development.

Keywords: Blastocyst complementation, Modeling immune system, Tumor microenvironment

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REAL-TIME MONITORING OF HOXA9 IN PLURIPOTENT STEM CELL DERIVED MODELS OF HAEMATOPOIESIS

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The homeodomain-containing transcription factor HOXA9 has long been proposed to play a central role in hematopoiesis. Overexpression and knock-out mouse studies demonstrate it is the most important hoxa gene in hematopoietic stem cells (HSC), with roles in HSC self-renewal and in vivo engraftment. In human pluripotent stem cell (hPSC) differentiation to hematopoietic cells, expression of HOXA genes during mesoderm specification is proposed to replicate intra-embryonic hematopoiesis. Further, HOXA9 expression during differentiation follows the pattern of hematopoietic commitment, suggesting HOXA9 may act as a marker of the definitive hematopoietic program. Our current studies have similarly demonstrated HOXA9 is gradually upregulated post mesoderm, reaching highest levels in the hematopoietic CD34+ population. However, the mechanism, stage and extent to which HOXA9 influences hematopoietic cell fate, in particular definitive cell fate, remains unclear. CRISPR-Cas9 was used to generate endogenous HOXA9/mScarlet-H reporter (eA9m) and AAVS1-targeted inducible HOXA9/mScarlet-H (iA9) iPSC lines to monitor HOXA9 expression during differentiation. Both lines were molecularly validated by PCR and Sanger sequencing and functionally validated by confocal microscopy during embryoid body (EB) and optimised monolayer-based hematopoiesis. Endogenous HOXA9 expression increases by Day 4 and peaks at Day 7 of iPSC-derived hematopoiesis coincident with the loss of pluripotency and expression of mesoderm markers and expression of hemogenic endothelial markers respectively. Formation of hematopoietic stem and progenitor cells was confirmed by flow cytometry and methylcellulose colony assays. Preliminary data shows that either stimulation of iA9 cells with doxycycline or transduction of developing eA9m cells with oncogenic MLL-AF9 results in a marked increase in HOXA9 and mScarlet-H. Induced HOXA9 expression in mesoderm results in altered gene expression (RNAseq) and colony formation of derived HSPCs. Stage, level and duration of HOXA9 expression in iA9 cells influences

both hematopoietic cell formation and cell fate. Together, the data supports the potential use of parental, iA9 and eA9m iPSCs to generate isogenic models of disease involving dysregulated expression of HOXA9, including cancer.

Funding Source: The Joan Browne Legacy Fund (University of Nottingham School of Medicine) and The Nottinghamshire Leukaemia Fund.

Keywords: HOXA9 reporter model, iPSC-derived HSPCs, MLL-AF9

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MODELLING HEMATOPOIESIS AND HEART DEVELOPMENT IN BLOOD-GENERATING HEART-FORMING ORGANOIDS (BG-HFOS) DERIVED FROM HPSCS

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In embryogenesis, hematopoietic stem cells (HSCs) derive from the hemogenic endothelium in the dorsal aorta, in close spatial and functional proximity to the heart anlagen. However, despite the biomedical importance of HSCs, their in vitro induction and niche-like stabilization in proper developmental context is challenging, because of the complex and poorly understood signalling required for HSCs and stem-niche development. Human pluripotent stem cell (hPSC)-derived heart-forming organoids (HFOs) represent a complex and highly structured multi-tissue model recapitulating key aspects of native heart development, accompanied by foregut and vasculature anlagen including putative hemogenic endothelium, recently published by us (Drakhlis et. al., Nat Biotechnol, 2021). Modulating the HFO protocol, we here induce directed differentiation of hemogenic endothelial and hematopoietic stem progenitor cells (HSPCs), without disrupting the three-dimensional multi-tissue pattern of HFOs, thereby generating an advanced model termed blood-generating heart-forming organoid (BG-HFO). BG-HFOs are composed of an inner core of endothelial and mesenchymal cells, surrounded by a half shell-structured myocardial layer recapitulating human heart anlagen, which is enveloped by an outer layer. The latter is composed of proepicardial cells and, importantly, a distinct endothelial compartment harbouring HSPCs, further supported by mesenchymal cells, equivalent to the intraembryonic zone where the first HSCs and HSPCs arise in native embryogenesis. The multilineage potential of BG-HFO-derived HSPCs has been revealed by flow cytometry and single-cell RNA-sequencing, and functionally proven via assays demonstrating erythro-myeloid potential. To determine presence of definitive HSCs within the BG-HFO-derived HSPCs cluster, lymphoid potential assay and in vivo engraftment in irradiated mice is underway. Together, the novel BG-HFO model recapitulates stabilised haematopoiesis, thereby recapitulating important aspects of native hematopoietic niche formation and HSPCs maintenance in vitro. Moreover, thanks to its multi-tissue complexity, the BG-HFO model might serve as a valuable system for advanced disease modelling, including the utility of patient-specific iPSCs.

Keywords: human pluripotent stem cells (hPSCs), hematopoietic stem cells (HSCs) development, cardiac development

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MODELING THE PATHOGENESIS OF MYELODYSPLASTIC SYNDROME IN BONE MARROW FAILURE

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The fundamental mechanisms underlying the pathogenesis of human myelodysplastic syndrome (MDS) are unknown. This is due to the limited availability of primary patient specimens, poor adaptability of MDS hematopoietic stem and progenitor cells (HSPCs) in culture, and low potential of these cells for xenotransplantation. There is a need to better define the molecular pathobiology of MDS, particularly in high-risk subtypes, to improve survival. MDS frequently develops in the setting of inherited bone marrow failure (BMF) disorders, where inherently defective HSPCs bearing germline mutations acquire secondary somatic mutations that confer aberrant clonal expansion and inhibit effective differentiation. Advances in genetic engineering and directed differentiation of induced pluripotent stem cells (iPSCs) offers the opportunity to develop novel human-based models of BMF and the pathogenesis of MDS. Here, we leveraged our iPSC-based model of the BMF of Fanconi anemia (FA), a congenital disorder of DNA repair with a predisposition to MDS, to understand how secondary somatic mutations drive the transition from failing HSPCs to aberrantly expanding MDS HSPCs. To do this, we performed simultaneous CRISPR-based editing of MDS mutational hotspots in iPSCs followed by directed differentiation to definitive HSPCs. By selecting HSPC subclones based on the acquisition of serial self-renewing of clonogenic potential, we identified RUNX1 mutations as increasing FA HSPC fitness and thus as candidate drivers of the MDS phenotype in FA. This aligns with sequencing studies of human FA MDS, where RUNX1 mutations are highly recurrent. We find that RUNX1 mutations increase the fitness of FA HSPCs through two mechanisms: direct interference with the p53/p21-triggered G1/S cell cycle checkpoint that is hyperactivated in the failure state and triggering of innate inflammatory signaling. Inflammatory signaling recruits downstream compensatory DNA repair mechanisms, enhancing HSPC fitness and promoting clonal expansion in the absence of somatic reversion. Innate inflammatory signaling is a vulnerability of FA MDS cells in culture and in vivo. We corroborate our results in primary patient FA MDS cells. Together, our studies demonstrate the utility of iPSCs in dissecting the basic stem cell biology of BMF disorders and MDS.

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Keywords: disease modeling, hematopoietic stem and progenitor cells, myeloid malignancies and bone marrow failure

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MICROGLIAL HYPERACTIVATION SHAPES THE CENTRAL NERVOUS SYSTEM PATHOLOGY IN AICARDI-GOUTIÈRES SYNDROME CAUSED BY IFIH1 MUTATION

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Aicardi-Goutieres syndrome (AGS) is a devastating infantile encephalopathy caused by overproduction of type I interferon (IFN). Recent progress in disease modeling techniques enabled us to elucidate the underlying mechanisms; however, previous reports have mainly focused on the interaction between neurons and astrocytes, and the role of microglia in the disease pathogenesis has not been well investigated. AGS is classified as an interferonopathy in which the chronic activation of the innate immune system plays a critical role; thus we generated microglia and central nervous system (CNS) cells from patient-derived induced pluripotent stem (iPS) cells aiming to elucidate the role of microglia in CNS pathogenesis. We established iPS cells from two AGS7 patients with IFIH1 mutation and derived CNS cells and microglia from both control- and patient-derived iPS cells. There was no significant difference in differentiation capacity. In CNS cells, AGS7

cells showed hyperactivation of the type I IFN pathway as well as other cytokine signal cascades, which was consistent with those reported previously. Microglia, on the other hand, showed hyperactivation of type I IFN pathway predominantly, and the changes were much greater than those in CNS cells. Next, to investigate the effect of humoral factors secreted by microglia, the CNS cells from control-iPS cells were treated with culture supernatant of control- and disease-microglia. Western blotting confirmed enhancement of STAT1 phosphorylation and increases in interferon inducible genes such as RIG-I and MDA5 in the CNS cells treated with AGS-microglia supernatant. Apoptosis was also observed in neurons treated with AGS-microglial supernatant. Recently, using iPS cell models, astrocyte overactivation has drawn attention as a cause of neuronal cell death in AGS1 (TREX1 deficiency) and AGS2 (RNASEH2B deficiency). Our result also showed slight increase in neuronal cell death in CNS cell culture containing both neurons and astrocytes. However, the effect of microglia had more impact on neuronal apoptosis, indicating overproduction of type I IFN from microglia plays an important role in CNS pathogenesis.

Funding Source: AMED: 20ek0109387h0003

Keywords: Interferonopathy, Aicardi-Goutières syndrome, Microglia

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AGING-ASSOCIATED CHANGES IN PLASMA PROTEOME AND HIPPOCAMPAL GENE EXPRESSION ARE REVERSED BY TREATMENT WITH IPSC-DERIVED MONONUCLEAR PHAGOCYTES

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A number of studies have shown that administering young plasma or bone marrow to aging mice has rejuvenating effects, including better performance on learning and memory tasks, and improvements in markers of neural health. The aim of the current study was to evaluate the potential of using human induced pluripotent stem cells (iPSCs) as a therapeutic product in order to achieve similar benefits. iPSCs were differentiated into mononuclear phagocytes (iMPs), as these cells are known to become less effective with age, and were administered to aging, genetically immunocompromised NOD scid gamma mice. iMP treatment significantly improved cognitive performance and neural health markers in aging mice. To begin to elucidate potential mechanisms underlying these effects, we performed a proteomic analysis of plasma as well as single nucleus RNA sequencing on hippocampus. Interestingly, we find that pathways that show aging-associated changes in plasma overlap with those in hippocampus, suggesting that these alterations are global. Notably, iMP treatment reverses the aging-associated phenotypes in both plasma and hippocampus, such that aging mice treated with iMPs are more similar to young mice. These findings identify an aging signature that is conserved between the periphery and the brain and, critically, suggest that iMPs provide a novel personalized medicine strategy for aging-associated diseases.

Keywords: Aging-associated cognitive decline, Neural health, Hippocampus

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INNOVATIVE PRE-TRANSPLANT CONDITIONINGS TO IMPROVE THE IMMUNOLOGICAL RECONSTITUTION IN IMMUNE DYSREGULATION DISORDERS

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Hematopoietic stem cell transplantation (HSCT) is the only treatment for severe immune disorders. Although the elimination of resident hematopoietic stem/progenitor cells (HSPCs) is crucial to allow engraftment of donor HSPCs, current conditioning regimens based on chemo/radiotherapy are associated with significant toxicities. Antibody-based conditioning, as anti-CD45 mAb conjugated with saporin (CD45-SAP), is a promising approach for safety improvement, however, there is a need to maximize its efficiency for various HSCT settings. We set out to test feasibility and efficacy of conditioning protocols based on CD45-SAP combined with a low dose of clinically relevant chemotherapies,

treosulfan and fludarabine (Treo/Flu), in distinct preclinical models of immune disorders. We assessed the depletion activity, engraftment and immune reconstitution achieved by CD45-SAP combined with a low dose of Treo/Flu, comparing it with CD45-SAP alone, full dose of Treo/Flu and total body irradiation (TBI), in Rag1-knock-out (KO) mice modelling T-B- severe combined immunodeficiency, Rag1-F971L mice recapitulating combined immunodeficiency with granulomas and autoimmune manifestations, and Wiskott-Aldrich Syndrome (Was)-KO mice characterized by immunodeficiency, thrombocytopenia and autoimmunity. Full Treo/Flu dose and TBI regimens efficiently deplete HSPCs and lymphoid progenitors in bone marrow resulting in high myeloid chimerism and immune cell reconstitution in all mouse models transplanted with WT HSPCs. The combination of CD45-SAP with low dose of Treo/Flu was well tolerated by all mice and showed a superior depletion effect in central and lymphoid organs as compared to CD45-SAP alone. Mice conditioned with the combination of CD45-SAP with low dose of Treo/Flu showed a myeloid chimerism and immune recovery higher than mice treated by CD45-SAP alone and similar to mice conditioned with full Treo/Flu dose or TBI. Our preliminary data show that CD45-SAP combined with low dose of Treo/Flu allows robust immune reconstitution in mouse models of diseases with a spectrum of immunodeficiency and autoimmunity. These data may eventually broaden the applicability of CD45-SAP in allo- and autologous HSCT when high chimerism or the clearance of autoreactive cells are necessary to ensure the curative outcome.

Funding Source: Italian Ministry of Health (GR-2019-12369050 to M.C.C.)

Keywords: Hematopoietic stem cell transplantation, Anti-CD45-saporin, Conditioning

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RECREATING ALVEOLAR CAPILLARY COMPLEXITIES IN VESSEL ORGANOIDS TO UNCOVER MECHANISMS UNDERLYING VASCULAR MALDEVELOPMENT CAUSED BY FOXF1 PERTURBATIONS

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Foxhead box F1 (FOXF1) is a transcription factor that is indispensable for proper pulmonary alveologenesis. Mutations in the FOXF1 gene cause a rare and severe congenital lung disorder called Alveolar Capillary Dysplasia with Misalignment of Pulmonary Veins (ACD/MPV). ACD/MPV is characterized by vasculo-pulmonary growth defects including immature lobular development and a sizeable decrease in number of capillaries in alveolar walls. Individuals with ACD/MPV typically die within the first few weeks of life due to respiratory failure. At present, it is unclear how mutations in FOXF1, a gene predominantly expressed in the developing lung mesenchyme, cause alveolar capillary malformations that ultimately lead to abnormal alveologenesis in humans. The lack of therapeutic or pharmaceutical interventions for ACD/MPV is perpetuated by this gap in knowledge. Here, we report the derivation of vessel organoids (VOs) with alveolar capillary complexities from iPSCs that were generated from ACD/MPV patients with different FOXF1 mutations (ACD-VOs). ACD-VOs displayed distinct vascular defects such as a significant reduction in endothelial cell population, diminished cell migration capacities, and poorly formed vascular networks that lack lumen structures. Also, differentiating ACD-VOs exhibited clear developmental aberrations and altered FOXF1 expression. In addition, we observed that ACD-VOs were highly hypoxic, which corresponds to what was observed in ACD/MPV patients. We were also able to recapitulate phenotypic changes in alveolar EC subtypes that were reported in ACD lung tissues. By introducing the patient-specific FOXF1 mutations via site-directed mutagenesis and performing luciferase assay, disrupted FOXF1 activity caused by the mutations was confirmed. By employing CUT&Tag, we were able to discern molecular mechanisms underlying capillary maldevelopment caused by FOXF1 perturbations. Utilizing a 3D vascular organoid system, coupled with the use of patient iPSCs harboring unique FOXF1 mutations, we present a strategic approach to carefully decipher the role of FOXF1 in human alveologenesis.

Funding Source: NIH LungMAP2, AHA Pre-doctoral Fellowship

Keywords: Vessel organoids, Alveolar Capillary ECs, Vascular development

TOPIC: KIDNEY

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REVOLUTIONIZING RENAL RESEARSCH: MICROFLUIDIC KIDNEY TUBULOIDS-ON-A-CHIP FOR IMPROVED DRUG TRANSLATION

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Current 2D in vitro models of renal epithelium lack the key features of the in vivo setting, such as tubular structure and perfusion, resulting in low translatability to human situations and failure in clinical translation. Microfluidic techniques are becoming increasingly recognized as a valuable tool for adding physiologically relevant cues to traditional cell cultures. These cues include long-term gradient stability and continuous perfusion. Microfluidic technology also allows for the patterning of cell layers as stratified co-cultures that are free from artificial membranes, enabling the capture of complex tissue architectures found in vivo. Here, we introduce the OrganoPlate microfluidic platform, which can accommodate up to 64 independent microfluidic chips in a microtiter plate format, allowing the growth of 64 independent kidney tubuloid-derived barrier tissues in the form of perfused tubules. These renal tubules can be formed in just four days of culture in the device showing rapid and reproducible cell polarization, tight junction formation and proper expression of renal markers. When integrated into an OrganoPlate system, kidney tubuloids

form leak-tight, perfusable tubes with stable Trans Epithelial Electrical Resistance (TEER), and are suitable for high-throughput screening of compound effects through assessment of barrier integrity by use of OrganoTEER and by real-time imaging of transport. OrganoPlate grown kidney tubes treated with Pgp inhibitor Verapamil show significant reduction of Rhodamine123 transport through kidney tubule barrier which confirms show stable activity of Pgp transporter and usability of the model in studying renal drug clearance. Our results demonstrate the suitability of our in vitro microfluidic kidney tubuloid-on-a-chip model in mimicking key physiological aspects of the kidney and offer new ways for studying organ physiology and renal disease mechanisms and drug toxicity.

Keywords: Perfused kidney organoid tubuloids, Improved drug translation, 3D human disease model

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REVEALING THE MOLECULAR NUANCES OF TRPC6 IN FOCAL SEGMENTAL GLOMERULOSCLEROSIS IN AN IN VITRO HUMAN PODOCYTE MODEL

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Focal segmental glomerulosclerosis (FSGS) disease is caused by mutations in the transient receptor potential cation channel-6 (TRPC6) gene, which is inherited as an autosomal dominant pattern. With predominance in podocytes, TRPC6 is involved in the proper intracellular calcium level, which maintains the normal physiological function of podocytes. Excessive or shortage of calcium influx in these cells may result in apoptosis, foot process effacement, and nephron degeneration. Here, we aim to unravel the mechanism behind podocyte loss in FSGS that is proposed to be caused by the gain of function (GOF) or loss of function (LOF) mutation in TRPC6, expressed in podocytes and supplement the lost podocytes by producing them in vitro. We have established a systematically optimized inducible FSGS model system in human induced pluripotent stem cells (hiPSCs) by targeting the dual genomic safe harbor (GSH) locus. The Tet-ON-controlled system allowed the controlled expression of the GOF / LOF TRPC6 proteins. Furthermore, these cell lines were induced to differentiate into podocytes. In order to assess the effects of mutation–dependent intracellular Ca+2 perturbations in podocytes, we measured the calcium influx in combination with TRPC6 agonists and inhibitors. Our data showed comparable responses of calcium influx that are consistent with the GOF and LOF phenotypes. In addition, we want to use transgenic TRPC6-induced podocytes in a microfluidic chip-based model system. The novel glomerulus platform offers a deeper understanding of the TRPC6-associated mechanism of podocyte loss by allowing the comparison between healthy and diseased podocytes in their morphology and behavior. We strongly believe that this humanized and personalized system can provide helpful insights for future therapeutic interventions that can slow down or even abolish the progress of FSGS and other CKDs.

Keywords: FSGS, TRPC6, Podocytes

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SPECIFYING PROXIMAL NEPHRON CELLS IN SYNCHRONIZED KIDNEY ORGANOIDS

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The function of the kidney is to receive one fifth of the cardiac output and to filter and maintain blood composition. Compounds that are required by the body are reabsorbed while unwanted metabolites are excreted. Renal function is performed by proximal tubule cells (PT). These cells are acutely vulnerable to damage and toxicity, and PT-related pathologies are the primary reason patients need dialysis and kidney transplants. While stem cell-based systems hold great promise for providing regenerative therapies alleviating kidney disease and developing safe therapeutics with reduced nephrotoxic side effects, current kidney organoids do not meet this demand. Regardless of the protocol used to generate them, PT organoid cells do not mature beyond early nephron stages and display differentiation abnormalities. A reason for this is that organoid nephrons (nephroids) do not receive signals that in vivo nephrons normally do. To address this bottleneck, we have developed a strategy utilizing timed addition of small-molecule inhibitors that, with precision, mimic cell-fate decisions. This drives normal developmental programs to proximally bias nephroids in our new system of synchronized nephroids. Our preliminary experiments show that transient application of small-molecule inhibitors drives a Notch-dependent response in early nephroids and generates proximal cell identities. Notch ligands and target genes are upregulated in response to early treatments and the nephroids gradually activate transcription factor HNF4A, which is necessary for normal PT maturation and function. Sequencing analyses of FAC sorted proximalizing cells show treated organoids increase early HNF4A target genes that impart physiological function to the PT, such as CUBN and LRP2. This work provides insights into the signaling networks that underpin the formation of PT cells, contributing to the development of high-fidelity in vitro models of PTs. These protocols are a strong stepping-stone for our efforts to build functional proximal cells.

Keywords: kidney organoid, proximal tubule, organoid nephrons

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HUMAN BILIARY ORGANOID MODEL TO EVALUATE ADD3 GENE PREDISPOSITION IN BILIARY ATRESIA

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Biliary atresia (BA) is a neonatal fibroinflammatory disease, resulting in the obstruction of biliary trees. To date, one of the putative risk factors – ADD3 gene was discovered in genome-wide association studies although the mechanisms of ADD3 have not yet been delineated in human specimens. In this study, we aimed to unearth the roles of ADD3 in biliary development, as well as the underlying pathogenesis in BA. We combined the state-of-the-art techniques of human induced-pluripotent stem cell (hPSC) and CRISPR-Cas9 to generate control, ADD3+/- and ADD3-/- stem cells. Organoids were derived in a stepwise manner to recapitulate key biliary development, which started with the differentiation of hPSCs into endoderms, foregut progenitors, hepatoblasts, cholangiocyte progenitors and eventually mature cholangiocytes. Knock out of ADD3 perturbed biliary development, given that markers of hepatoblasts (alpha fetoprotein and SOX9) and those of cholangiocyte progenitors (cytokeratin19 and SOX9) were downregulated. ADD3+/- and ADD3-/- biliary organoids also demonstrated aberrant morphology and retarded growth, which expressed fewer differentiation markers (hepatocyte nuclear factor 1 homeobox B [HNF1B], cytokeratin 19, SOX9, and γ glutamyl transferase [GGT]) and proliferation marker (Ki67). Interestingly, loss of ADD3 disrupted apical – basal organization through impairing the localizations of F-actin, tight junction protein zonula occludens-1 (ZO-1), claudin, radixin, β catenin and cystic fibrosis transmembrane conductance regulator (CFTR), which indicated the disruption of cell polarity. Last but not least, loss-of-function mutation of ADD3 gene can inactivate Notch, Wnt and YAP/TAZ pathways which governed biliary development. Together, these results suggest that downregulation of ADD3 hinders biliary development and disrupts apical–basal organization, recapitulating the phenotype of BA patient liver organoids.

Funding Source: Theme-based Research Scheme (T12-712/21-R) RGC Hong Kong SAR Government, Hong Kong SAR, China

Keywords: hPSC – derived biliary organoids, ADD3, Biliary Atresia

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EXPLORING THE MECHANISTIC BASIS OF A GENETIC VARIANT-MEDIATED RISK TO NONALCOHOLIC FATTY LIVER DISEASE IN A DISSECTIBLE HPSC-DERIVED LIVER CULTURE

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A number of genetic polymorphisms (SNP) have been associated with the pathogenesis of nonalcoholic fatty liver disease (NAFLD), but their underlying mechanisms remain elusive. Here, we focused on the rs738409 C>G, and the encoded patatin-like phospholipase-domain containing protein 3 (PNPLA3) I148M variant, which shows a strong association with NAFLD. We created a human pluripotent stem cell (hPSCs)-derived multicellular liver culture and modeled NAFLD by adding lipotoxic risk factors derived from patients to co-cultured hepatocytes, hepatic stellate cells and macrophages. Using a pair of isogenic liver cultures differing only at the rs738049 locus, we observed enhanced development of NAFLD phenotypes by the I148M variant. These differences were associated with elevated IL6/STAT3 activity, which is also observed in patient liver biopsies. Dampening IL6/STAT3 activity alleviated the I148M-mediated susceptibility to NAFLD, while boosting it in wild-type liver cultures led to an enhanced development of NAFLD phenotypes. Our study thus reveals a potential causal link between elevated IL6/STAT3 activity and 148M-mediated susceptibility to NAFLD. Our liver culture is therefore a useful platform through which isogenic pairs of hPSCs can be utilized to dissect the mechanistic contributions of genetic variants to NAFLD.

Keywords: Nonalcoholic fatty liver disease, Genetic variant, Multicellular liver model

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cAMP SIGNALLING IMPROVES IPSC-DERIVED HEPATOCYTE POLARITY AND MATURITY TO GENERATE IMPROVED MODEL FOR STUDYING CHOLESTASIS

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Cholestasis is a liver disorder characterised by impaired bile formation and flow. It is caused by genetic mutations in the canalicular transporters ABCB11, ABCB4 or ATP8B1, or by drugs that inhibit them. The lack of in vivo and in vitro cholestasis models limits our understanding of the disease and ability to predict drugs that cause drug-induced cholestasis (DIC) or treat genetic cholestasis (GC). Consequently, DIC remains a main cause of drug failure in clinical trials and withdrawal of drugs from the market. There are also no approved medicines to cure GC, so patients rely on liver transplants. There is therefore a critical need for better cholestasis models. The aim of this project is to generate a new physiologically relevant iPSC-derived hepatocyte (iHEP) model where the iHEPs form functional bile canaliculi (BC), express BC transporters and produce bile which can be used for modelling cholestasis. We found that stimulation of cAMP signalling in a Matrigel sandwich culture during a 25-day differentiation protocol significantly improved iHEP functionality, polarisation and BC formation. Electron microscopy revealed that the iHEPs were ultrastructurally more hepatocyte-like, with spherical mitochondria and nuclei. The iHEP BC expressed the appropriate ABC transporters, were sealed by junctional complexes, developed a brush boarder and appeared very similar to normal liver. BC functionality was confirmed by their ability to actively excrete a fluorescent dye into the BC lumen. Using CRISPR-Cas9, we generated iHEPs with cholestatic ABCB4null and ABCB4S320F mutations and showed that ABCB4S320F expression at the BC membrane was reduced to ~50%, as expected. ABCB4S320F expression at the BC membrane could be increased to wild type levels using cyclosporin A, demonstrating the utility of this model to screen for compounds that increase BC expression of ABC transporters in cholestatic patients. We also induced cholestasis via bile acid overload and ABCB11 inhibition and showed that the iHEPs mimicked key cholestatic changes in BC morphology, lipid composition, ultrastructure and function. In conclusion, we generated an advanced iHEP model that shows mature hepatic functionality and polarisation which has allowed us to model characteristic changes associated with cholestasis for the first time in an iHEP model.

Funding Source: BBSRC and AstraZeneca

Keywords: Hepatocyte Differentiation, Cholestasis, Liver Disease Modelling

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SCREENING PLATFORM FOR GALNAC-CONJUGATED THERAPEUTICS USING IPSC-DERIVED HEPATOCYTE-LIKE CELLS

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RNA-based therapies represent an attractive solution to modulate clinically relevant targets that are considered unattackable via traditional approaches. They have the potential to easily adapt to the rapid evolution of mutation rate and spectrum for genetic disorders. Safe, effective, and selective in vivo delivery of RNA-based therapies to the liver has shown to be achievable by conjugating the RNA molecule to a carbohydrate moiety, N-acetylgalactosamine (GalNAc), that facilitates receptor-mediated uptake of the molecule. In particular, the abundant and specific hepatic ASGR receptor is capable of mediating uptake and internalization of GalNac-conjugated molecules allowing this class of therapeutics to overcome the struggle of cellular barriers, maximize delivery to on-target cells and minimize exposure to off-target cells. In this context, a physiologically relevant in vitro system that can model a specific disease phenotype and simultaneously provide a platform for screening GalNac-conjugated RNA-based therapeutics could be a powerful tool within the framework of pre-clinical efficacy studies. Our platform allows us to differentiate successfully induced pluripotent stem cells (iPSC) into hepatocyte-like cells (HLCs) that show expression levels of key hepatocyte markers close to primary human hepatocytes (PHHs) and can resemble specific disease phenotypes making it a versatile technology for disease modelling. In this study we evaluate the ability of iPSC-derived HLCs to be used as a screening platform for GalNac-conjugated RNA-based therapeutics. Despite differences in the gene expression levels of ASGR1 compared to PHHs, we demonstrated that iPSCs-derived HLCs effectively respond to GalNac-conjugated siRNAs by measuring their specific ASGR-mediated intracellular uptake and silencing effect on the targeted genes in addition to showing an increased uptake versus non-conjugated siRNA. The results were consistent across iPSC-derived HLCs lines from different donors, showing the robustness of the screening platform regardless of the genetic background. This together with the great potential of iPSCs to model different human diseases offer a unique opportunity for personalised medicine to develop faster and to finally shine a light on the unmet medical needs of liver diseases.

Keywords: GalNac conjugated RNA-based therapeutics, Liver disease modelling, Screening platform

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A NOVEL LIVER-HUMANIZED RAT MODEL WITH FUMARYLACETOACETATE HYDROLASE DEFICIENCY AND SEVERE COMBINED IMMUNODEFICIENCY

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To mimic human metabolism and test the drug efficacy and toxicity, animal models and human /rat liver microsome are wildly used over the past decades. Among them, humanized-liver generated from mouse showed superior advantages for a great variety of applications. However, mouse can only provide limited biological samples for analyses. Since rat is much bigger, and has been proved to recapitulate human in various physiological and pathological aspects, humanized rat models would be a preferred one over mouse. By CRISPR/Cas9 technique, we generated Fah-/-Rag2-/-IL2rg-/- (FRG) rat model, in which the progressive liver damage status could be controlled by treatment of 2-(2-Nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC). Moreover, FRG rats showed extremely low level of CD3+ T cells, CD45RA+ B cells, and CD161a+ NK cells, which offer a superior tolerance for xenogeneic transplantation. Under the controlling of NTBC, FRG rats were highly repopulated with human hepatocytes and achieved human albumin levels >1.5 mg/ml 7 months after transplantation. Over 10% human hepatocytes were co-expressed Ki67, indicating a continuously proliferative status. Moreover, the hepatocytes in vivo could maintain the expression of human-specific metabolism marker genes. This model not only offers a reliable platform for generating humanized liver, benefiting the prediction of the specific pharmacokinetics and drug–drug interactions in humans, but would also be applied in hiPSC-derived hepatocytes and hepatic organoid transplantation, as well as liver disease modeling.

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Keywords: Humanized liver, FRG rat, Human drug metabolism

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LOSS-OF-FUNCTION MUTATION OF KIF3B CAN CAUSE A DEFECTIVE BILIARY DEVELOPMENT IN BILIARY ATRESIA: EVIDENCE FROM IPSC-DERIVED BILIARY ORGANOID

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Biliary Atresia (BA) is a poorly understood devastating fibro-obliterative biliary disease of newborns. Limited access to primary biliary tissue, difficulties in culturing primary biliary cells (cholangiocytes) and inadequate animal disease model have led to a slow advancement in unravelling the patho-mechanisms, diagnosis and treatment for BA. Human iPSC-derived biliary organoids provide us an unprecedented cellular model to study BA. We have conducted whole exome sequencing on 85 BA trios, identified deleterious loss of function (LOF) mutations in cilia-related genes including KIF3B in 31.5% non-syndromic BA patients. KIF3B encodes Kinesin-like protein KIF3B that is a subunit of the anterograde intraflagellar transport (IFT) motor protein kinesin-II in cholangiocyte cilia. Functional analyses demonstrated absence of cilia in the BA livers with KIF3B mutation and knockdown of KIF3B in human fibroblasts resulted in reduced number of cilia. Additionally, CRISPR/Cas9-engineered zebrafish knockouts of KIF3B displayed reduced biliary flow. In this study, we generated KIF3B+/- & KIF3B-/- human iPSC cells and differentiated them into biliary organoids to investigate the impacts of the KIF3B LOF mutation in biliary development in BA.Single-cell-RNA-seq analysis and immuno-staining showed that KIF3B+/- and KIF3B-/- iPSCs are less capable in the differentiation of hepatoblast and cholangiocyte progenitors (CPs). Individual cell AUC revealed down-regulation of Wnt, Notch and TGF-beta pathway activity, while cell-cell interaction analysis showed a defective cell-cell interaction mediated by TGAV and ITGB8 (integrin αvβ8) in the KIF3B+/- and KIF3B-/- CPs. Furthermore, KIF3B+/- & KIF3B-/- biliary organoids were few, tiny and with abnormal or no cilia. Bulk-RNA-seq and immunostaining analysis of biliary organoids revealed a shift from cholangiocyte to hepatocyte differentiation in KIF3B+/- & KIF3B-/- biliary organoids. Taken together, our data indicate that KIF3B plays a key role in cholangiocyte differentiation, which demonstrates that the human iPSC-derived biliary organoid is a valuable disease model for patho-mechanistic study of BA.

Keywords: Biliary Atresia, KIF3B, iPSC-derived biliary organoids

TOPIC: MESENCHYMAL STROMAL CELLS, ADIPOSE, AND CONNECTIVE TISSUE

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A TARGETED MEDICINE APPROACH FOR DERMAL CLINICAL THERAPIES USING WHARTON JELLY MESENCHYMAL STEM CELL (WJ-MSC) SECRETOME

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Mesenchymal stem cells obtained from the stroma of the human umbilical cord (WJ-MSC) are particularly advantageous for stem cell therapy due to their easy obtaining, hypo-immunogenicity, high proliferation, and differentiation characteristics. WJ-MSCs secrete bioactive factors, stimulating the patient's cells for angiogenesis and tissue repair. Although the WJ-MSC secretome, applied directly or in combination with biomaterials, has a prominent therapeutic effect acting locally and systemically, its regenerative, anti-inflammatory, and wound-healing properties have not been fully elucidated. We aimed to identify proteins present in the secretome of WJ-MSCs by LC-MS/MS combined with Exponentially modified Protein Abundance Index (emPAI) analyses. Our results showed several secreted proteins, some potentially carried by exosomes, which is relevant when addressing the proper mechanism of secretome delivery in clinical trials. Functional enrichment analysis showed that most proteins were related to structural support networks associated with the extracellular matrix, organization, and assembly; there was also an enrichment for angiogenesis and blood vessel development functions. When analyzing only secreted proteins, the processes related to ossification and skeletal system development appear significantly enriched. Moreover, the analysis of proteins potentially present in exosomes highlights the wound-healing process. These observations may be relevant to enhance the therapeutic efficacy by obtaining a selected core of bioactive factors from WJ-MSC that reflect a personalized formulation for patients with specific disease profiles in the healing of cutaneous wounds. Current biosafety studies on individuals are considering these WJ-MSC's secretome selection criteria when used for specific dermal treatments.

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Keywords: Wharton Jelly Mesenchymal Stem Cells, Secretome, Wound Skin Repair

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HYPERINSULINEMIA CAUSES OXIDATIVE STRESS IN ADIPOCYTES BLOCKING MITOCHONDRIAL METABOLISM AND INSULIN RESPONSE

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Adipocytes are key regulators of whole-body metabolism, and they play a central role in metabolic disease such as type II diabetes mellitus (T2D) if their insulin signaling is disrupted. However, the progression of cellular insulin resistance (IR) towards full-blown T2D is still poorly understood. Our progress has in part been hampered because traditional models fail to recapitulate the human physiology of insulin signaling. We previously published a protocol to derive hPSC-adipocytes that mimic in vivo cell function, primarily by adapting the adipocytes to a medium resembling human physiology. By chronically exposing cells to 3nM insulin we can model diabetic hyperinsulinemia, leading to IR as demonstrated by reduced insulin response in protein phosphorylation, glucose uptake, and lipolysis, plus altered gene expression. The gene expression results implied dysregulated oxidative phosphorylation (oxphos) and glycolysis, which we confirmed functionally through Seahorse assays. Here we find IR adipocytes show impaired metabolism and absent acute insulin response. This is paired with increased oxidative stress and decreased mitochondrial membrane potential. Metabolomic profiling shows decreased overall abundance of glycolysis-related metabolites, and several key mis-regulated metabolites in the oxphos pathway. Alleviating oxidative stress by use of MitoTEMPO – an antioxidant specifically targeted to the mitochondria – normalizes IR adipocytes in Seahorse and metabolomics. More importantly, MitoTEMPO rescues insulin-stimulated AKT2 phosphorylation after hyperinsulinemia exposure. Altogether this reveals that IR leads to oxidative stress and impaired mitochondrial function, which we can therapeutically target. We hypothesize the mitochondrial dysfunction leads to a feedback mechanism on insulin signaling through protein post-translational modifications (PTMs), hinted at by altered levels of PTM-related oxphos metabolites such as acetyl-CoA. We are further trying to clarify the mechanism underlying insulin resistance in this setting, as well as leverage other metabolic insults including inflammation and hyperglycemia to study adipocyte metabolic disease. We have also had success with adapting this insulin signaling and IR model to a number of other stem cell-derived cell types.

Funding Source: This study was supported by Novo Nordisk.

Keywords: Diabetes, Adipocytes, Insulin resistance

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DEVELOPMENT OF NANOBODY-MEDIATED SPECIFIED TARGETING OF OSTEOSARCOMA CANCER STEM CELLS

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Osteosarcoma is a malignant bone tumor typically presenting in childhood and adolescence. The dense extracellular matrix (ECM) and the heterogeneity of osteosarcoma cells make developing targeted therapy for these tumors extremely difficult. Nanobody, or single-domain antibody, is an antibody fragment consisting of a single monomeric variable antibody domain. Nanobodies show excellent promise for treating osteosarcoma due to their high specificity and small size, allowing them to efficiently target specific surface markers on these tumor cells while penetrating through the dense ECM surrounding them. We hypothesized that nanobody-mediated specified targeting of osteosarcoma cancer stem cells (OCSC) could overcome these pitfalls and provide a new paradigm for solid tumor treatment. In the present study, we engineered an osteosarcoma cell line by labeling the cell membrane with GFP protein, which can be recognized by anti-GFP nanobody, to test the hypothesis. In vitro co-culture of GFP labeled and non-labeled tumor cells was used to evaluate GFP nanobody specificity. In vivo mouse models of GFP labeled and non-labeled tumor cells derived osteosarcoma were generated to test nanobody-mediated specific targeting and penetration. Cellular barcoding technique and single-cell RNA-sequencing were used to track lineage cell output of transplanted human primary osteosarcoma and to identify specific markers for OCSC. Novel nanobodies were then screened and tested to target OCSC. We observed that anti-GFP nanobody specifically binds to GFP-labeled osteosarcoma cells in the in vitro co-culture system. In the in vivo model, anti-GFP nanobody showed higher affinity and longer persistence in GFP-labeled tumors. Several specific markers for OCSC have been identified and are under testing for nanobody targeting. In conclusion, our results proved the concept of nanobody-drug conjugate for osteosarcoma cancer stem cell targeted therapy.

Keywords: Osteosarcoma, Nanobody, Cancer Stem Cells

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A NOVEL MITOCHONDRIAL HOMING DRUG, MITOCHONIC ACID5, MITIGATES A DYSFUNCTIONAL PHENOTYPE IN BARTH SYNDROME PATIENT-DERIVED IPSC-MYOBLAST

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Barth syndrome (BTHS) is an X-linked recessive multisystem disorder caused by a mutation in Taffazin (TAZ) gene. TAZ is highly expressed in both cardiac and skeletal muscle and catalyzes cardiolipin (CL) into its mature form at the mitochondrial inner membrane. Thus, the mutation in TAZ causes mitochondrial dysfunction followed by dilated cardiomyopathy and skeletal myopathy. However, there is currently no definitive treatment other than interventions to ameliorate acute symptoms. We recently developed a novel mitochondria-homing drug, Mitochonic Acid (MA)-5 (under phase 1 clinical trial), which can alleviate mitochondrial dysfunction and can facilitate ATP production by ATP synthase oligomerization and super complex formation through binding to a mitochondrial inner membrane protein. In this study, we investigated the therapeutic effect of MA-5 on BTHS using human iPSCs-derived myocytes and drosophila BTHS model with TAZ mutation. We first differentiated control (PGP1-TAZWT) and TAZ (PGP1-TAZ517delG) mutant human iPSCs into myoblast and examined their mitochondrial function and oxidative stress tolerance. As a result, the mitochondrial respiratory function, ATP production, and tolerance to oxidative stress deteriorated in TAZ mutated iPSC-myoblast compared to their control. Treatment with MA-5 rescued the mitochondrial dysfunction and increased cell survival in TAZ-mutated iPSC-myoblasts. We also examined the effect of MA-5 on the BTHS model drosophila. Administration of MA-5 improved the motor dysfunction measured by climbing assay in BTHS model drosophila. By electron microscopy, the muscle of BTHS model drosophila showed abnormal mitochondria accumulation that was significantly improved by MA-5 treatment. We also measured the cardiolipins by lipidomics and found that the CL composition was not changed by MA-5, suggesting that structural change of mitochondrial membrane by MA-5 would ameliorate the symptom of BTHS. In conclusion, these data suggest that MA-5 is a potential therapy for BTHS patients.

Keywords: Barth syndrome, Mitochondria, Myopathy

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THE BIG BAG3 THEORY: INSIGHTS FROM HIPSC MODELS FOR BAG3P209L - MYOFIBRILLAR MYOPATHY

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Patients with an amino acid substitution at position 209 in the Bag3 gene (p.P209L; c.626C>T) suffer from severe myofibrillar myopathy (MFM6) and neuropathy. The MFM6 manifests as skeletal muscle dystrophy and restrictive cardiomyopathy, reducing average life expectancy to about 20 years. BAG3 plays as a member of the CASA complex (chaperone-assisted selective autophagy) a key role in the turnover of muscle proteins. Our goal is to gain a mechanistic understanding of BAG3-associated muscle diseases and to develop new experimental therapeutic approaches. To this end, we generated a human induced pluripotent stem cell (iPSC) line carrying the BAG3P209L mutation both heterozygously and homozygously by gene editing from a well-characterized iPSC line. In addition, we use an iPSC line derived from a patient with BAG3P209L MFM6 and a BAG3-/- iPSC line together with the isogenic controls. These iPSC lines could be successfully differentiated into twitching skeletal muscle cells using an established differentiation protocol, and phenotypically analyzed. Interestingly, both the BAG3P209L mutation and deletion of the BAG3 gene lead to morphological abnormalities, such as the formation of thinner skeletal myotubes and an increase in apoptosis rate compared with controls. Western blot analyses and immunohistochemical stainings for structural components (e.g., desmin, actin, MF20) indicated differences, such as more prominent cross striations in controls compared to mutant myotubes. Members of the protein quality control system (e.g., BAG3, HSPB8, LC3B) were found to be upregulated in the patient-specific myotubes when compared with controls. In addition, we analyzed the effects of physical stress induced by electrical stimulation, which resulted in a significant increase in protein levels of BAG3, p62, and LC3B. Neuromuscular organoids (NMO) were generated to investigate both skeletal muscle and neuronal phenotypes of MFM6. Initial results showed that deletion of the BAG3 gene led to morphological changes as the BAG3-/--NMOs are larger. We also detected changes in the twitch pattern, both in spontaneous and electrically stimulated activity of the BAG3-/--NMOs. We are currently investigating whether these BAG3-associated pathomechanisms in the organoids can be attributed to a dysfunctional innervation.

Keywords: BAG3, skeletal muscle cells, iPSC

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EFFICIENT GENERATION OF PLURIPOTENT STEM CELL-DERIVED MYOGENIC PROGENITOR CELLS AND MYOFIBERS

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Driving efficient and pure skeletal muscle cell differentiation from pluripotent stem cells has been challenging. Here, we report an optimized protocol that generates skeletal muscle progenitor cells with high efficiency and purity, in a short period of time. Human induced pluripotent stem cells (hiPSC) and mouse embryonic stem cells (mESC) were specified into the mesodermal myogenic fate using distinct and species-specific protocols. We used a specific Maturation Medium to promote terminal differentiation of both human and mouse myoblast populations, and generated myotubes associated with a large pool of cell cycle-arrested PAX7+ cells. We further show that myotube maturation is modulated by the dish coating properties, cell density and percentage of myogenic progenitor cells. Given the high efficiency in the generation of myogenic progenitors and differentiated myofibers, this protocol provides an attractive strategy for tissue engineering, modeling of muscle dystrophies and evaluation of new therapeutic approaches in vitro.

Keywords: myogenic differentiation, hiPSC, muscle progenitor cells

TOPIC: NEURAL

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LOSS-OF-FUNCTION MUTATION IN KCNQ5 FAILS TO PROTECT FROM NEURONAL HYPERACTIVATION AND LEADS TO AN EPILEPTIFORM PHENOTYPE IN IPSC-DERIVED NEURONS

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Developmental and epileptic encephalopathy (DEE), as well as intellectual disability, have been linked to the malfunction of members of the KCNQ channel family. While the role of KCNQ2/KCNQ3 channels in epilepsy was first described in 1998, less is known about the function and malfunction of KCNQ5. First de novo mutations in the KCNQ5 gene have been described recently. Here, we search for novel KCNQ5 gene variants and aim to analyze the pathomechanism in a human iPSC-derived neural culture. Next-generation sequencing was applied to identify novel KCNQ5 variants in DEE patients. Two electrode voltage clamp re-

cordings, patch clamp, and disease modeling in human iPSC-derived neural cultures were conducted. We identified a novel heterozygous missense variant in the channel's distal N-terminus associated with epileptic encephalopathy. The missense variant displayed a strong loss-of-function in heterologous expression. Mutant channels exerted a significant dominant-negative effect on wildtype KCNQ5 and KCNQ2/KCNQ3 channels. Immunofluorescence microscopy revealed a reduced plasma membrane expression of the variant. Further, the missense variant failed to limit burst-like neuronal activity in human iPSC-derived neural culture, thereby leading to an epileptiform phenotype on the cellular level. Here, we identify a novel KCNQ5 variant in association with an epileptic encephalopathy phenotype in a patient. Loss-of-function, reduced trafficking of mutant channels to the plasma membrane, and dominant-negative effects on wt channels are detected in heterologous expression. The reduced KCNQ5 M-currents limiting function in human iPSC-derived neurons, indicated by increased burst-like behavior, suggests a pathomechanism of the variant to induce generalized seizures in patients.

Keywords: Epilepsy, Electrophysiology, Potassium channels

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MODELING PARKINSON'S DISEASE IN 2D AND 3D: EVALUATING ALPHA-SYNUCLEIN AGGREGATION, AUTOPHAGY, AND NEURAL NETWORK ACTIVITY

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Discovery and validation of new treatments for Parkinson's Disease (PD) can greatly benefit from the creation of advanced, biologically relevant human cellular models. In partnership with the Parkinson's Progression Markers Initiative (PPMI) and The Michael J. Fox Foundation (MJFF), FUJIFILM Cellular Dynamics generated iPSC lines from clinically symptomatic PD patients carrying known risk-associated gene mutations. These human iPSC lines were differentiated into midbrain dopaminergic neurons (iCell® DopaNeurons). The etiology of dopaminergic neuron cell death in PD is complex involving multiple factors that include mitochondrial dysfunction, impaired endosomal/lysosomal protein degradation, α-synuclein and tau aggregation, and neuroinflammation. In this study, we utilized dopaminergic neurons generated from apparently healthy normal (AHN) and PD donor-derived iPSCs harboring either the LRRK2 G2019S or GBA N370S mutations to investigate multiple mechanisms thought to underly PD. Dopaminergic neurons were cultured either in 2D (as a mono-culture or in co-culture with iCell Astrocytes) or as 3D spheroids (iCell NeuroSpheres). Mono-cultures of iPSC-derived dopaminergic neurons were evaluated for multiple cell functions related to neurodegeneration, including cell metabolism, neurite outgrowth and degeneration, α-synuclein aggregation, and lysosomal activation. Using α-synuclein preformed fibrils to seed the cultures, elevated α-synuclein aggregation was observed in LRRK2 G2019S and GBA N370S dopaminergic neurons compared to AHN controls. Interestingly, no striking differences were observed in any of the cells, with only a small change in puncta area for the GBA N370S mutant, when assessing engagement of autophagic pathways via LAMP-1 expression and puncta formation. We also evaluated neural network activity in both 2D, using multi-electrode array (MEA), and in 3D using calcium oscillation assays of dopaminergic neuron-containing iCell NeuroSpheres. Functional assays revealed diverse phenotypes for the PD patient-derived cells in both 2D and 3D systems compared to AHN controls. This study expands the characterization of LRRK2 G2019S and GBA N370S PD dopaminergic neurons and shows proof-of-concept data for 3D disease modeling of PD using multi-cellular neurospheres.

Keywords: Parkinson's disease, human iPSC-derived dopaminergic neurons, synuclein

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SARS-COV-2 INFECTION CAUSES DOPAMINERGIC NEURON SENESCENCE

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COVID-19 patients commonly present with neurological signs of central nervous system (CNS) and/or peripheral nervous system dysfunction. However, which neural cells are permissive to infection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been controversial. Here, we show that midbrain dopamine (DA) neurons derived from human pluripotent stem cells (hPSCs) are selectively permissive to SARS-CoV-2 infection , and that SARS-CoV-2 infection triggers a DA neuron inflammatory and cellular senescence response. A high-throughput screen in hPSC-derived DA neurons identified several FDA approved drugs, including riluzole, metformin, and imatinib, that can rescue the cellular senescence phenotype and prevent SARS-CoV-2 infection. RNA-seq analysis of human ventral midbrain tissue from COVID-19 patients, using formalin-fixed paraffin-embedded autopsy samples, confirmed the induction of an inflammatory and cellular senescence signature and identified low levels of SARS-CoV-2 transcripts. Our findings demonstrate that hPSC-derived DA neurons can serve as a disease model to study neuronal susceptibility to SARS-CoV-2 and to identify candidate neuroprotective drugs for COVID-19 patients. The susceptibility of hPSC-derived DA neurons to SARS-CoV-2 and the observed inflammatory and senescence transcriptional responses suggest the need for careful, long-term monitoring of neurological problems in COVID-19 patients.

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Keywords: SARS-CoV-2, midbrain dopamine neuron, senescence

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THE ROLE OF FATTY ACID BETA-OXIDATION IN HUMAN BRAIN DEVELOPMENT

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Neural stem cells (NSCs) are the stem cells that give rise to the entire brain and even continue to form new neurons throughout life. Understanding what regulates NSC behavior is thus important for development and adulthood. Recently, metabolism has been shown to have an important role in the regulation of stem cell activity/fate in different tissues. Previously, Knobloch and colleagues specifically described the importance of lipid metabolism for NSC quiescence, proliferation and integration of their progeny in the mouse brain. However, whether lipid metabolism plays a similar role in the regulation of human NSCs remains poorly understood. Therefore, we aim to assess the importance of lipid metabolism, specifically the breakdown of lipids, for human NSCs and during human brain development. To do so, we are targeting carnitine palmitoyl transferase 1a (CPT1A), the rate-limiting enzyme of fatty acid beta-oxidation (FAO) using the pharmacological FAO inhibitor etomoxir and shRNAs against CPT1A. We are using monocultures of in vitro derived humans NSCs to assess the effect of FAO blockage for NSC proliferation, apoptosis, quiescence and differentiation capacities. To better understand the

effect of FAO inhibition during brain development, we are also using cerebral organoids and neural rosettes, which model early brain development. Our results show that CPT1A is highly expressed in NSCs during brain development. While blocking FAO in human NSC monocultures only showed subtle effects, blocking FAO in cerebral organoids strongly reduced NSC proliferations and increased cell death, suggesting that FAO is indeed an important metabolic pathway for human NSCs.

Keywords: lipid metabolism, brain development, neural stem cell

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MODELLING NEUROEPITHELIAL BEHAVIOURS RELEVANT TO SPINA BIFIDA CAUSATION IN IPSC-DERIVED SHEETS

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Mammalian morphogenesis requires force-generating cell behaviours, such as apical constriction. During closure of the neural tube, neuroepithelial apical constriction is enhanced by non-canonical Wnt/Planar Cell Polarity (PCP) signalling. Failure of apical constriction can stall neural tube closure in mice, causing neural tube defects (NTDs) such as spina bifida, which affect 1:1,000 human pregnancies globally. Mutations in core PCP components including VANGL2 (e.g. R353C) have been identified in individuals with NTDs. What is lacking is experimental evidence that human NTD-associated mutations impair apical constriction. Human stem cell-based neural tube organoid platforms which model neural tube closure have been developed, but their neuroepithelial apical domain is inaccessible due to their complex geometry. Here, we used the well-established dual-SMAD inhibition protocol to differentiate iPSCs into neuroepithelial sheets. We validate that these sheets faithfully replicate the morphology of the neuroepithelium observed in vivo, and undergo apical constriction synchronised with their cell cycle. Screening for PCP components reveals differentiation-related activation of the pathway via apical enrichment of VANGL2. We compared VANGL2-R353C knock-in lines with congenic controls, and two lines derived from individuals with spina bifida versus a panel of six non-congenic equivalents. VANGL2 remains homogenously apically enriched in neuroepithelial sheets generated from VANGL2-R353C knock-in lines, but apical recruitment of its interacting partner SCRIBBLE is reduced compared to congenic controls. VANGL2-R353C knock-in neuroepithelial cells have significantly larger apical areas, and reduced apical recoil following laser ablation, compared with congenic lines. Similarly, one of the two patient-derived lines has larger apical areas than control lines, which are all comparable to each other. Our bedside to bench approach provides a highly reproducible modelling strategy to identify genetic failures of neuroepithelial apical constriction underlying spina bifida.

Keywords: Neural tube defects, Disease model, Planar Cell Polarity

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UNDERSTANDING GENETIC RISK FACTORS FOR NEUROPSYCHIATRIC DISORDERS IN CORTICAL INTERNEURON DEVELOPMENT USING HUMAN PLURIPOTENT STEM CELL DIFFERENTIATION

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Reliable cellular model systems are an invaluable tool for studying the neuropsychiatric disorders. We have revised existing protocols to generate cortical interneurons from human pluripotent stem cells (hPSCs) in 2D culture, resulting in consistent production of PV+ alongside other cortical interneuron subtypes which are implicated in neuropsychiatric disorders. Analysis of single cell transcriptomes of cortical interneuron differentiation revealed enrichment of both common and rare genetic risk variants for several disorders including SZ, ASD, neurodevelopmental delay (NDD) and major depressive disorders (MDD) in cortical interneuron development. This interestingly emphasised the concentrated risk in very early stage of development, i.e. transition from precursors to neurons. We have applied the protocol to hESC lines lacking DLG2 (de novo deletion found in SZ, ASD & bipolar disorder), revealing deficits in neurogenesis and enriched rare mutations for NDD and ASD in knockout cells during the neurogenesis. This suggests neuropsychiatric pathological processes may start much earlier than the currently postulated.

Funding Source: Medical Research Council, UK The Waterloo Foundation Royal Embassy of Saudi Arabia in the UK

Keywords: Cortical interneuron, Pluripotent stem cell neural differentiation, Common and rare genetic risk factors

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PHENOTYPIC ANALYSIS OF HUMAN RELEVANT HETEROZYGOUS RET POINT MUTATION IN MOUSE AS A NEW ANIMAL MODEL FOR HIRSCHSPRUNG DISEASE THERAPY

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Hirschsprung's disease (HSCR) patients show functional bowel obstruction and impaired gastrointestinal motility due to the absence of enteric neurons and glia in the distal colon. To date, several genetically-modified animal models have been developed as HSCR-like models but these have limitations for use in preclinical studies mainly due to their short lifespan and severe disease phenotypes. Recently, the study by Sunardi M et al. demonstrated that a single Ret missense mutation induced colonic aganglionosis in mice. In this current study, we further investigated the disease phenotype of this human-relevant heterozygous Ret missense-mutated mouse as a new animal model for HSCR. C57BL6/N back-Ret mutant (B6-Ret mt) mice and wild type (B6-WT) mice aged 6 to 10 weeks were used for all experiments. Electric field stimulation (EFS)-induced colonic motility was measured by ex vivo organ bath assay. Enteric nerve-related genes were analyzed by RT-qPCR. Histological analysis was performed by immunohistochemistry (IHC) and hematoxylin-eosin (HE) staining. Functional in vivo assay regarding colonic motility was evaluated by bead expulsion assay. B6-Ret mt mice showed obvious stool congestion in the distal colon compared with B6-WT mice. The nerve-related motility of distal colon was significantly reduced in B6-Ret mt mice compared with B6-WT mice. The expression of enteric nerve-related genes including Tubb3, Nos1 and Chat were significantly reduced in the distal colon of B6-Ret mt mice than B6-WT mice. Failure of enteric nerve formation in the distal colon was also observed in B6-Ret mt mice as revealed by whole-mount IHC staining. Gut dysfunction assessed by bead expulsion latency was sustained until 18 weeks of age in B6-Ret mt mice which grew normally. While loss of normal enteric ganglia was observed in distal colon from B6-Ret mt mice compared with B6-WT mice, smooth muscle and mucosal layer thickness were significantly increased in B6-Ret mt mice as compared to B6-WT mice. This novel Ret point mutation mouse has a HSCR-relevant disease phenotype while showing prolonged survival. This mouse will therefore be a very valuable animal model for the testing of new cell-based therapies for restoration of the enteric nervous system in HSCR.

Keywords: Hirschsprung's Disease, Ret mutation, Animal model

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PATIENT STEM CELL-DERIVED MODELS OF BRAIN DEVELOPMENT WITH PATHOGENIC DNMT3A MUTATIONS EXHIBIT ALTERED PHYSIOLOGY, GROWTH, AND FUNCTION

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Mutations in DNMT3A cause Tatton-Brown Rahman Syndrome, a neurodevelopmental disorder characterized by overgrowth, macrocephaly, intellectual disability and a high prevalence of autism. While mouse models have provided insight into the developmental role of DNMT3A, mouse models carrying pathogenic DNMT3A mutations fail to recapitulate key aspects of Tatton-Brown-Rahman Syndrome, such as macrocephaly. We have developed patient derived pluripotent stem cells and genetically engineered models of DNMT3a mutations. Using these models, we have investigated human cortical development utilizing both developmental patterning and direct differentiation paradigms, to generate GABAergic cortical interneurons, glutamatergic cortical neurons, and astrocytes. To understand the developmental origins of deficits associated with DNMT3A mutations, we examined neural progenitor, immature and mature neuronal populations. In progenitor populations, we observed increased proliferation rates, likely directly relevant to the macrocephaly common among Tatton-Brown Rahman Syndrome patients. Transcriptomic analysis at later time points revealed significant changes in the expression of genes associated with metabolism, which was manifested by differences in metabolite uptake and production. Neuronal function was significantly affected by DNMT3A mutations, as assessed by calcium imaging and electrophysiology. Investigating the temporal kinetics of these phenotypes using inducible CRISPR inhibition technology, we have defined the developmental origin of these phenotypes. Identification of differentially methylated regions across these models has highlighted the developmental mechanisms by which DNMT3A mutations cause these phenotypes. Ongoing work focuses on the interplay between astrocytes and neurons to identify additive or synergistic effects on neuronal function. These results identify critical time periods and sensitive cell populations with respect to the origin of Tatton-Brown-Rahman Syndrome. Using these insights, novel therapeutic avenues can be explored to advance future treatments for Tatton-Brown-Rahman Syndrome.

Funding Source: Engelhart Family Foundation/Intellectual and Developmental Disabilities Research Center Pilot Award

Keywords: Neurodevelopment, DNMT3a, Epigenetics

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LOW-IMMUNOGENIC HUMAN IPSCS-DERIVED DOPAMINERGIC PROGENITORS SUCCESSFULLY ENGRAFT IN IMMUNE-INTACT RATS OF PARKINSON'S DISEASE MODEL

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Parkinson's disease (PD) is one of the progressive neurodegenerative disorders which causes a heavy burden to patients and their families and society worldwide including Taiwan. Progressive degeneration symptoms of PD patients mainly result from dopaminergic neurons dead in the substantia nigra and lead to cognitive and motor function impairment. Current clinical treatment such as supplementation of dopamine may improve motor functions. However, the drug effects fade with disease progression and over 80% of prolonged usage of these medications results in side effects including dyskinesias. Thus, it is pivotal to develop a new therapeutic strategy for PD. Autologous hiPSCs-derived dopaminergic neuron transplantation has been proposed as a promising approach for PD patients. However, whether the differentiated cells from patients are healthy and functional after transplantation is another important issue. The allogeneic grafts differentiated from low-immunogenic hiPSCs of healthy donors could alleviate the rejection reaction and provide individuals with healthier cell sources. Previously, we generated low-immunogenic human iPSC (Li-iPSC) by gene editing with the HLA-I component, Beta 2 Microgloublin (B2M) knockout. In this study, we utilized Li-iPSC and developing progenitors of the midbrain to differentiate the progenitors of dopaminergic (DAP) neurons for in vivo PD animal transplantation and in vitro assessments. Our Li-iPSC-DAP protocols established highly dopaminergic progenitor cell characteristics such as FoxA2, LMX1A, and Nestin bio-markers. Besides, we further evaluated the in vivo functions of grafted dopaminergic neurons by transplant day 16 Li-iPSC-DAP in immune-intact 6-OHDA injured rat models to monitor the behavior outcomes and histology of brain section. Our results established that Li-iPSC-DAP could survive and grow in the striatum of immune-intact SD rats. The present study demonstrated that low-immunogenic iPSCs-derived dopaminergic neurons successfully engraft in immune-intact rats of the Parkinson's disease model, which provided proof of concept that could have benefits for allogenic PD therapeutics.

Keywords: Low-immunogenic, induced pluripotent stem cells, Dopaminergic progenitors

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THE LIMBIC-CORTEX AXIS IN 3D: MODELING HUMAN NEURODEVELOPMENT AND DISEASE.

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The limbic system is a collection of structures, including the hippocampus and amygdala, involved in processing emotion and memory. Alterations in these brain structures and their interaction with the cerebral cortex within the limbic-cortex axis have been highlighted as hallmarks of neurodevelopmental and neuropsychiatric disorders, such as autism spectrum disorder, anxiety, depression, and schizophrenia. However, the neuroanatomy and functionality within the limbic-cortex axis and how alterations may lead to the development of neuropsychiatric disorders remain largely unknown. Stem cell-derived 3D organoid models provide an excellent opportunity to expand our understanding of human brain diseases. Many organoid models have been generated for different brain regions; however, these are unable to replicate the long-range connections among distinct brain regions. In particular, current methods to generate hippocampus organoids involve the generation of dorsomedial telencephalic tissue with broad regional identities, therefore are inadequate to investigate aspects of development and function specific to the hippocampus. Here we describe a novel method to generate hippocampus organoids. By leveraging patterning signals to induce an internal asymmetry along the rostral-caudal axis within iPSC aggregates and adapting the cultures to spinner-flask bioreactors, we achieved the long-term development of an extensive compendium of hippocampal cell types. To recreate the mutual interactions between the human cortex and hippocampus, we will use previously characterized cortical organoids and our newly developed hippocampus organoids to generate a novel 3D hiPSC-derived assembloid model. We will leverage iPSC lines engineered to express the enhanced tandem dimer tomato (TdTomato), and blue fluorescence protein (BFP), to investigate the connectivity visually between defined brain regions with long-term live imaging and functional assays. The development of an in vitro assembloid model where the interactions between the cerebral cortex, hippocampus and amygdala are established, will be instrumental for investigating circuit function during brain development and disease.

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Keywords: Brain Organoids, Assembloid system, Limbic system development

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MODELING NEUROTROPIC VIRUS INFECTION IN REGION-SPECIFIC NEURAL SPHEROIDS FOR HTS ANTIVIRAL COMPOUND SCREENING

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Neural spheroid models derived from differentiated human induced pluripotent stem cells (hiPSCs) provide a reliable tool for development of high-throughput screening (HTS) compatible assays for drug discovery. The continuous public health threat from emerging and re-emerging viruses has highlighted the need for the development of virus-specific or pan-viral therapies and the importance of designing new approaches to efficiently screen antiviral compounds that have a higher success rate down the antiviral drug development pipeline. In this study, we utilize our previously established functional neural spheroid model comprised of hiPSC-neurons and astrocytes at different cell type compositions that mimic specific regions of the brain to evaluate a panel of neurotropic viruses with high pandemic potential, including Rift Valley Fever virus (RVFV) and Chikungunya virus (CHIKV) and further characterize this model as an antiviral drug screening platform for hit compounds. Here we describe how intracellular calcium oscillations of the neural spheroids can be used as a functional readout for HTS of selected compounds in addition to classical viability and infection readouts. We show that the use of these brain region-specific spheroids is a robust model for virus infection studies and a reliable platform for HTS for antivirals.

Funding Source: This research was supported in part by the Intramural/Extramural research program of the NCATS, NIH.

Keywords: hiPSC derived neural spheroids, antiviral development, neurotropic viruses

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THE ROLE OF ZNF292 IN HUMAN CORTICAL INTERNEURON DEVELOPMENT AND ITS DISRUPTION TO CAUSE NEURODEVELOPMENTAL DISORDERS

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Pathogenic mutations in the transcription factor, ZNF292 cause multiple neurodevelopmental disorders (NDDs) including intellectual and developmental disabilities and autism spectrum disorder, constituting a new ZNF292 syndrome. However, the role of ZNF292 in the development of sensitive neuronal populations, such as cortical interneurons, and how this process is disrupted by pathogenic mutation or deficiency, remained entirely unknown. To address this question, we generated human pluripotent stem cell-derived models with pathogenic, patient-specific mutations in the ZNF292 gene by variant knock-in (ZNFVKIs), performed CRISPRi-based knockdown (ZNF292 KD), and differentiated these models into cortical interneurons to characterize the molecular, cellular and functional consequences. Related cellular phenotypes in both ZNFVKI and ZNF292 KD cellular models suggested a critical role for ZNF292 in the early aspects of interneuron development, leading to precocious differentiation of neuronal progenitor cells (NPCs) into neurons with compromised maturation potential and corresponding transcriptomic changes. Analysis of the genome-wide binding profile of ZNF292 indicated which dysregulated genes were direct transcriptional targets of ZNF292 and corroborated the findings above. Moreover, electrophysiology-based functional analysis of interneurons derived from these compromised NPCs indicated significant alteration in their functional properties, and further supported their failure to mature appropriately. Together, this work revealed substantial alterations of interneuron development, physiology, and function that stem from ZNF292 pathogenic mutation or loss of function. These may contribute to the etiology of NDDs involving ZNF292 mutation and provide information regarding the temporal requirements for ZNF292 mutation during brain development. These data also indicate ZNF292 direct transcriptional targets that may provide leads for developing interventions to normalize neuronal function in individuals with ZNF292 mutation.

Keywords: Neurodevelopmental disorders, ZNF292 pathogenic variants, Pluripotent stem cell-derived neurons

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DEVELOPING A MACULAR REGION IN STEM CELL DERIVED RETINAL ORGANOIDS

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The macula is a specialised region of the human retina, distinguished by its dense population of cone photoreceptor cells responsible for our central, colour vision and visual acuity and is the source of many retinal diseases and degenerations. Non-primate animal models lack a macula, making the study of this region difficult. Retinal organoids derived from pluripotent stem cells (PSCs) offer a human model although they do not yet develop a typical macular region. In chicks, FGF-8 and retinoic acid (RA) signalling has been implicated in the development of retinal zones where cones are the prevalent population, a region 'analogous' to the human fovea. Here we used PSC-derived retinal organoids to characterise the early stages of retinogenesis as well as the differentiation and maturation of retinal ganglion and cone cells, the first-born retinal cells. First, we characterised FGF8 and RA signalling during key time points in vitro, demonstrating a dynamic expression of related signalling proteins and molecules. Organoids receiving various regimens of RA and FGF-8 were examined for retinal differentiation. Modulation of these signalling pathways and other culture conditions during the development of retinal organoids led to significant differences in their cellular

composition, specifically the photoreceptors and ganglion cells. Retinal organoids deprived of RA showed an increase in the number of cone cells and an earlier genesis of cone photoreceptor outer segments, which were also more numerous in mature retinal organoids, indicating a potential accelerated maturation of cone cells. Additionally, we altered our media culture conditions to investigate the effects of neuronal maturation media on the development of our organoids and saw an increase in BRN3B ganglion cells in our late-stage organoids with no differences in the number of photoreceptor cells. This data highlights how these signalling pathways are integral to retinal development and contribute to our understanding of cone photoreceptor cells, the most prevalent cell type in the macula. These are the first steps into creating a better model for studying human maculopathies.

Funding Source: Macular Disease Foundation Australia Luminesce Alliance (PPM1 K5116/RD274)

Keywords: Retinal Organoid, Macular Development, Cone Photoreceptors

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IPSC-DERIVED CHOROID PLEXUS ORGANOIDS REVEAL APOE4 EFFECTS ON BLOOD-CEREBROSPINAL FLUID BARRIER

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APOE4, the most significant genetic risk factor for sporadic Alzheimer's disease (AD), has been shown to disrupt the blood-brain barrier in animal models (Bell et al., 2012) and iPSC-derived cell models (Blanchard et al., 2022). The blood-cerebrospinal fluid (CSF) barrier, formed by tight junctions of epithelial cells at the choroid plexus (ChP), also expresses the APOE protein (Ruzali et al., 2012), but whether APOE4 affects ChP cell state and barrier function has not yet been studied. Based on a published protocol (Pellegrini et al., 2021), we generate ChP organoids from APOE3/3 and APOE4/4 isogenic iPSC lines. As expected, the ChP organoids develop transthyretin (TTR)–positive epithelium by day 25 and display more mature cuboidal epithelia with fluid-containing compartments or "cysts" by d50. Due to the developmental heterogeneity within ChP organoids, we focus our immunohistochemistry analysis on the "cysts" and their surrounding epithelial layer. We detect aberrant expression of tight junction proteins in the APOE4/4 ChP organoids, consistent with their altered epithelial permeability measured by fluorescently labeled dextran. Single-nuclei RNAseq on ChP organoids derived from APOE3/3 and APOE4/4 iPSCs reveals diverse cell types, including immature and mature epithelial cells and stromal cells. Notably, we detect a reduced expression of genes essential to ChP function (APOE, LUM, TTR, CLDN18, DCN) in APOE4/4 ChP organoids. We also observe a decrease in the expression of genes involved in mitochondrial metabolism, ribosomal biosynthesis, and innate immunity and an increase in genes related to the extracellular matrix and cell adhesion. Our data demonstrates that APOE4 alters the blood-CSF barrier in ChP, providing an additional layer of complexity on APOE4 contribution to AD pathogenesis at brain border regions.

Funding Source: NIH

Keywords: Choroid plexus, Alzheimer's Disease, APOE

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JOINT DECOMPOSITION IDENTIFIES CONSERVED TRANSCRIPTOME DYNAMICS ACROSS SINGLE-CELL ATLASES OF THE DEVELOPING NEOCORTEX

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Single cell transcriptomic and epigenomic analyses of neurogenesis have been used to generate cellular atlases of mammalian neocortical development in rodent, primate and human brain tissue. Similar maps have been generated in cerebral organoid models, but understanding of the exact elements of development that are modeled in vitro is lacking. To harness the collective discovery power of these rich data resources, we integrated these atlases into a single analytical environment for both computational biologists and cell biologists without coding expertise. We use structured joint decomposition (SJD: doi.org/10.1101/2022.11.07.515489 & chuansite.github.io/SJD) to define dynamic gene expression modules which are shared across species as well as transcriptional programs which are unique to human cortical development. We map these conserved and human specific transcriptomic elements of neocortical neurogenesis onto the genetics of human brain disorders, revealing links between particular elements of mammalian neurogenesis and specific disorders. We interrogate these foundational in vivo transcriptomic programs in cerebral organoid systems to chart developmental dynamics that are recapitulated with high fidelity in vitro. This high resolution map of transcriptomic dynamics in neurogen-

esis can be leveraged to explore and manipulate precise cellular mechanisms underlying risk for common complex brain disorders in tractable in vitro systems. We invite the research community to explore these integrated public data resources along with the transcriptomic elements of human neurogenesis that we have defined and their projection into in vitro stem cell models at https://nemoanalytics.org/p?l=NeocortexEvoDevo&g=FEZF2 & https://nemoanalytics.org/p?l=NeocortexDevoVivoVitro&g=EOMES.

Funding Source: NIMH 1R24MH114815-01A1; JHU Discovery Award

Keywords: Neocortex, Development, Cerebral Organoids

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INVESTIGATING THE ROLE OF MICROGLIA IN AUTISM SPECTRUM DISORDER

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Autism spectrum disorder (ASD) is one of the most common genetic and heterogenous neurodevelopmental disorders, with consistent incidence of disproportionate megalencephaly (ASD-DM) or enlarged brains among 15% of boys with ASD during their first postnatal year. Increase in brain size often precedes the first clinical signs of ASD, suggesting that understanding the mechanisms leading to brain overgrowth could provide a window of opportunity to intervene and possibly prevent disease onset. Here, we used human induced pluripotent stem cells (hiPSCs) to investigate the mechanisms regulating brain overgrowth in ASD, with a focus on the neuroimmune system. We generated hiPSCs from well-phenotyped and genotyped autistic individuals with megalencephaly (ASD-DM), autistic individuals with normal sized brains (ASD-N), typically developing (TD) individuals with megalencephaly (TD-DM), and TD individuals with normal sized brains (TD-N). We subsequently differentiated the iPSCs into microglia, the main immune cells in the brain that maintain homeostasis through phagocytosis of damaged cells and pruning synapses during normal development. A growing body of evidence indicates microglial dysfunction in ASD; however, the molecular mechanisms remain unknown. Using STEMdiff™ kits from STEMCELL Technologies, we derived microglia expressing microglial-specific markers (IBA1, TMEM119, and P2YR12) using quantitative PCR and immunostaining. We show that microglia generated from ASD-N and ASD-DM individuals exhibit morphologic changes relative to TD individuals. Our preliminary evidence shows that ASD-N microglia display limited processes and have a more immature phenotype, while ASD-DM microglia have a highly activated phenotype with increased expression of CD68, CD11b, and CD206 compared with ASD-N and TD-N microglia. We also observe a greater abundance of microglia in the ASD-DM conditions. We also illustrate how these structural and phenotypic changes affect cellular phagocytosis and microglial activity in ASD using various functional assays. Furthermore, we shed light on the transcriptional profiles distinguishing microglia in ASD-N and ASD-DM and how these transcriptional changes impact brain growth and neuronal health in ASD.

Funding Source: NICHD grant P50 HD093079

Keywords: Microglia, iPSCs, idiopathic autism

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MODELING GALACTIC COSMIC RAY-INDUCED NEURAL PATHOLOGY WITH HUMAN BRAIN ORGANOIDS AND IDENTIFICATION OF OPTOGENETIC FGF SIGNALING MODEL AS A COUNTERMEASURE

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Exposure to space radiation is one of the main challenges to human health during deep space missions. Galactic cosmic rays (GCR), which are a dominant source of space radiation, are heavy, high-energy ions of elements and have been considered as one of major limiting factors for long-duration human spaceflight. Although active shielding technologies are currently being explored, there has been no notable progress in developing a countermeasure to prevent GCR-induced cellular damages. Here, as a part of the Translational Research Institute for Space Health (TRISH) research program, empowered by the NASA Human Research Program, we measured the dose-response effects of simplified GCR simulation (SimGCRsim, a specific model for GCR research in the earth) exposure on the cell survival/death and differences in susceptibility in human 3D brain organoids. In addition, we hypothesized an optogenetically-regulated FGF2 signaling (opto-FGFR) to reduce the damages of human pluripotent stem cells (hiPSCs)-derived brain organoids from simGCRsim exposure. Interestingly, GCR-induced neural damages were significantly rescued in opto-FGFR-expressing brain organoids via the activation of FGF2 downstream signaling, suggesting that opto-FGFR could possess the neuroprotective effects in brain organoids. The expression levels of collagen-extracellular matrix (ECM) components were up-regulated in simGCRsim-exposed opto-FGFR-expressing group, which may be associated with neuroprotection in the brain organoids. In conclusion, our data demonstrate that human brain organoids can be invaluable models for study GCR, and suggest a possibility that simGCRsim-activated opto-FGFR module may induce the de novo synthesis of collagen-ECM components, as a neuroprotective countermeasure against GCR in the human neural tissue in future space mission.

Funding Source: This work is supported by the Translational Research Institute for Space Health through NASA Cooperative Agreement NNX16AO69A.

Keywords: Space radiation, development of countermeasure, human brain organoids

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GENERATION OF AN ISOGENIC STEM CELL MODEL TO STUDY SYNGAP1 HAPLOINSUFFICIENCY IN HUMAN NEURONS

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SYNGAP1 syndrome is a rare neurodevelopmental disorder caused by a heterozygous mutation in the SYNGAP1 gene leading to symptoms like intellectual disability and epilepsy. The SYNGAP1 gene encodes for the 'Synaptic Ras/Rap-GTPase-activating-Protein' (SynGAP), which is mainly restricted to the postsynaptic density. In its basal state it is thought to act as GTPase activator protein leading to inactivation of proteins like Ras, Rap, Rac and Cdc42. Upon electrical stimulation, SynGAP gets phosphorylated by CamKII and dispersed from the postsynaptic density, leading to a release of Ras inhibition with consecutive Erk phosphorylation as well as increased surface AMPA receptor expression and bigger spines. SYNGAP1 haploinsufficiency would be predicted to result in decreased Ras inhibition, enhanced AMPA receptor expression at the surface and bigger spine morphologies. To elucidate the impact of SYNGAP1 haploinsufficiency on early human neural development and synapse formation, we generated SYNGAP1+/- human pluripotent stem cells (hPSCs). Controlled generation of excitatory or inhibitory neurons was achieved via transcription factor-mediated forward programming employing doxycycline-inducible NGN2 or ASCL1/DLX2 transgenes. Data from Sholl analyses suggest that SYNGAP1+/- neurons are larger and more complex compared to isogenic controls. We also analyzed dendritic spines as changes in their shape (mushroom, stubby, thin, branched), size and number are correlated with functional changes in excitatory synapses. Our findings point to a higher proportion of spines with mushroom-like morphology in SYNGAP1+/- neurons, which may reflect premature neuronal differentiation. Electrophysiological analyses using multi-electrode-arrays suggest hyperactivity of SYNGAP1+/- NGN2 neurons. Congruent with the proposed role of SynGAP in Ras signaling, soluble fractions of SYNGAP1+/- neuronal lysates showed higher Ras-GTP and pERK levels. Our data reveal morphological, biochemical and functional consequences of SYNGAP1 haploinsufficiency in human neurons. Translated into an in vivo scenario, the premature differentiation and altered Ras signaling observed in cultured SYNGAP1+/- neurons might lead to disrupted circuit formation as a potential cause of ID and other symptoms of SYNGAP1 syndrome.

Funding Source: This project was supported by the BMBF under the umbrella of the ERA-NET NEURON (German grant agreement 01EW1812A9) within the TREAT-SNGAP consortium.

Keywords: SYNGAP Syndrome, Isogenic stem cell model, Forward programmed neurons

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UNTANGLING THE ROLE AND CONTRIBUTION OF NEURONS AND MICROGLIA TO KCNQ3 GAIN-OF-FUNCTION ENCEPHALOPATHY IN IMMUNOCOMPETENT FOREBRAIN ORGANOIDS

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Gain-of-Function (GoF) variants in KCNQ3 lead to severe early-onset neurodevelopmental disorders (KCNQ3-GoF-Encephalopathy), characterized by neurodevelopmental delay (NDD), autism spectrum disorder (ASD) and, less prevalent, epileptic seizures. Kv7.3 subunits, encoded by KCNQ3, form homo- or hetero-tetrameric voltage-gated potassium channels (Kv7). Kv7 channels expressed in neurons produce a well characterized M-current that is a critical regulator of neuronal excitability. To date, the disease mechanism of KCNQ3-GoF-Encephalopathy is not well understood and there is no treatment available. However interestingly, single nuclei RNA sequencing databases of the human brain have revealed that KCNQ3 expression is not limited to inhibitory and excitatory neurons, but that this gene is also highly expressed in microglial cells. Together with the emerging evidence that microglia dysfunction is involved in the development of NDD and ASD, we hypothesize that KCNQ3-GoF variants affect microglia function, which contributes to the already dysfunctional neuronal network. To explore the role of neurons and microglia within the underlying disease pathology, we are generating immunocompetent forebrain organoids (IFOs) from iPSC lines carrying KCNQ3-GoF-variants and their respective isogenic controls. These IFOs recapitulate the in vivo developing brain more closely

compared to traditional brain organoids as they also include microglia. The generated IFOs will be characterized on three levels: 1) transcriptomics (single-cell RNA sequencing), 2) morphology (immunohistochemistry combined with confocal microscopy) and 3) electrophysiology (on the single cell level using patch clamping and the network level using multielectrode arrays). By combining read outs going from the molecular to the phenotypic level, we expect to obtain a detailed overview of dysfunctional pathways in KCNQ3-GoF-Encephalopathy. Simultaneously, we will create a phenotypic fingerprint for mutant and wildtype IFOs, which can be used as a drug screening platform in a later stage. When successful, this approach will provide a better understanding in the disease pathology of rare KCNQ3-GoF-Encephalopathy, it will potentially expose new therapeutic targets and it will provide a comprehensive, in vitro drug-screening platform.

Funding Source: Marguerite-Marie Delacroix fund

Keywords: Neurodevelopment, Immunocompetent organoids, KCNQ3

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CHARACTERIZING THE ROLE OF ASTROCYTES IN HYPEREXCITABLE NEURAL NETWORKS ASSOCIATED WITH ALZHEIMERS DISEASE

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Alzheimer's Disease (AD) is the most prevalent aging-related neurodegenerative disorder. The incidence of AD is forecasted to drastically increase and by 2050, over 12.7 million people aged 65 and older are projected to be diagnosed with AD. AD requires significant long-term care and currently there are only two disease-modifying treatments available, both undergoing post-approval trials by the FDA, and no preventative therapeutics. Decades of reliance on animal models fail to yield translational results as these models do not faithfully recapitulate the progressive pathophysiology present in humans. Cortical organoids generated from AD patient derived human induced pluripotent stem cells (hiPSC) act as a controllable disease-relevant platform as they retain the same genetic background as the affected individual. Our lab has generated AD cortical organoids from patients with a duplication in the amyloid precursor protein (APP) which increases the propensity of these cells to generate amyloid-beta accumulation, a hallmark of AD. These cortical cultures contain multiple cell types including neurons, astrocytes and oligodendrocytes and show cardinal features of human AD brains, including hyperexcitability and calcium ion disequilibrium. To explore new treatment avenues for AD, we investigated the role of astrocytes in the establishment and maintenance of neural circuitry in AD cortical cultures and examined how astrocytic phenotype and morphology contributed to network hyperexcitability in the early stages of AD. We used immunocytochemistry to characterize the identity of the cell populations in the AD-derived cortical organoids. The ratio of astrocytic phenotypes present and morphology observed was monitored over the developmental timeline. Calcium imaging and multi electrode array were used to characterize network formation and function. AD-derived cortical organoids were found to have a greater proportion of reactive astrocytes than unaffected controls, and the number of reactive astrocytes in AD cortical cultures was found to increase with culture age. Mechanisms of astrocytic involvement in hyperexcitable network formation and function should be further elucidated to identify new avenues for treating hyperexcitable network phenotypes in aging-related neurodegenerative diseases.

Keywords: Alzheimer's Disease, hyperexcitability, astrocytes

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TRANSCRIPTOMIC ANALYSIS OF INDUCED NEURONS IN RESPONSE TO ANTISENSE OLIGONUCLEOTIDE TREATMENT (ASO) IN AUTISM

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Many genes with de-novo copy number variants (CNVs) and single nucleotide variants have been discovered and associated with autism spectrum disorder (ASD), a neurodevelopmental disorder with neocortical/cognitive defects. Antisense Oligonucleotide (ASO) therapy is emerging as a powerful drug platform that can target disease at the pre-mRNA level, eliciting its effect without changing the genome. To accelerate development of treatments for monogenic forms of ASD, we designed and tested ASOs for PPP2R5D, a known autism gene with toxic gain of function mutations. To assess the genome-wide transcriptional alterations, we performed RNAseq on differentiated induced neurons (iNs) before ASO treatment, and observed increased expression of neuronal genes (MAP2, PSD95, SYNAPSIN and DCX), and reduced expression of precursor genes (Ki67 and SOX2). We next applied 5-10-5 2′-O-methoxyethylribose (MOE) 'gapmer' ASO against alpha actinin compared with scrambled ASO (negative control) in wildtype cultures to assess the knockdown efficacy and specificity. We obtained an 85% knockdown from our positive control compared to our scrambled and untreated samples, suggesting a robust knock-down. We hope that we will be able to achieve reversal of preclinical endpoints in patients derived cells carrying a mutation in PPP2R5D. In the future, we will continue this strategy for SYNGAP1, another ASD risk gene, aiming to provide personalized treatment to patients with ASD caused by genetic mutations, aligning with the mission of the California Institute for Regenerative Medicine and the n-Lorem Foundation.

Funding Source: CIRM Bridges to Stem Cell Research Training Grant Program California State University, Channel Islands

Keywords: Autism Spectrum Disorder, Antisense Oligonucleotide Therapy, Neuronal Differentiation

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PERVASIVE ENVIRONMENTAL CHEMICALS IMPAIR OLIGODENDROCYTE DEVELOPMENT

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Exposure to environmental chemicals can impair neurodevelopment. Oligodendrocytes that wrap around axons to boost neurotransmission may be particularly vulnerable to chemical toxicity as they develop throughout fetal development and into adulthood. However, few environmental chemicals have been assessed for potential risks to oligodendrocyte development. Here, we utilized a high-throughput developmental screen and human cortical brain organoids, which revealed environmental chemicals in two classes that disrupt oligodendrocyte development through distinct mechanisms. Quaternary compounds, ubiquitous in disinfecting agents, hair conditioners, and fabric softeners, were potently and selectively cytotoxic to developing oligodendrocytes through activation of the integrated stress response. Organophosphate flame retardants, commonly found in household items such as furniture and electronics, were non-cytotoxic but prematurely arrested oligodendrocyte maturation. Chemicals from each class impaired human oligodendrocyte development in a 3D organoid model of prenatal cortical development. In analysis of epidemiological data from the CDC's National Health and Nutrition Examination Survey, adverse neurodevelopmental outcomes were associated with childhood exposure to the top organophosphate flame retardant identified by our oligodendrocyte toxicity platform. Collectively, our work identifies toxicological vulnerabilities specific to oligodendrocyte development and highlights common household chemicals with high exposure risk to children that warrant deeper scrutiny for their impact on human health.

Keywords: oligodendrocyte, neurodevelopment, toxicology

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A NOVEL DUAL INDUCIBLE SYSTEM IN HUMAN IPSCS FOR AGEING INEURONS REVEALS CHANGES IN THE EPIGENETIC LANDSCAPE OF THE AGED CELLS

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Modeling late-onset disorders using induced pluripotent stem cells (iPSCs) technology holds a challenge due to iPSCs cellular rejuvenation during reprogramming. Hallmarks of ageing are often difficult to distinguish from disease-related degenerative processes but are usually associated with a gradual deterioration in structure and function. Extreme perturbations of cellular ageing occur in the context of genetic progeria syndromes, especially in Hutchison-Gilford Progeria Syndrome (HGPS). These provide unique opportunities to investigate the ageing program. Here we generated a cellular model system of human ageing based on progerin overexpression. We harnessed our inducible reprogramming system, OptiOX, and engineered human iPSCs to overexpress progerin in parallel with neurogenin 2 (NGN2). This enables direct conversion of iPSCs into cortical glutamatergic neurons by overexpressing NGN2, while ageing the cells by overexpressing progerin. Aged, induced neurons (iNs) show phenotypic changes in neuronal process outgrowth, connectivity, and in morphology. Selected hallmarks of physiological ageing were observed, among these downregulation of peripheral heterochromatin markers and increased double strand DNA breaks. In addition, the epigenetic clock of the aged iNs was studied. These findings confirm our base hypothesis that our system enables independent control of progerin expression and cellular reprogramming. The resulting neurons remain viable and demonstrate select hallmarks of ageing, which provides a cellular model for both physiological and chronological ageing.

Funding Source: Evolution Education Trust bit bio LTD

Keywords: Ageing, Aging, Neurons, Reprogramming

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ALTERED NEURONAL NETWORK DYNAMICS IN BRAIN ORGANOIDS FROM MECP2 DUPLICATION SYNDROME PATIENTS

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MECP2 duplication syndrome (MDS) is a devastating neurodevelopmental disorder caused by duplication of the Xq28 region in the long arm of the X-chromosome, encompassing the methyl-CpG-binding protein 2 (MECP2) gene. MECP2 is a dosage-dependent gene: gain-of-function leads to MDS seen mostly in boys and loss-of-function causes Rett syndrome seen mostly in girls. Despite MeCP2-dosage-related disorders being sex-dependent, their clinical presentation is similar and encompasses a spectrum of autistic features, learning deficits, epilepsy, and impaired motor function. Importantly, the mechanisms and affected cellular processes underlying these disorders remain poorly understood and disease-modifying treatments are still lacking. To begin to disentangle the MeCP2-dosage-dependent mechanisms, we generated human dorsal forebrain organoids derived from molecularly confirmed MDS patients as well as typically developing controls. To study the patterns of neuronal network activity in these organoids, we recorded from multiple electrode arrays and calculated the mean firing rates across electrodes, synchrony, oscillations, and network bursts. Analysis of the frequency of spiking activity collected from continuous voltage measurements revealed a reduced number of spikes and mean firing rate in organoids from MDS patients compared to healthy controls from day 100 to 118 in vitro, indicating that these neurons have impaired electrophysiological function. Moreover, at this stage MDS organoids exhibited asynchronous pattern of firing with no notable differences in network oscillations, indicating altered synaptic strength but relatively normal network functionality. Remarkably, application of the potassium channel blocker 4-AP resulted in network hyper-synchrony accompanied by lightning-bursts of network activity not seen in organoids from healthy controls. These findings shed light onto novel neuron network dynamics in MDS that may help identify potential therapeutic targets or prognostic markers for disease progression, toxicity, and therapeutic efficacy.

Funding Source: NICHD 1P50HD103555

Keywords: Brain-organoids, MECP2, Electrophysiology

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TRANSPLANTED HUMAN SPINAL INTERNEURONS FUNCTIONALLY INTEGRATE WITH THE INJURED SPINAL CORD

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Advances in cell therapies offer new promise for some of the most devastating neural injuries like spinal cord injury (SCI). One particular type of neuron – the spinal V2a interneuron – has been implicated as a key component in plasticity and therapeutically driven recovery post-SCI. In this study, we engineered V2a spinal interneurons (SpINs) from human induced pluripotent stem cells and tested their ability in forming functional synapses with injured motor networks. We used a clinically-relevant cervical contusion SCI in adult rats that results in damage to the phrenic motor network and impaired breathing to test integration, functional connectivity and overall contribution to respiratory recovery mediated by transplanted human V2a SpINs. Neuroanatomical tracing and immunohistochemistry were performed to demonstrate transplant integration and synaptic connectivity with injured host tissue, and diaphragm electromyography was used to assess functional recovery of the injured phrenic network. Optogenetic activation of transplanted human V2a SpINs revealed functional synaptic connectivity to injured host circuits and improved diaphragm activity. Optogenetic activation of host supraspinal pathways revealed functional innervation of host neurons with transplanted cells. These studies are the first to 1) engineer human spinal V2a interneurons as an intended therapeutic product, and 2) demonstrate functional integration of human SpINs with injured respiratory pathways post-SCI. Having rigorously established improvement in diaphragm muscle activity with objective metrics, this strategy holds great promise to establish motor recovery post-SCI.

Funding Source: NIH NINDS F32 NS119348, R01 NS104291, Roddenberry Foundation

Keywords: spinal interneurons, spinal cord injury, transplantation

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MODELING MULTI-STEP RETINOBLASTOMA GENESIS IN VITRO WITH CONE REPORTER RETINAL ORGANOIDS

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Retinoblastoma, the most prevalent childhood intraocular malignancy, originates from maturing cone photoreceptor precursors with biallelic RB1 inactivation. Rb1 mutant animal models fail to recapitulate retinoblastomagenesis likely due to human-specific cone development features. In explanted fetal retina, pRB-depleted post-mitotic cone precursors proliferate, followed by a 4-5 month premalignant indolent phase before re-entering cell cycle to form retinoblastoma-like masses at tissue ages similar to that of retinoblastoma in vivo. However, low tissue availability limits research with this disease model. RB1-/- retinal organoids (ROs) provide a potential alternative, as they demonstrate initial cone proliferation, but they deteriorate before entering indolence. Here, we produced and characterized an RB1-null cone reporter RO model that recapitulates multi-step retinoblastomagenesis. We generated a cone-reporter iPSC line through CRISPR knock-in of EGFP-P2A at the N-terminus of GNAT2 in WTC11-mTagRFPT-LMNB1. In ROs derived from these iPSCs, EGFP+ cone precursors first appear at d34 and adopt mature cone morphology at ~d120. Immunohistochemistry with cone markers ARR3 and RXRg confirmed cone-specific EGFP expression. A second round of CRISPR editing produced homozygous RB1 knockout. Chimeric organoids generated from RB1-null cone reporter iPSCs mixed with unedited parental iPSCs recapitulated pRB loss in a subset of retinal cells in an otherwise healthy retina and enabled live-imaging cell tracking in intact hydrogel embedded organoids. Bi-weekly live confocal imaging of EGFP+ RB1-/- cones from d85 to d238 captured their initial proliferation followed by a pre-malignant indolence phase. The majority of the initially proliferating cones remain quiescent, with some adopting mature cone morphology. Nascent retinoblastoma-like foci formed on several organoids after d281, a tissue age that equates to the first post-natal month when early retinoblastomas typically emerge and co-expressed EGFP, cone markers, and proliferation marker Ki67. In summary, we generated a human retinoblastoma organoid model that recapitulates the cell-of-origin and timing of multi-step retinoblastomagenesis, paving the way for mechanistic studies and therapeutic screening.

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Keywords: Retinoblastoma, Organoid, Cone reporter

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A MODULAR ASSEMBLOID MODEL OF THE OPTIC NERVE

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Physical damages but also pathologies such as glaucoma can lead to an irreversible destruction of the optic nerve and consequently to vision loss. A promising tool to study the underlying processes specific the human physiology is provided by induced pluripotent stem cell derived retinal organoids (RO). Their use for modelling the optic nerve is however hampered by a premature loss of ganglion cells and the absence of their main target neurons in the lateral geniculate nucleus of the thalamus. To overcome these limitations, we created an optic-nerve assembloid model (ONA) by a precise fusion of human induced pluripotent derived retinal organoids (RO) and thalamic organoids (TO). In contrast to previously described models and in order to acknowledge the importance of the nerve structure itself, we added a hydrogel-based sphere between RO and TO mimicking the optic nerve compartment. In this way, the integration of missing cell types and components as well as a direct manipulation the nerve structure is enabled. To mimic the architecture of the pathologically-relevant first millimeters of the optic nerve head, we added iPSC-derived astrocytes to the nervous compartment. We observed an efficient outgrowth of nervous structures of the RO towards the TO and an ensheathing of the nerve bundles by the astrocytes demonstrated by clearing-assisted 3D imaging. Furthermore, we mimicked the mechanical destruction of the nerve ("Optic nerve crush") which led to GFAP upregulation in astrocytes. In a further step, we also added iPSC-derived microglia (iMacs) creating an immune-competent model. iMacs in the ONA expressed canonical microglial markers, could be integrated more than 10 days and migrated within the nerve compartment towards RO and TO. In summary, the presented optic nerve assembloid model is a step towards a complete optic nerve in vitro model and could provide a valuable tool for nerve regeneration and disease modelling studies.

Keywords: Optic Nerve, Retinal and Thalamic Organoids, Multi-Assembloids

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THE AGING TECHNIQUE: STUDYING THE DIFFERENTIATION FROM IPSC TO NEURONS FOR 120 DAYS

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Alzheimer's disease (AD) is characterized by a progressive loss of memory accompanied by behavioral changes, with advanced aging as the primary disease risk factor. The pathological markers of Alzheimer's disease are the presence of amyloid plaques, phospho-tau (p-tau) aggregates and neuronal death. There are

no treatments available to stop its progression. The objective of this project is to compare side by side the differentiation of CTL or AD human induced pluripotent stem cells (iPSC) into differentiated neurons for an extended period and thus imitating the aging process that is crucial in the development of sporadic AD. This will allow us to have a better understanding of the molecular cascades that lead to the degenerative phenotype known in AD patients. Human induced pluripotent stem cells will be differentiated into neurons. The iPSCs are cultured on Corning Matrigel hESC-Qualified Matrix until they are 70% confluent. They were then seeded on Corning Matrigel GFR Membrane Matrix to start the differentiation. After 25 days, the neurons were reseeded on Corning Matrigel GFR Membrane Matrix. Every 10 days, the media containing Corning Matrigel hESC-Qualified Matrix concentrated 5 times was added to the cells to create a sandwich that prevented cell detachment. The cells were left in culture for 120 days. Along each step of the differentiation, western blot analyses were done specifically looking at the expression of MAP2 and BMI1, a protein known to have a lower expression in mature AD neurons (day 60 vs day 120). We also did a single-cell RNA sequencing with the two cell lines (CTL and AD). With the single-cell RNA sequencing and analysis, we are in the process of determining different cell types and determine the difference between the two cell lines. We have noticed different cell types that have decreased in the Alzheimer's disease cell line. This method using a cell model that is more representative of aging neurons in the brain of AD patients will allow us to better understand the onset of the disease.

Keywords: Alzheimer's disease, Aging, Neuronal differentiation

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CD99 EXPRESSION DYNAMICS IS ASSOCIATED WITH BRAIN OVERGROWTH IN AUTISM SPECTRUM DISORDER

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Autism spectrum disorder (ASD) is a complex condition characterized by important changes to the brain resulting in impaired behavior. Approximately 15-20% of individuals with ASD have disproportionate megalencephaly (ASD-DM) or enlarged brain relative to body size. An increase in brain size often precedes the first clinical signs of the disorder, yet the underlying mechanisms leading to brain overgrowth remain unknown. Here, we develop cellular models using patient-specific induced pluripotent stem cells (iPSCs) to understand the mechanisms contributing to brain overgrowth in genetic and idiopathic forms of autism. RNA-sequencing and transcriptome analyses of neural progenitor cells (NPCs) derived from patient-specific iPSCs show an important role for cancer related pathways (e.g., CD99) in contributing to brain overgrowth in ASD. While basal expression of CD99 is required for the differentiation of NPCs into well-defined functional neurons, overexpression of CD99 can lead to aberrant differentiation of NPCs into immature neurons. Here, we explore the role of CD99 in regulating proliferation and terminal differentiation of NPCs into neurons in both genetic and idiopathic forms of ASD with and without brain overgrowth. Furthermore, we show how genes impacted in genetic forms of autism (e.g., 16p11.2 deletion syndrome) directly interact and modulate the expression of CD99 to ultimately regulate cellular proliferation and differentiation. Thus, our study provides a mechanistic link for CD99 overexpression in brain overgrowth in autism and highlights genes and pathways as potential therapeutic targets in future work.

Keywords: Induced Pluripotent Stem Cells, Autism Spectrum Disorder, CD99

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MODELING SEX DIFFERENCES IN ALZHEIMER'S DISEASE USING ISOGENIC HIPSC LINES WITH DIFFERENT SEX CHROMOSOME COMPLEMENTS AND APOE ALLELES

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Late Onset Alzheimer's Disease (LOAD) is the most common neurodegenerative disorder. LOAD is genetically heterogeneous but carriers of an ε4 allele of the apolipoprotein E gene (APOE) have significantly increased risk of developing LOAD. LOAD is also strongly sex biased. Besides the well-established role of sex hormones, the impact of genetic sex combined with APOE genotypes has not been studied. Sex chromosome dosage (SCD), two X chromosomes in females or one X and one Y chromosome in males, is the main genetic difference between the sexes. X chromosome inactivation (XCI) silences most genes on one X chromosome in females, however ~15-25% of genes escape XCI and are expressed from the inactive X (Xi). Many of the escape genes are related to neurological and immune functions and could contribute to neuroinflammation in AD. To understand the genetic interactions between autosomal risk (APOE) and sex-bias in AD, we are generating isogenic sets of hiPSCs with various sex chromosome complements and APOE genotypes. We have derived XXY/XY, XY/X, XX/X, and XXX/X isogenic pairs by reprogramming cells from naturally mosaic individuals or by selective removal of the Xi in vitro. These lines uniquely position us to differentiate the effects of X chromosome dosage and/or presence of a Y chromosome, while minimizing genetic variability and environmental or hormonal confounders. Preliminary results in differentiated neural precursor cells show differential expression of escape genes in relation to SCD. We also observed genome-wide

changes in autosomal gene expression and DNA methylation. Subsets of autosomal sex-biased genes were differentially affected either by X chromosome dosage, presence of a Y, or both. To address the effects of SCD on cellular AD phenotypes, we are constructing APOE ε3 and ε4 alleles in our paired hiPSC lines with differing sex chromosome content using gene editing. Starting from an XXY/XY isogenic pair, we have generated an hiPSC series with six different isogenic genotypes of APOE ε3/ε3, ε3/ε4 or ε4/ε4 alleles with either the XXY or XY genotype. The new hiPSC lines are being differentiated to AD-relevant cell types, including neurons, microglia, and cortical organoids, for transcriptomic and functional analyses to elucidate how APOE alleles and genetic sex interact to modulate risk in LOAD pathology.

Keywords: Alzheimer's Disease, Sex differences, Isogenic hiPSCs

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DIRECTLY REPROGRAMMED FRAGILE X SYNDROME NEURAL PRECURSOR CELLS GENERATE CORTICAL NEURONS EXHIBITING IMPAIRED NEURONAL MATURATION

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The neurodevelopmental disorder Fragile X syndrome (FXS) is the most common monogenic cause of intellectual disability and autism spectrum disorder. Inaccessibility to developing human neurons is a major barrier to studying FXS. Direct reprogramming provides a unique platform to investigate the developmental profile of FXS-associated phenotypes throughout neural precursor and neuron generation, at a temporal resolution not afforded by post-mortem tissue and in a patient-specific context not represented in rodent models. Direct reprogramming also circumvents the protracted culture times and low efficiency of current induced pluripotent stem cell strategies. Using our chemically modified mRNA (cmRNA) -based direct reprogramming protocol, we generated dorsal forebrain precursors (hiDFPs) from FXS patient derived fibroblasts, with subsequent differentiation to glutamatergic cortical neurons. We observed differential expression of mature neuronal markers suggesting impaired neuronal development and maturation in FXS- hiDFP derived neurons compared to controls. FXS- hiDFP-derived cortical neurons exhibited dendritic growth and arborization deficits characterized by reduced neurite length and branching consistent with impaired neuronal maturation. Furthermore, a significant decrease in the density of pre- and post- synaptic proteins in FXS- hiDFP derived neurons suggests impaired excitatory synapse development. We also observed a reduced yield of FXS- hiDFP derived neurons with a significant increase in FXS-affected astrocytes. This study represents the first reported derivation of FXS-affected cortical neurons following direct reprogramming of patient fibroblasts to dorsal forebrain precursors and provides a unique platform for further study into the pathogenesis of FXS as well as the identification and screening of new drug targets for the treatment of FXS.

Funding Source: Supported by the Neurological Foundation of NZ.

Keywords: Direct reprogramming, Fragile X Syndrome, Human induced dorsal forebrain precursor cells

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DISTINCT PATTERNS OF NEURAL OSCILLATION ACTIVITIES AND DEFICITS IN CORTEX- AND HIPPOCAMPAL-GANGLIONIC EMINENCE ASSEMBLOID MODELS OF GENETIC EPILEPSY

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Many neurodevelopmental disorders are associated with impairments in multiple cognitive domains. For example, while epilepsy is defined by recurrent spontaneous seizures resulting from hyperexcitable neural circuits, it is also highly associated with comorbid memory impairment and depression. Though widespread neural circuit dysfunction is apparent, it remains unclear whether a single upstream pathological driver has shared or unique effects on circuit function in distinct brain regions. While regional divergence has important implications for both disease pathogenesis and treatment, modeling and isolating the development of brain region-specific pathology has been challenging. Here, we developed a pipeline for creating and comparing cortical-ganglionic eminence (GE) and hippocampus-GE assembloids. This approach allows for the intermixing of excitatory and inhibitory neurons and establishment of neural networks that exhibit distinct oscillatory activities. Like cortex, hippocampal assembloids display multifrequency neural oscillations, but additionally generated sharp wave ripple (SWR) complexes and stereotyped patterns of theta-gamma phase amplitude coupling (PAC). These distinct patterns of circuit activity are associated with hippocampal learning and memory in vivo and were not seen in cortical-GE preparations. We next generated assembloids from iPSC from a patient afflicted with developmental epileptic encephalopathy-13 (DEE-13) due to a pathogenic gain of function mutation in the SCN8A sodium channel. Extracellular recordings of local field potentials revealed substantial hyperexcitability as well as a loss of sustained oscillatory activity in the cortex-GE assembloids compared to isogenic controls. By contrast, DEE-13 hippocampus-GE assembloids did not show hyperexcitability and instead exhibited changes associated with impaired function of hippocampal learning and memory circuits including reduced SWR frequency and disordered patterns of theta-gamma PAC. The changes were associated with a selective loss of interneurons that were not seen in cortex-GE fusions and were partially rescued with optogenetic activation of

interneuron populations. These results suggest that interneuron dysfunction is a key contributor to memory impairment in DEE-13.

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Keywords: brain organoids, neurodevelopmental disorder, epilepsy

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REDUCED SH3RF3/POSH2 EXPRESSION PROTECTS AGAINST ALZHEIMERS DISEASE BY LOWERING MICROGLIAL PRO-INFLAMMATORY RESPONSES VIA MODULATION OF JNK AND NFKB SIGNALING PATHWAYS

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Understanding how some high-risk individuals are protected from developing Alzheimer's disease (AD) may illuminate novel therapeutic targets. We previously identified protective genetic variants in a Caribbean Hispanic cohort carrying the PSEN1 G206A mutation sufficient to cause early-onset AD, including a SNP in SH3RF3/POSH2 which was associated with delayed age at onset of AD for 9.2 years and predicted to have lower expression. We have now identified additional protective SNPs in SH3RF3 including a predicted damaging coding variant. SH3RF3 has the same domain structure as its better studied homologue SH3RF1/POSH (plenty of SH3 domains), which acts a JNK (c-Jun N-terminal kinase) pathway scaffold by binding multiple components of the pathway including the GTP-bound form of the upstream JNK activator RAC1. Overexpression of SH3RF family genes has also been reported to activate the NFKB pathway by an unknown mechanism. While the effects of SH3RF3 knockdown in hiPSC-derived neurons were subtle, knockdown in hiPSC-derived microglia significantly reduced inflammatory cytokine production and release in response to either a viral mimic (poly(I:C)) or oligomeric Abeta42. This was associated with reduced activation of both JNK and NFKB pathways in response to these stimuli. Pharmacological inhibition of JNK signaling at either the MAP3K level or RAC1, or inhibition of NFKB, phenocopied SH3RF3 knockdown in inflammatory cytokine production, consistent with SH3RF3 modulating inflammatory responses via activation of these pathways. In addition, we have preliminary evidence that PSEN1 G206A microglia have a reduced inflammatory response relative to isogenic control microglia, similar to what has been reported for PSEN1 delE9 mutant microglia. Thus, further reduction of the inflammatory response in PSEN1 mutant carriers by protective SNPs in SH3RF3 might serve to sever (or at least greatly reduce) the link between amyloid and neuroinflammation and subsequently delay the onset of disease. While there is debate over whether increasing or decreasing microglial activation might be better as a therapeutic strategy for late-onset AD, our data suggests that reduction of pro-inflammatory signaling in microglia such as by JNK pathway inhibition might be beneficial for early-onset familial AD caused by PSEN1 mutations.

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Keywords: Alzheimer's disease, Microglia, Neuroinflammation

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CEREBRAL ORGANOIDS REVEAL GENETIC DIFFERENCES ASSOCIATED WITH RESILIENCE IN AN IN VITRO STROKE MODEL

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Stroke is one of the leading causes of death. Although stroke is a multifactorial disease, the genetic differences among patients contribute significantly to variation in risk, survival, and recovery. Progress in establishing the genetic underpinnings of stroke has been slow, the genetic and mechanistic differences that distinguish between susceptibility (death or severe sequelae) and resilience (recovery) in stroke patients are not well understood and this limits our ability to identify targets that can be translated into new therapeutics. Thus, there is a critical need for an efficient approach to elucidate the genetic mechanisms that drive resilience to stroke so that novel therapeutic targets can be identified and exploited. We use a stem cell (SC)-based approach to rapidly analyze phenotypical differences among genetically diverse populations. We developed a protocol to differentiate neurons and cerebral organoids from genetically diverse mouse and human SCs, making readouts between mouse and human more comparable. We developed an in vitro stroke-like paradigm which we used to test for phenotypical differences among genetically diverse neurons using SCs from the founders of the collaborative cross mice, and human SCs from the iPSCORE collection. After measuring the stroke outcome, we found a varied response to the insult associated with genetic diversity. We conducted a transcriptional regulatory network analysis which revealed that resilience is linked to Vdr, Tfap2c, and Irf7 transcriptional factors (TFs). To validate the effect of these TFs using our in vitro stroke model, we incubated human cerebral organoids with commercially available molecules that activate these TFs. We found that a combination of these molecules can increase the survival of neurons after the insult. After generating CRISPRa human SCs to activate the endogenous TFs, we were able to corroborate the pro-survival effects of these TFs in cerebral organoids. The present model demonstrates how complex genetic factors modify differential responses to stroke. The transcriptional adaptation associated with a favorable response to stroke can lead to much-needed new therapeutics.

Keywords: stroke, neuronal differentiation, cerebral organoids

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NOVEL CANDIDATE AGE-RELATED REGULATORY RISK TRANSCRIPTIONAL FACTORS (ARTFS) FOR ALZHEIMER'S DISEASE

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Intense research is focused on Alzheimer's disease (AD) but robust therapy has not been found yet, which highlights the need of better understanding the disease and provide new druggable targets for diagnosis and therapeutics. Using fibroblast-directly induced neurons (iNs) from a well characterized cohort of 16 AD and 19 age-matched donors, we identified a set of specific age-related regulatory candidate risk transcription factors (arTFs), including RUNX1, YY1, NFIB and PPARG, that may drive the development of the AD neuronal phenotype. These TFs are important cancer-related factors, whose change in expression has also been associated with neuronal development. To test if these TFs may act upstream of the transcriptional and epigenetic changes occurring in neurons from AD patients, we performed RNA-seq on a homogenous cell population of healthy iNs overexpressing (OE) the TFs individually. Strikingly, gene set enrichment analysis of iNs OE RUNX1 indicated a major depletion of genes promoting neuronal identity enriched for terms regarding central nervous system development, ion channels and synapses, as well as energy metabolism, such as thermogenesis and oxidative phosphorylation, which are the major energy sources for mature neurons. RNAseq analysis of the other individual datasets from YY1, NFIB and PPARG OE iNs revealed a similar gene expression signature induced by each of these TFs, with the most significant downregulated genes enriched for mitochondria and energy metabolism related terms, and upregulation of gene categories enriched for a broad semantic area around stem cell signaling pathways and cancer. We then asked if the expression of each TF changed when OE any of the others. Interestingly, we found that RUNX1 was the only TF upregulated in all datasets, suggesting that it might act as a downstream factor. Notably, comparison with AD iN RNAseq data showed a major overlap with RUNX1 OE iN RNAseq data. While further investigation is required, our data currently suggest that these arTFs might contribute to the disease by priming neuroins to develop an AD cellular phenotype, by downregulating fundamental maturation neuronal genes and upregulating de-differentiation pathways, possibly driving neuronal fate loss, which could make these arTFs appealing promising targets for therapeutic intervention.

Keywords: Alzheimer's Disease, induced neurons (iNs), arTFs

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MODELING GENETIC CREUTZFELDT-JAKOB DISEASE USING HUMAN IPSC-DERIVED CEREBRAL ORGANOIDS

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Creutzfeldt-Jakob Disease (CJD) belongs to a family of human and animal diseases known as transmissible spongiform encephalopathies or prion diseases. CJD is a rapidly progressive, fatal neurodegenerative disease caused by the accumulation and aggregation of a misfolded form (PrPsc) of prion protein (PrPc). Genetic CJD caused by the E200K mutation on the PRNP gene encoding PrPc is the most common subtype of genetic prion disease worldwide. The process by which the infectious prion protein causes neuronal damage has remained mysterious. In order to study prion mediated neural disease, we established a CJD E200K-specific iPSC library, including carriers and non-carriers, and tested their differentiation towards cerebral organoids (COs). The COs are similar in cell composition and anatomical structure of the brain and can reflect its developmental process, as well as its physiology, pathology, and pharmacology. By generating a stable 3D model of the human cerebral cortex, we aim at characterizing E200K PrP in terms of its expression levels, cellular localization, and infectivity, as well as its effects in synaptic signaling. Here we describe the generation of hiPSC-derived cerebral organoid cultures from nine individuals, five carriers for the E200K mutation and four non-carriers. They revealed variations in organoids size in which the mutant organoids were significantly smaller compared with the non-mutants. We then performed single-cell RNA sequencing (scRNA-seq) of 6 months old COs. The transcriptomic profile from E200K carriers revealed several differentially expressed gene signatures relevant to neuronal and astrocytic function that may be involved in the inflammatory process. Complementary histologic analysis of the COs together with neuronal and astrocytic cultures will provide a comprehensive cellular and molecular characterization of E200K PrP-mediated pathogenicity. Our study shows that reproducible hiPSC-derived cerebral organoids expressing endogenous levels of mutant PrP can model certain aspects of human prion disease, offering an extra dimension and a powerful platform for investigating subtype pathologies and testing alleged therapeutic compounds.

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Keywords: CJD/Prions, hiPSC, Cerebral Organoids

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EFFECTS OF PRENATAL GABAPENTINOIDS EXPOSURE ON HUMAN CORTICAL NEURONS

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Prenatal substance exposure is a major public health concern associated with many detrimental fetal consequences. Unfortunately, polysubstance use in pregnancy is common. Gabapentinoids are widely used as treatments in psychiatry and neurology; however, they have been increasingly reported as having potential for misuse. Moreover, gabapentinoids can cross the placental barrier. Due to difficulties in accessing fetal brains exposed to gabapentinoids, we used the human embryonic stem cell (hESC) line H9 to generate early, intermediate cortical progenitors and cortical neurons to modulate prenatal gabapentinoid exposure in vitro. Since the cortex is responsible for cognition and behavior, we focused on cortical development. We analyzed treated (10uM) and untreated (control) cultures for gene expressions, neurogenesis, and morphogenesis. At the early patterning stage, there was a significant increase in Tbr2+ intermediate progenitors in pregabalin- and gabapentin-treated cultures. In addition, there was a significant increase in the expression of cortical related genes Pax6, Foxg1, and Tbr2 in pregabalin-treated cultures, whereas gabapentin significantly increased Tbr2 expression solely. At the maturation stage, the number of mature cortical neurons was unchanged in pregabalin-treated cultures. At early maturation, gabapentin significantly increased Tbr1+ neurons, but not Ctip2+ neurons. At the genetic level, we screened the effects of pregabalin on different cortical layer related genes. Pregabalin significantly increased expression of Brn2 without significant effects on other screened genes. Meanwhile, gabapentin did not alter any cortical layer related genes. Regarding morphogenical analysis, both pregabalin and gabapentin significantly decreased neurite length, branches, and neurites of human cortical neurons. Our data also shows that the effects of pregabalin and gabapentin on the morphogenesis of cortical neurons differ based on the presence of maturation factors, such as GDNF and BDNF, suggesting a possible interaction mechanism. Our study demonstrates that exposure to gabapentinoids during early brain development may interfere with the neurogenesis and morphogenesis of various neuronal subpopulations.

Keywords: Embryonic stem cells, Gabapentinoids, Cortical development

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INVESTIGATING THE MOLECULAR SIGNATURES OF ASH1L ASD MUTATIONS IN HUMAN IPSC-DERIVED NEURONS.

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Autism spectrum disorder (ASD) is characterized by impairments in social communication and social interactions as well as the presence of restrictive and repetitive behaviors. Chromatin and transcriptional regulators are among the class of genes with the most high-risk disease variants associated with ASD. Absent, Small, Or Homeotic-Like (ASH1L) has high risk variants associated with ASD, and it encodes for a histone methyltransferase that deposits two methyl groups on lysine 36 on histone H3 (H3K36me2). Previous work from our lab suggests that ASH1L modulates molecular mechanisms governing neuronal morphogenesis. However, the molecular signatures that are regulated by ASH1L in human neuronal development are unknown. Here, we generated disease variants in ASH1L that are associated with a range of phenotypes including ASD, seizures and with variable degrees of intellectual disability (ID). We used genome editing to generate nonsense pathogenic mutations in the chromatin binding domain (R2426*) and the catalytic domain (E2143*) of ASH1L in human induced pluripotent stem cells (iPSCs). Through bulk RNA-sequencing (RNA-seq) of iPSC-derived cortical excitatory neurons containing the ASH1L mutations, we observed widespread dysregulation of multiple gene expression programs. The differential expressed genes (DEGs) are enriched in pathways that include: extracellular matrix organization, neurotransmitter secretion, and modulation of chemical synaptic transmission. In addition to changes in gene expression, we also observed extensive differential isoform usage and alternative splicing changes in the ASH1L mutants. Lastly, we performed chromatin immunoprecipitation approaches followed by sequencing or CUT&TAG to determine how levels and positioning of specific histone modifications are altered in the ASH1L mutant iPSC-derived neurons. Integration of the transcriptomic and epigenomic datasets in this study will allow us to uncover the molecular underpinnings associated with ASH1L dysfunction in human neurons.

Funding Source: National Institute of Mental Health (NIMH) R01MH127081

Keywords: autism spectrum disorder, epigenetics, chromatin

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MATRIX STIFFNESS REGULATES THE TIGHT JUNCTION PHENOTYPES AND LOCAL BARRIER PROPERTIES IN TRICELLULAR REGIONS IN AN IPSC-DERIVED BBB MODEL

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The blood-brain barrier (BBB) can respond to various mechanical cues such as shear stress and substrate stiffness. In the human brain, the compromised barrier function of the BBB is closely associated with a series of neurological disorders that are often also accompanied by the alteration of brain stiffness. In many types of peripheral vasculature, higher matrix stiffness decreases the barrier function of endothelial cells through mechanotransduction pathways that alter cell-cell junction integrity. However, human brain endothelial cells are specialized endothelial cells that largely resist changes in cell morphology and key BBB markers. Therefore, it has remained an open question how matrix stiffness affects barrier integrity in the human BBB. To gain insight into the effects of matrix stiffness on BBB permeability, we differentiated brain microvascular endothelial-like cells from human induced pluripotent stem cells (iBMEC-like cells) and cultured the cells on extracellular matrix-coated hydrogels of varying stiffness. We first detected and quantified the junction presentation of key tight junction (TJ) proteins. Our results show matrix-dependent junction phenotypes in iBMEC-like cells, where cells on softer gels (1 kPa) have significantly lower continuous and total TJ coverages. We also determined that these softer gels lead to decreased barrier function in a local permeability assay. Furthermore, we found that matrix stiffness regulates the local permeability of iBMEC-like cells through the balance of continuous ZO-1 TJs and no junction regions ZO-1 in tricellular regions. Together, these findings provide novel insights into the effects of matrix stiffness on TJ phenotypes and the local permeability of iBMEC-like cells.

Keywords: iPSC-derived BBB, matrix stiffness, tight junctions

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INVESTIGATING THE ROLE OF NF1 DURING CORTICAL NEUROGENESIS

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Neurofibromatosis type 1 (NF1) is a neurodevelopmental disorder characterized by macrocephaly and cognitive deficits. Despite extensive research, the underlying pathological mechanism remains poorly understood. Previous studies on Nf1 heterozygous inactivation in mice revealed changes in progenitor cell expansion and differentiation, leading to cognitive impairments similar to those observed in NF1 patients. Of note, NF1 is expressed by outer radial glial progenitors (oRGs), which are thought to be responsible for the increased cortical size and complexity of human brains. However, oRGs are largely absent in rodent brains, indicating limitations in using animal models to study complex neurological conditions. We propose that NF1 haploinsufficiency caused by disease-relevant mutations dysregulates the proliferation and differentiation of human oRGs, ultimately leading to impaired cell lineage and function in the brain. To address this hypothesis, we investigated the molecular and cellular role and disease-associated dysfunction of Neurofibromin 1 during early cortical development using a human induced pluripotent stem (iPS) cell-derived 3D organoid model. Specifically, we generated organoids from cell lines with a conditional NF1 heterozygous null allele and genetically engineered patient-identified NF1 mutations. We then analyzed these organoids to characterize disruptions in progenitor cell proliferation and differentiation at 1.5 and 3 months in vitro. Additionally, we employed single-cell RNA sequencing to identify cellular composition and gene co-expression relationships that are perturbed in cells with mutations found in NF1 patients. Our work aims to deepen our understanding of the neurobiology and pathophysiology of Neurofibromin 1 in humans and potentially illuminate cellular and molecular phenotypes associated with distinct NF1 patient mutations.

Keywords: Neurofibromin1, NF1, neurofibromatosis 1, 3D cortical forebrain organoids, single-cell RNA-sequencing

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TRANSCRIPTIONAL CONSEQUENCES OF TRISOMY 21 ON NEURAL INDUCTION

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Trisomy 21 (T21) is the cause of Down syndrome, a developmental disorder that affects multiple organ systems, including the nervous system. A fundamental question is how an extra copy of human chromosome 21 (HSA21) translates into the organ-specific pathologies that characterize individuals with Down syndrome. Structural pathologies, such as brain size and heart malformation, are present at birth, reflecting embryonic origins that are generally associated with smaller organs or reduced growth. We tested the hypothesis that T21 exerts effects on human neural development as early as neural induction. We assessed the transcriptional effects of T21 on the process of neuroepithelial differentiation from human pluripotent stem cells (PSCs) that resembles in vivo neural induction in the temporal course, morphogenesis, and biochemical changes. We used T21 induced PSCs (iPSCs) and isogenic controls generated from individuals mosaic for T21 to reveal previously unknown molecular mechanisms influenced by T21 during neural development. We performed bulk transcriptome analysis of cells along PSC differentiation to early neuroepithelia at Day 6 and definitive neuroepithelia at Day 10. We identified ~1500 differentially expressed genes in T21 neural progenitors at each timepoint. Less than 3% of the gene expression changes included upregulated HSA21-encoded genes at Day 6 and Day 10 and most of the same HSA21 genes were upregulated at both timepoints. Gene ontology indicated changes in gene expression throughout the genome in T21 cells in pathways related to neuron development and extracellular matrix at both Day 6 and Day 10. Gene set enrichment analysis (GSEA) across the time course revealed changes in tightly controlled stage-specific molecular

programs in T21 cells. Results show that genes involved in specific growth factor signaling pathways (Hedgehog, Wnt, Notch), metabolism (including interferon response and oxidative stress) and extracellular matrix were changed in T21 cells. These data show that T21 has effects on discrete developmental pathways at the earliest stages of neural development. Further, the results suggest that metabolic dysfunction arises early in development in T21 and may thus affect development and function more broadly.

Funding Source: This work was supported by NIH grant 1R01HD106197, funding from University of Wisconsin-Madison and the Wisconsin Alumni Research Foundation and in part by a NIH-NICHD core grant to the Waisman Center (P50HD105353).

Keywords: Down syndrome, neural differentiation, transcriptomics

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GENERATION OF OLIGODENDROCYTES-ENRICHED 3D HUMAN BRAIN ORGANOIDS FOR THE STUDY OF GLOBOID CELL LEUKODYSTROPHY

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Globoid Cell Leukodystrophy (GLD) is a lysosomal storage disorder due to genetic deficiency of galactosylceramidase (GALC), a key enzyme in sphingolipid metabolism. GLD manifests with rapid and relentless demyelination/neurodegeneration of the central and peripheral nervous system. To date, the pathogenic mechanisms leading from the primary defect to overt tissue damage are not fully understood. Our work on human patient-specific iPSC (hiPSC)-derived neural progenitor cells (NPCs) and mixed neuronal/glial 2D cultures showed that a timely regulated GALC expression is critical during neural commitment and highlighted a defective neuronal and oligodendroglial differentiation in GLD patients' cells. Still, the 2D culture conditions did not support the full maturation of hiPSC-derived neurons and oligodendrocytes (OL), the latter cell type being primarily affected by GALC deficiency. We envisaged that 3D OL-enriched spheroids allowing extensive maturation of both neuronal and glial cell populations may represent a more suitable experimental model to investigate the early neurodevelopmental defects linked to the GLD pathology as well as the impact of GALC deficiency during OL maturation and myelination. Here, we generated 3D OL-enriched spheroids from healthy donor (HD) and GLD hiPSC and newly established GALC knock-out (KO) and knock-in (KI) hiPSC obtained by CRISPR-Cas9-mediated gene editing. We characterized the spheroids through molecular and immunophenotypical analysis. Our results suggest that: i) OL-enriched 3D spheroids can be generated with comparable efficiency from HD, GLD, and GALC isogenic hiPSC lines; ii) Mature MBP+ OL, MAP2+ neurons and GFAP+ astrocytes are present in both HD and GLD organoids; iii) OL in 3D spheroids contact axons and produce myelin. We are currently evaluating the kinetics of neuronal and glial differentiation/maturation as well as the presence of GLD pathological hallmarks using biochemical and molecular techniques coupled with advanced imaging approaches. The use of single-cell sequencing will further clarify the role of specific neuronal and glial populations during GLD pathogenesis. The use of this novel experimental platform will shed new light on the complex pathophysiology of the disease and on the role of GALC during the maturation of neural cell types.

Keywords: Brain organoids, Oligodendrocytes, Myelin disorders

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ANTISENSE OLIGONUCLEOTIDE THERAPY FOR SCHINZEL-GIEDION SYNDROME

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Schinzel-Giedion syndrome (SGS) is an autosomal dominant gain-of-function (GOF) disease characterized by severe mental retardation, neuroepithelial spinal tumors, bone and renal abnormalities and neurological degeneration. It is caused by missense mutations in a hotspot region of SETBP1 (SET binding protein 1), which changes a B-TrCP degron motif within SETBP1. There is currently no treatment for SGS and symptoms are so severe that affected children usually die within the first decade of life. We made forebrain neural progenitor cells (NPCs) from 4 patients with SGS through the use of induced pluripotent stem cells. We demonstrate that the mechanism of disease stems from the lack of degradation of SETBP1, leading to its accumulation in the cell. We designed and tested several AntiSense Oligonucleotides (ASOs) to reduce SETBP1 protein in SGS NPCs and SGS neuroepithelial tumour cells. We propose selected ASOs as a treatment for Schinzel-Giedion syndrome. These data provide a realistic therapeutic avenue for the treatment of SGS.

Funding Source: - CONACYT - Government of Mexico - FRQ - Fonds de Recherche du Québec - European Joint Programme on Rare Diseases

Keywords: Antisense oligonucleotides, Rare disease, Therapy

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CELLULAR MATURATION OF OLIGODENDROCYTES IS GOVERNED BY TRANSIENT GENE MELTING

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Pluripotent stem cells (PSCs) provide an unlimited source for generating somatic cell types. However, generating fully mature cells constitutes a bottleneck for realizing their full potential in research and medicine. Here, we report a transcriptional mechanism that governs the timing of cellular maturation in post-mitotic oligodendrocytes. During differentiation of PSCs to oligodendrocytes, the transcription factor SOX6 redistributes from nearly all super enhancers in proliferating oligodendrocyte progenitor cells to cluster across specific gene bodies in immature oligodendrocytes. These sites exhibit ‘gene melting’, a process of extensive chromatin decondensation and transcription, which abruptly turns off upon maturation. Suppression of SOX6 deactivates these immaturity loci, resulting in rapid transition to mature myelinating oligodendrocytes. Cells harboring this immature oligodendrocyte SOX6 gene signature are specifically enriched in multiple sclerosis patient brains, suggestive that failed maturation may contribute to limited myelin regeneration in disease. Collectively, our finding that maturation rate is controlled by transient transcriptional clusters may inform approaches to accelerate the generation and regeneration of mature cell types.

Keywords: Cellular maturation, Glial biology, Epigenetics

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MODELING SCHIZOPHRENIA ASSOCIATE D STRUCTURAL VARIANTS IN A NEOCORTICAL ASSEMBLOID MODEL UNCOVERS DEFECTS IN CORTICAL INTERNEURON DEVELOPMENT

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Though schizophrenia tends to manifest in adolescence, a substantial body of evidence indicates an early neurodevelopmental origin for the disorder . Data from patients has pointed to the frequent disruption of GABAergic cortical interneurons in schizophrenia, however, how such defects are acquired during cortical development is poorly understood. This is in part due to the cortical interneuron subtypes likely most relevant to the disease, fast-spiking PVALB+ cortical interneurons, having a protracted development and being difficult to derive in vitro. While iPSC models of schizophrenia have provided some interesting insights into the early stages of the disease, the complex genetics of schizophrenia present challenges for genetic background control and establishing isogenic systems. To address these issues, we have developed a human neocortical assembloid system that models early cortical interneuron development and supports the maturation of fast-spiking PVALB+ cortical interneurons on a reduced timescale. By using CRISPR to generate isogenic lines harboring distinct schizophrenia-associated genomic structural variants, we have applied this assembloid system to model early neurodevelopmental defects in schizophrenia while controlling for genetic background. Using whole organoid clearing and imaging, we found that the 22q11 deletion, which is among the most highly associated of genetic lesions with schizophrenia, imparts a defect in cortical interneuron tangential migration. With a combination of single cell RNA-sequencing and Seahorse-based assessment of mitochondrial function, we were able to show that immature cortical interneurons have a mitochondrial defect, possibly owing to haploinsufficiency of the two 22q11 genes SLC25A1 and MRPL40. Finally, we show evidence that inhibition of SLC25A1 in wild type cortical interneurons similarly leads to a disruption of migration rates. These findings suggest a plausible mechanism for which deficits in cortical interneurons during development could lead to a disruption of their function within the cortex and acquisition of schizophrenia. Additionally, our successful generation of fast-spiking PVALB+ cortical interneurons offers the exciting possibility to track later stages of cortical interneuron development in an isogenic system.

Funding Source: This research was supported by the NIMH of the NIH under the award number: 5F32MH116590-03.

Keywords: Cerebral Organoids, Cortical Interneurons, Schizophrenia

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THE INTRACELLULAR CALCIUM OSCILLATION PROFILING OF NEURAL STEM CELLS DERIVED HUMAN STEM CELLS FROM APICAL PAPILLA UNDER THREE-DIMENSIONAL NEUROSPHERES INDUCTION

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Transplantation of neural stem cells (NSCs) has emerged as a promising approach for neurodegenerative disease. Human stem cells from apical papilla (hSCAPs) originated from migratory neural crest stem cells which indicated the superior capacity for neural differentiation. This study aims to investigate the neural induction of hSCAPs into NSCs under three-dimensional (3D) neurospheres induction and characterize their functionality via intracellular calcium oscillation. The hSCAPs were isolated from healthy impacted third molar teeth of Thai donors and characterized as mesenchymal stem cells (MSCs). Consequently, the characterized hSCAPs were induced into neurospheres via a specific neural induction medium with an ultra-low attachment culture vessel. Additionally, the functional ability of these NSCs was demonstrated by intracellular calcium oscillation. Importantly, the characterized hSCAPs were successfully induced into neurospheres that exhibited the 3D-floating spheroid shape and consisted of several intra-neurospheral cells. Whereas the hSCAPs presented a fibroblast-like shape and were attached to plastic culture vessels as a monolayer (two-dimensional, 2D). Interestingly, the intra-neurospheral cells obviously revealed an intense fluorescent signal which indicated the calcium ions during the neurotransmitter-releasing activity of neuronal cells. On the other hand, The hSCAPs weakly expressed fluorescent signal. Moreover, these intra-neurospheral cells presented a dynamic change of calcium ions intensity that showed high peak and interval, while the lower and narrow dynamic change of calcium ions intensity was observed at the hSCAPs. In conclusion, this study demonstrates the typical patterns of the intracellular calcium oscillation of functional NSCs. Thus, these finding suggests the potential of using hSCAPs to generate functional NSCs as a stem cell-based therapy for further transplantation in the treatment of neurodegenerative disease.

Funding Source: This study was supported by a grant from Mahidol University Faculty of Dentistry Grant (2022), Faculty of Dentistry, Mahidol University, Thailand.

Keywords: Human stem cells from apical papilla, Three-dimensional neurospheres induction, Neural stem cells

TOPIC: PANCREAS

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ARACHIDONIC ACID PROMOTES THE DIFFERENTIATION OF PLURIPOTENT STEM CELLS INTO PANCREATIC BETA CELLS

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Pluripotent stem cell (PSC) derived pancreatic β islets are a promising source of cells for transplantation for patients with diabetes. Various protocols for differentiating PSCs into functional β islets have been established, which require stage-specific growth factors and small molecules. The quality and quantity of the functional β islets and the production cost matter the most when it comes to translational research. Optimizing the protocol with new molecules that can improve the differentiation process is the need of the hour. In this study, we used an ω-6 fatty acid, Arachidonic acid (AA), to differentiate induced Pluripotent Stem Cells (iPSCs) into pancreatic β islets. AA is involved in signalling pathways that drive cell fate decisions and is a major component of cellular membranes. Previous studies have reported the anti-diabetic activity of AA in vitro and in vivo. We expected AA to modulate the differentiation of iPSCs in a stage-specific manner. In the early stage, the 3D embryoid bodies of iPSCs differentiated into a good population of definitive endoderm (DE) cells in the presence of AA, expressing the DE marker SOX17. The changes in cellular morphology were evident from the phase contrast images of cells from various stages of differentiation. After 30 days of differentiation, cellular clusters that resembled the islet-like clusters were formed, with inter-islet connections protruding out. These clusters expressed NEUROG3, which is needed for the development of endocrine cells in the pancreas. There was a high expression of the MAFB gene, which is critical for β cell terminal differentiation, in these clusters. Our results suggest that the supplementation of AA is a promising strategy for improving the efficiency of pancreatic β cell differentiation from iPSCs.

Funding Source: EMR/2017/003114, Science and Engineering Research Board, Department of Science and Technology, Govt. of India

Keywords: Pluripotent Stem Cells, Arachidonic acid, Pancreatic beta islets

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LOSS-OF-FUNCTION OF WFS1 LEADS TO DELAYED B CELL DIFFERENTIATION VIA BOOSTING STRESS GRANULE FORMATION

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Loss-of-function mutation (LOF) of WFS1 leads to human Wolfram Syndrome (WS), resulting in juvenile-onset diabetes mellitus characterized by genetic lesion-caused β cell failure. However, due to the scarcity of human samples and ethical issues, the pathological mechanism underlying this β cell loss remains elusive, especially

during the development of pancreatic β cells, which limits drug discovery for WS. With human embryonic stem cell derived pancreatic β cells (SC-β cells), we recapitulated the β cell maldevelopment and β cell failure caused by WFS1 deficiency. We found that WFS1-LOF not only leads to β cell dysfunction, characterized by impaired insulin synthesis and secretion, but also delays β cell differentiation from pancreatic progenitor cell to β cell. To further understand the mechanism that WFS1 mediating pancreatic development, we performed the single-cell-RNA-sequencing of wildtype and WFS1-LOF SC-β cells, and it suggested that activated integrated stress response (ISR) with elevated stress granule formation is a key mechanism resulting in pancreatic β cell loss in SC-β cells with WFS1-LOF. Consistently, combined with experimental validation, SC-β cells with WFS1-LOF suggest activated ISR that contributes to stress granule formation with the significantly upregulated expression level of G3BP1. Most importantly, we test ISRIB as a potential drug to mitigate ISR and reduce the formation of stress granules, and finally mitigates delayed development and rescued cell death in SC-β cells. Altogether, our study has mechanistically and therapeutically provided novel insights into the pathogenesis and treatment of β cell loss for WS.

Funding Source: This work was supported by the National Key *Research and Development* Program of China. W.L. is also supported by the National Natural Science Foundation of China and the Key Project of the Science and Technology Commission of Shanghai Municipality.

Keywords: WFS1, Islet development, Integrated stress response

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ASSESSING THE IMMUNE RESPONSE TO STEM CELL-DERIVED ISLETS IN MICE USING SPATIAL TRANSCRIPTOMICS AND 3D IMAGING

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Stem cell-derived islets (SC-islets) therapy offers opportunities to treat type-1 diabetes. However, immune rejection of the transplanted islets is a major obstacle. Previous strategies for protecting the cells from the immune cells, such as long-term immunosuppression and graft encapsulation, have certain drawbacks. To determine the mechanism that drives immunogenicity of SC-islets, we performed time-course analysis study on humanized mice transplanted with SC-islets and allogeneic peripheral blood mononuclear cells (PBMCs). We used FFPE-based single-cell RNA seq (FcsRNAseq) combined with spatial transcriptomic (slideseq), 3D spatial biology and spatial proteomics at different time points with a single cell resolution. While single cell spatial proteomics allows proximity, nearest neighbor and Infiltration analysis, FcsRNAseq and spatial transcriptomic allow the detection of differently expressed genes and gene networks in both SC-islets and immune cells. Our results emphasize the role of B and T cells in SC-islets rejection and the beneficial use of genetically modified SC-islets for immune protection. Finally we suggest the use of single cell spatial transcriptomics/proteomics approach as a way of finding specific interaction between immune cells and the SC-islets which may allow encapsulation-free SC-islets transplantation.

Keywords: Stem cell-derived islets (SC-islets) therapy, Allogeneic rejection, Spatial transcriptomic (slideseq)

TOPIC: NO TISSUE SPECIFICITY

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ALLOGENIC NAÏVE AND PRIMED EMBRYONIC STEM CELLS ELICIT A CANCER-SPECIFIC IMMUNOTHERAPEUTIC RESPONSE IN MICE

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Recently patient-specific iPSCs have been reported as an efficient source of tumor-specific antigens (Kooreman et al., 2018). Pluripotent cells share an overlapping expression profile with cancer cells, suggesting that some surface markers may serve to prime the immune system to target cancers. Indeed, artificial reprogramming has been compared to the biogenesis of cancer stem cells (CSCs). We have previously demonstrated that Zeb2, a factor involved in epithelial mesenchymal transition (EMT), is an important barrier limiting the reprogramming of aged cells until the acquisition of epithelial characteristics (Bernardes de Jesus et al., 2018; Goossens et al., 2011). Further exploring the similarities between cancer and ESC, we demonstrate that sub-optimal culturing conditions, through the withdraw of the 2 inhibitors (CHIR99021 and PD0325901) (Bernardes de Jesus et al., 2018) results in the acquisition of characteristics related with cancer, such as the higher expression of oncogenes or (novel) cancer antigens, such as Claudin (CLDN) 6 (Reinhard et al., 2020) or the Wilms' tumor 1 transcript (WT1) (Van Tendeloo et al., 2010). When left in suboptimal culture conditions, metastable ESC rapidly undergoes EMT as observed though the co-expression of stemness factors and EMT factors (such as Zeb2) in the vicinity, a process resembling oncogenic EMT. Metastable ESC-based vaccines demonstrates an in vivo immunotherapeutic potential in an orthotopic and syngeneic breast cancer model, eliciting a specific response further supporting the similarity in antigenic repertoire. In fact, using mass-spectrometry we demonstrated that metastable ESC share several peptides with cancer cells, sharing several biological processes. These results support ESC as an innovative tool to derive specific cancer vaccines.

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Keywords: mouse ESC, immunotherapy, primed

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INCREASING EFFICIENCY AND CELL VIABILITY WHEN REPROGRAMMING SOMATIC CELLS INTO IPSCS

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Patient-specific induced pluripotent stem cells (iPSCs) are a valuable resource in the development of models for studying unique disease or drug responses. Donor somatic cells are reprogrammed into iPSCs, then differentiated into target cells for treatment or testing purposes. Despite the potential of these cells, reprogramming has low efficiency (< 1%), instability of pluripotency, and higher chance for mutations. Reprogramming is long, labor intensive and manual, and requires additional screening to derive a monoclonal population. To robustly deploy iPSCs in clinical settings, it is necessary to overcome these limitations. We hypothesized that the novel CellRaft® AIR System Technology could accelerate reprogramming by improving efficiency, automation, and cell viability. Reprogrammed fibroblasts (Epi5 Reprogramming Kit, Thermo Fisher) were seeded in either a traditional 6-well plate or in a CellRaft® Array. In both cases, the electroporated cells were immediately seeded in bulk. However, the CellRaft® Array provided flask-like population cell culture conditions with the added benefit of single-cell segregation, allowing for interrogation of individual cells. Cells settled into microwells and attached to a CellRaft® tissue culture growth surface. The 6-well plate was manually examined on a microscope, while the Array was automatically scanned on the CellRaft® AIR System for 15 days. Each Array scan generated time course images, allowing for precise monitoring of reprogramming efficiency, viability, and clonality. On day 15, pluripotency was confirmed by live TRA-1-60 staining. The Array contained 1457 individually segregated TRA-1-60+ colonies, whereas the 6-well plate contained 440 TRA-1-60+ foci amongst a lawn of non-reprogrammed fibroblasts. Using the CellRaft® AIR, positive CellRafts® were individually automatically isolated into 96 well plates for downstream analysis. Isolated clones were expanded into a bankable colony at least a week sooner than the 6-well plate, with a significantly higher success rate of outgrowth. These data demonstrate that the CellRaft® AIR System provided a gentle solution to isolating fully reprogrammed iPSCs without the need for manual perturbation, while also overcoming major common bottlenecks such as efficiency and time that complicate these efforts.

Funding Source: 1R43GM143978-01

Keywords: Reprogramming, Viability, Clones

834

A DISEASE-SPECIFIC IPS CELL RESOURCE FOR STUDYING RARE AND INTRACTABLE DISEASES

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Disease-specific iPSCs are useful tools for pathological analysis and diagnosis of rare diseases. Given the limited available resources, banking such disease-derived iPSCs and promoting their widespread use would be a promising approach for untangling the mysteries of rare diseases. Herein, we comprehensively established iPSCs from patients with designated intractable diseases in Japan and evaluated their properties to enrich rare disease iPSC resources. We established 1,532 iPSC clones from 259 patients with 139 designated intractable diseases. The efficiency of iPSC establishment did not vary based on age and sex. Most iPSC clones originated from non-T and non-B hematopoietic cells. All iPSC clones expressed key transcription factors, OCT3/4 (range 0.27–1.51; mean 0.79) and NANOG (range 0.15–3.03; mean 1.00), relative to the reference 201B7 iPSC clone. These newly established iPSCs are readily available to the researchers and can prove to be a useful resource for research on rare intractable diseases.

Keywords: iPS cells, designated diseases, rare and intractable diseases

836

WELLREADY: ENABLING THE ROOM TEMPERATURE SHIPMENT OF CELL-BASED IN VITRO MODELS FOR DRUG DISCOVERY

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Advanced physiologically relevant in vitro model systems drive progress in drug discovery by predicting the efficacy and safety of drug candidates. With high attrition rates of new drugs and the phasing out of animal models, accurate models for drug discovery have never been so important. Whilst this is well accepted, barriers exist for the adoption of advanced models due to the high level of skill required for culture, long culture times and high failure rates. Concomitantly, high complexity generally leads to high fragility restricting the ability to ship cell models from their site of manufacture to the end-user and making cryopreservation impossible. WellReady™ enables the room temperature storage and shipment of in vitro models by protecting them from the associated physiochemical and mechanical damage. It does this through enveloping biological samples in alginate-based hydrogels. Once the gel layers are removed, models can be returned to physiological temperature and be “assay-ready” for drug screening. We have demonstrated the utility of WellReady™ in storing and shipping a range of model systems including iPSC-derived cells, transfected cell lines, and primary human cells/stem cells. iPSC-derived neurons in 96-well plates stored and shipped at room temperature over a 3-day period in medium exhibited considerable destruction to the axonal network whilst this was prevented by WellReady™ with a 2.6±0.7-fold higher viability compared to medium alone. WellReady™ also maintained the viability of iPSC-derived cardiomyocytes over 7 days at room temperature with no drop in viable cell number compared to non-stored cells (109±9%) and regained beating activity upon return to 37°C. HEK293 cells transiently transfected with cDNA could be stored at room temperature in WellReady” for 5 days and respond to drug treatment in kinase-based target engagement assays with an EC50 comparable to non-stored cultures. Finally, primary human airway epithelial monolayers could be stored for 7 days at refrigerated or room temperature in WellReady” without any loss of viability whilst all cells were dead after storage in medium alone. These examples on a range of different cell models demonstrate WellReady” to be a highly effective tool in enabling the room

temperature storage and shipment of cell-based models for drug discovery.

Keywords: Preservation, Shipping, In vitro model

838

PATIENT IPSC-BASED IN VITRO MODEL TO STUDY NF1 MUTATION IN GLIAL CELLS

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Neurofibromatosis type 1 (NF1) is one of the most common genetic tumor predisposition conditions. Individuals with germline mutations in NF1 are susceptible to tumor development in both the central and peripheral nervous systems (CNS and PNS). In the CNS, NF1-associated tumors are most commonly detected in the optic pathway, which often leads to progressive visual loss. Several studies have suggested that NF1 mutations also lead to dysfunction of the glial cells in the CNS. However, the effect of loss of NF1 function in the human glial cells is not well characterized. To address this limitation, we are using NF1 patient-derived iPSC lines and differentiating them into oligodendrocytes (OLs), OL precursor cells (OPCs) and astrocytes. To enable scalable production of the iPSC-derived glial cells and to use them for biochemical studies, we generated dual knock-in reporter lines of NF1 mutant (NF1+/-) and CRISPR-corrected isogenic control (NF1+/+) hiPSC lines. The dual reporters are 1) td-tomato and a cell-surface protein THY1.2 are expressed under the control of the OPC-enriched PDGFRα gene, and 2) a secreted Nanoluc (secNluc) is driven by the OL-specific MBP gene. This reporter scheme allows the OPCs to be purified using Thy1.2 antibody-conjugated magnetic microbeads following 75 days of differentiation, and the maturation of OPCs into OLs can be quantified by measuring the Nluc activity, which corresponds to the MBP expression. We find that the NF1+/- hPSCs, potentially due to the loss of NF1 tumor suppressing function, proliferate 1.5 times faster than their control counterparts. However, the NF1+/- hPSCs differentiate into OPCs significantly less efficiently than the WTs. Interestingly, the purified mutant OPCs show a higher level of secNluc activity and significant enrichment of MBP and PLP1 transcript expression when compared to the WT OPCs. Furthermore, our preliminary data suggests that the NF1+/- hiPSC-derived astrocytes, upon the inflammatory cytokine (TNFα, IL1α and C1q) treatment, express significantly higher levels of reactive astrocyte markers such as C3, VCAM1 and CXCL10 than the NF1+/+ astrocytes. Taken together, the patient iPSC-based cell lines that we have generated serve as a physiologically relevant human cell model, which will aid in studying the outcome of NF1 mutations in human glial cells.

Funding Source: Gilbert Family Foundation

Keywords: Neurofibromatosis Type 1, Oligodendrocytes, Astrocytes

840

IMPROVING GENETIC STABILITY IN HUMAN PLURIPOTENT STEM CELLS MAINTAINED AS SINGLE CELLS USING A NOVEL MAINTENANCE MEDIUM

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To improve reproducibility and rigor of human pluripotent stem cell (hPSC) research, there has been a renewed focus on establishing defined quality standards and guidelines. An important quality attribute being scrutinized is genetic stability, with the acquisition of cytogenetic abnormalities widely reported in hPSCs. Recurrent cytogenetic abnormalities in hPSCs can confer a strong selective advantage through different mechanisms including increased growth rates and resistance to cell death. Long-term single-cell passaging has been linked with increased genetic instability and is generally not recommended for the maintenance of hPSCs. To address this, we have developed a novel hPSC culture medium (nSCM) specifically formulated to support hPSCs when maintained as single cells. To assess genomic stability, 48 independent clones from 2 hPSC lines (H1 and H9) were single-cell passaged for 20 weeks in either nSCM or two control media. Selected control media were developed and optimized for use with traditional aggregate passaging protocols. After 20 weeks, clones were screened for 8 common recurrent abnormalities using a qPCR-based method and then confirmed using FISH. A total of 53% (25/47) and 46% (22/48) of the hPSC clones maintained in control media were found to have recurrent abnormalities. In contrast, abnormalities detected in hPSCs maintained in nSCM were significantly lower with only 7% (3/46) of clones abnormal after 20 weeks. This marked difference was characterized by the absence of 20q gains detected in hPSC clones maintained in nSCM (0/4 abnormal clones) versus control media (45/47 clones). hPSCs cultured as single cells in the nSCM display characteristic hPSC morphology, express high levels of undifferentiated cell markers, and differentiate efficiently to all three germ layers. hPSCs passaged as single cells in nSCM displayed an improved average expansion rate of 104-fold over 5 days, and when seeded at 1 cell/well in nSCM supplemented with CloneR™2 displayed cloning efficiencies of 41 ± 5% (mean ± SD, n = 4 cell lines). In summary, we demonstrate the utility of a novel hPSC culture medium that improves genetic stability of hPSCs when maintained long-term using single-cell passaging protocols. hPSCs maintained in nSCM

retain key hPSC quality attributes ready for use in downstream applications.

Keywords: Pluripotent, Genetic stability, single cell

842

DIRECTED DIFFERENTIATION OF FUNCTIONAL VASCULAR SMOOTH MUSCLE CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Smooth muscle cells (SMCs) consist of the wall in variety of tissues including blood vessels, which plays a pivotal role in vascular homeostasis and disease. The need for smooth muscle cells applications is increasing in the field of biomedical and biotechnological with the development of regenerative medicine and disease modeling for studying vascular disease pathophysiology. Currently, the most reliable source of human SMC scan be obtained from umbilical cord and bone marrow. However, those cells are shown the high variability among donors and culture senescence, limiting their proliferative capacity. The human induced pluripotent stem cells (iPSCs) can self-renew indefinitely in culture and can be differentiated into the cells of three germ layers, which provides a platform to generate a large number of cells desired. We recently have developed a method for generating SMCs from human iPSCs. Those cells display the phenotypically and transcriptionally similar properties as primary SMCs which expression α-smooth muscle actin, calponin, and SM22. Furthermore, the cells can be induced into mature and functional SMC. Our results exhibit that the iPSC derived SMCs have the potential to be used as high-throughput invitro assays for evaluating compound toxicity and compound screening for drug discovery and development. Moreover, SMCs generated from patient-specific iPSCs can model the disease pathophysiology and be used in personalized medicine development.

Keywords: Smooth muscle cells, Differentiation, INDUCED PLURIPOTENT STEM CELLS

844

DIRECTED DIFFERENTIATION OF BIPOTENTIAL GONADAL CELLS GIVES RISE TO EITHER EARLY TESTICULAR OR EARLY OVARIAN CELLS

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Reproductive development can be disrupted by both environmental and genetic factors, with Differences of Sex Development (DSDs) affecting about 1.7% of babies. There is an urgent need for an in vitro model to study human embryonic gonadal development and DSDs. We have addressed this by establishing a world-first protocol to differentiate human induced pluripotent stem cells (iPSCs) into early gonadal cells cultured as testis-like organoids. Our stepwise differentiation protocol uses small molecules to mimic developmental signalling, inducing iPSCs to develop into the bipotential gonad by day 7. Culturing these cells as 3D organoids and treating them with masculinising growth factors results in significant testis gene expression by day 21. In these early testis-like organoids, cells expressing testis markers reside within tissue structures delineated by basement membrane. These "tube-like structures" are reminiscent of cord-like assemblies in re-aggregated mouse testes. Transcriptomic profiling of our testicular organoids shows clusters with gonadal and reproductive tissue identities, and distinct testicular cell lineages, which overlap with those found in human fetal gonads. Furthermore, using feminising factors in our differentiation protocol instead of masculinising factors, leads to the upregulation of female marker genes and the expression of proteins crucial for female reproductive development.

Funding Source: National Health and Medical Research Council Ideas Grant GNT2012250 2022-2025

Keywords: gonadal development, gonadal differentiation, organoids

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ALTERED NEUROVASCULAR CROSSTALK IN SCHIZOPHRENIA: AN IPSC-DERIVED NEURODEVELOPMENTAL STUDY

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Schizophrenia (SZ) is a chronic debilitating neuropsychiatric disorder affecting around 1% of the population worldwide. To date, SZ has no cure, and more than one-third of patients describe little to no progress after treatment, for which, an expanded knowledge and comprehension of SZ physiopathology is imperative. Increasing evidence is tracing SZ's origin to embryonic neurodevelopment, where the brain is formed concomitantly with a vast and complex vascular network, structuring the neurovascular unit (NVU). Recently, we have worked in the modeling of early neurovascular interactions in SZ using induced pluripotent stem cell (hiPSC) reprogramed from healthy controls (HC) and SZ patients into the main cellular components of the NVU: Neural stem cells (NSC), neurons, astrocytes and brain endothelial cells (BEC). We have studied their functional and molecular alterations and the effect of their secretomes on angiogenesis and brain barrier formation. Our results show changes in functional connectivity dynamics of SZ hiPSC-derived long-term neuronal cultures suggesting that alterations in neuronal communicational dynamics are already present during early development in SZ and may contribute to the brain functional connectivity anomalies described in SZ patients. SZ-derived BEC show a decreased response to angiogenic stimuli and present alterations in their barrier capacities. The overall secretome of SZ-derived NVU cultures is less neurotrophic than HC, inducing a decreased angiogenic response and an increased permeability both in vitro and in vivo. Our findings suggest the presence of inherent deficiencies in both neural and vascular components resulting in defective crosstalk in the formation of the NVU that possibly contributes to the phenotype described in SZP. These results broaden the spectrum of possible therapeutic targets for SZ treatment and posit new possibilities for biomarkers for disease diagnosis and stratification.

Funding Source: Fondecyt Grant # 1221522

Keywords: induced pluripotent stem cells, schizophrenia, neurovascular unit

FRIDAY, 16 JUNE 2023

TRACK: NEW TECHNOLOGIES (NT)

Session 4: Odd

9:30 AM – 10:15 AM

TOPIC: CARDIAC

849

USING AUTOMATED PATCH CLAMP FOR HIGH THROUGHPUT PHARMACOLOGICAL CHARACTERIZATION OF CARDIAC ACTION POTENTIAL IN HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED CARDIOMYOCYTES

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Drugs interfering with cardiac electrophysiology is of important concern and is the leading cause for either attrition or withdrawal from the market. Thus, there has been an increased prioritization to assess the cardiac safety liability of the drugs quite early in the drug development process in human relevant model systems. Human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) recently emerged as a valuable in vitro tool for screening of drug-induced cardiotoxicity as they well replicate human adult cardiac electrophysiological features. However, the low diastolic resting potential, low throughput and the tediousness with the gold standard manual patch clamp measurements has limited its applicability in drug discovery projects. To address these limitations, we have developed an assay using our automated patch clamp (APC) system the QPatchII, with the state-of-art feature Adaptive Current Clamp - or IAdapt - which allows users to automatically clamp each individual iPSC-CM to the holding potential of interest and evoke a cardiac action potential with duration and amplitude well mirroring the human adult cardiac action potential. With the use of IAdapt feature, we observed a significant improvement in the upstroke velocity and action potential shape, which are critical parameters for evaluating the proarrhythmic screening of drugs. Furthermore, we also benchmarked our assay with generic ion channel inhibitors, such as E4031, Dofetilide, Nifidipine and BayK8644. We observed the alterations of action potential duration (APD) with each respective drug, due to their modulation of either potassium or calcium conductance. The possibility to clamp each iPSC-CM cell individually to hyperpolarized potential with the IAdapt feature is efficient, robust, and reliable in supporting the characterization of the cardiac cellular electrophysiology, which is essential for cardiac safety liability studies.

Keywords: iPSC derived cardiomyocytes, ion channel, Automated patch clamp

851

ISOLATION OF MATURE HUMAN IPSC-DERIVED VENTRICULAR CARDIOMYOCYTES USING SYNTHETIC MICRO-RNA SWITCH

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For disease modeling and drug screening using induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs), mature and subtype-specific iPSC-CMs are required to mimic the disease pathology, to understand the underlying mechanism of diseases, and to confirm the candidate drug's deleterious features. We have shown that synthetic mRNAs encoding a fluorescent protein tagged with complementary sequences against specifically expressed microRNA (miRNA-switch) can efficiently purify the target cells with the specific miRNA. To purify iPSC-CMs, miR-208a-responsive mRNA (miR-208a switch) was reported to isolate iPSC-CMs efficiently. However, the miR-208a switch cannot purify each subtype-specific iPSC-CMs, such as ventricular, atrial, and nodal types. We differentiated iPSC-CMs using ventricular, atrial, and nodal protocols, respectively. Using purified iPSC-CMs with miR-208a switch in those three protocols, we found miR-V expressed more highly in iPSC-CMs generated by ventricular protocol than the others. MiR-V-responsive mRNA (miR-V switch) reacted to iPSC-CMs only in the ventricular protocol and not in the atrial and nodal protocols. The proportion of miR-V positive iPSC-CMs increased with relatively long-term culture only in the ventricular protocol (9.2±3.8% on day 20, 35±8.8% on day 40). MiR-V positive iPSC-CMs showed significantly higher expression of ventricle- and maturation-related genes such as MYL2 and CD36 than miR-V negative iPSC-CMs. In electron microscopy observation, miR-V positive iPSC-CMs showed M-bands, while miR-V negative iPSC-CMs did not. We also showed that the previously reported maturation protocol increased miR-V-positive iPSC-CMs.

In conclusion, we demonstrated that the combination of miR-V and miR-208a switches efficiently isolated mature ventricular iPSC-CMs, enabling its application in disease modeling and drug screening.

Keywords: miRNA, mature cardiomyocyte, Induced Pluripotent Stem Cell

853

MULTI-LINEAGE HEART-CHIP MODELS DRUG CARDIOTOXICITY AND ENHANCES MATURATION OF HUMAN STEM CELL-DERIVED CARDIOVASCULAR CELLS

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Currently prescribed chemotherapeutic drugs inhibiting tyrosine kinase (TKI) pathways account for major cardiotoxic events in some cancer patients. In the field of personalized medicine, on-chip devices are a gold standard for gathering preliminary results on drug effects on a patient specific cell line, given a comprehensive and reproducible model is developed. In this work, we have developed a cardiovascular on chip model for monitoring the cardiotoxic effect of sorafenib as a TKI on human stem cell-derived cardiomyocytes and endothelial cells. Human iPSC cardiomyocytes are cultured in the top channel of an Emulate® organ-on-chip device, over a porous membrane that separates them from the bottom channel hosting human iPSC endothelial cells. Continuous medium flow and mechanical stimulation on chip has shown advancements in cellular maturity of both cell types. Furthermore, the application of sorafenib has shown measurable cardiotoxicity through structural and functional characterization of both cell lines. Uniqueness of each individual in our world mandates the use of personalized medicine for future patients and a goal to be set for today's healthcare industries. Our study shows today's capabilities of developing in-clinic personalized medicine, which can be faithful physiological models for novel and specific drug testing, amongst other healthcare applications.

Keywords: Organ-chips, cardiotoxicity, stem cell

855

SPATIAL TRANSCRIPTOMICS REVEALED LONG-TERM ENGRAFTMENT AND FUNCTIONAL MATURATION OF THE HUMAN GRAFTS IN MYOCARDIAL INFARCTED PIGS' HEART

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Cellular therapy could potentially replace damaged heart muscle for regenerative cardiology. However, there has been a lack of understanding of the molecular mechanisms involved in the engraftment and maturation of these transplanted cells. Therefore in this study, we utilized the spatial capture technology to get a holistic understanding of the complex transcriptional landscapes within their spatial context. Previously, we have shown the safety and efficacy of transplanting our laminin221-derived cardiovascular progenitors (CVPs) into myocardial-infracted (MI) pig hearts. Here, we continue to gain a deeper understanding of these cells by performing 10x spatial transcriptomics at 1-, 4- and 12- weeks post-transplantation in both acute and chronic MI pig models. We are able to separate the transcripts from the pigs and humans in the infarcted region and confidently identify the regions that contain human cells. This in silico prediction has been validated by immunohistochemistry staining of the pig tissue samples with human-specific anti-Ku80 antibodies. Results demonstrated the engraftment and viability of the CVPs into the infracted region across the time points. Interestingly, we observed an increase in ribosomal and mitochondria activity at 12 weeks as compared to 1- and 2- weeks. These findings fit into the cardiac maturation model where cells will switch their metabolic energy source from glucose to fatty acid which increases the ribosomal and mitochondrial activities as they switch from immature fetal-like cells to mature cardiomyocytes (CMs). We also confirmed the mature status of the human grafts by the ratio of cardiac-specific genes such as MYH6 and MYH7. Furthermore, we also uncovered novel upstream regulators that are highly expressed in mature grafts, and these genes will be studied to validate cardiac maturation. We also compared the differences in acute and chronic MI models and these findings will be presented. In conclusion, we have shown the successful engraftment and maturation of human CVPs toward CMs using spatial technology. These data will be used to understand other aspects of regenerative cardiology such as the mechanism of VTs and in vivo cellular repair mechanisms.

Keywords: Spatial transcriptomics, Cardiac maturation, Regenerative cardiology

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MACHINE LEARNING-BASED SCREENING SYSTEM USING HPSC-DERIVED CARDIOMYOCYTES FOR THE DISCOVERY OF NOVEL THERAPIES TO ATTENUATE DOXORUBICIN-INDUCED CARDIAC DAMAGES

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Cardiotoxicity is a life-threatening side effect in Doxorubicin (DOX) treatment for patients with cancers. However, there are currently no effective methods to attenuate DOX-induced cardiac damages. Here we have developed an in vitro platform to evaluate the effect of DOX on cardiomyocytes and established a high-throughput screening assay by the machine learning-based evaluation of the DOX-induced cardiac damage using hiPSC-derived cardiomyocytes. 2-dimensionally cultured mature ventricular cardiomyocytes revealed the DOX-induced sarcomere disorganization and 3-dimentional mature ventricular cardiac tissues enabled to quantify the DOX-induced contractile impairment and reveal the cellular damage in non-cardiac cells. Using the sarcomere imaging in 2-dimentional DOX-treated cardiomyocytes, we established the efficient method to quantify the DOX-induced sarcomere disorganization by supervised machine learning system. This machine learning-based system can precisely score the sarcomere damage in cardiomyocyte, which allowed to quantify the DOX-induced cardiomyocyte damage in the dose-dependent manner. Using this quantification method, we have established high-throughput screening (HTS) system. We are currently performing HTS to discover candidate compounds to attenuate DOX-induced sarcomere damages and validating their cardio-protective effects using 3D mature cardiac tissues. Collectively, this study highlights the power of the combination of the disease modeling using iPSC-derived mature cardiac tissues and the machine learning-based evaluation of cellular structures to investigate human heart disease.

Keywords: machine learning-based screening, doxorubicin-induced cardiomyopathy, iPSC-derived cardiomyocyte

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A SIMPLE AND EFFICIENT CULTURE SYSTEM FOR THE LARGE-SCALE EXPANSION OF HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

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The in vitro expansion of human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) is limited, and the ability to generate large numbers of cardiomyocytes would benefit drug discovery, toxicology, and cell therapy research. Here, we present STEMdiff™ Cardiomyocyte Expansion Kit, the first commercially available and optimized culture system amenable to large-scale expansion of hPSC-CMs. STEMdiff™ Cardiomyocyte Expansion Kit includes STEMdiff™ Cardiomyocyte Passaging Supplement and Expansion Medium. To initiate the hPSC-CM expansion workflow, hPSCs were differentiated to CMs using STEMdiff™ Ventricular Cardiomyocyte Differentiation Kit. A confluent beating monolayer of early-stage hPSC-CMs was observed on day 11 of differentiation and harvested using STEMdiff™ Cardiomyocyte Dissociation Kit. The Day 11 hPSC-CMs expressed > 80% cardiac troponin T, as measured by flow cytometry, thereby confirming their CM identity. Day 11 hPSC-CMs were replated onto Matrigel®-coated cultureware at a low density using STEMdiff™ Cardiomyocyte Passaging Medium. After 24 hours, a full-medium change was performed with STEMdiff™ Cardiomyocyte Expansion Medium, and every 2 days onwards. After 7 days in culture, a confluent beating monolayer of expanding hPSC-CMs was observed. The expanding hPSC-CMs were then dissociated and replated as described above for passage 1. After 5 passages, the hPSC-CMs had an average cumulative fold expansion of 72 ± 12 (n = 15), ranging from 33- to 103-fold across 4 hPSC lines. The expanded hPSC-CMs expressed high levels of cardiac troponin T (90 ± 2%, n = 15, 4 hPSC lines). After expansion, hPSC-CMs were cultured for one week using STEMdiff™ Cardiomyocyte Maintenance Kit. Microelectrode array electrophysiology readings confirmed that expanded hPSC-CMs had a stable beating profile (12 ± 1 bpm, n = 14, 4 hPSC lines). STEMdiff™ Cardiomyocyte Expansion Kit provides a simple and standardized workflow for efficient and scalable expansion of hPSC-CMs for use in drug discovery, toxicology studies, and cell therapy models.

Keywords: Cardiomyocyte, Expansion, hPSC-Derived Cardiomyocytes

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END TO END AUTOMATION FOR LARGE SCALE DRUG DISCOVERY USING PATIENT DERIVED 3D COLORECTAL CANCER ORGANOIDS

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Many oncology drugs fail at the later stages of the drug development pipeline and in clinical trials, despite promising data in vitro. 3D cell models, such as patient-derived organoids (PDOs), offer a promising solution to this problem. Cells grown in 3D can better mimic cell-cell interactions and the tissue microenvironment. Studies show that patients and their derived organoids respond similarly to drugs, suggesting the value of using PDOs to improve therapeutic outcomes. However, challenges such as assay reproducibility, scalability, and cost have limited the use of PDOs in mainstream drug discovery pipelines. To address challenges associated with the use of PDOs in large scale applications, a semi-automated bioprocess was developed for the controlled production of standardized PDOs at scale. The bioprocessor maintains an environment that ensures constant delivery of nutrients and growth factors to the culture while preventing the accumulation of toxins. This method results in the large-scale production of assay-ready organoids that are uniform in size and have high viability. To demonstrate the utility of these PDOs for downstream drug discovery applications, we previously carried out a pilot study using patient derived colorectal cancer (CRC) organoids for a compound screen. The complete workflow was done manually in 96W and 384W microtiter plates. Here, we sought to improve assay throughput by developing an end-end, automated workflow starting with assay-ready CRC organoids. CRC organoids were established in culture, maintained, and screened in an automation enabled workcell consisting of an incubator, high content imager, liquid handler and robotic plate handler. For maintenance, the liquid handler was used for media changes with routine imaging to monitor organoid growth (pre and post treatment). CRC PDOs were treated with selected anti-cancer compounds and analyzed using deep learning-based image segmentation. A viability assay was carried out using high-content imaging. We find that PDOs treated with romidepsin and trametinib showed the most significant reduction in size, with a greater number of dead cells compared to the other compounds and controls. Overall, our results show the utility of PDOs in high throughput drug discovery applications using automation with high-content imaging.

Keywords: Colorectal cancer, organoids, screening

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PRODUCING OF INDUCED PLURIPOTENT STEM CELLS AND MESENCHYMAL STEM CELLS FROM ONE PLUCKED HAIR

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Induced pluripotent stem cells (iPSCs) have been reprogrammed into an embryonic-like pluripotent state. They theoretically enable to differentiate into any type of human cell. Mesenchymal stem cells (MSCs) are mesenchymal stromal cells that differentiate into various cell types. Due to of their multipotency, iPSCs and MSCs are encouraging options for cell treatment and the reconstruction of human tissues. However, the application of these cells to therapies has been limited due to the highly invasive procedures needed for their collection. iPSCs are generally produced from skin fibroblasts or mononuclear cells, while MSCs are isolated from tissues such as adipose tissue, bone marrow and umbilical cord. We herein describe a minimally invasive method to collect iPSCs and MSCs from only one plucked hair follicle. All experimental procedures conformed to ethical standards. iPSCs were obtained by reprogramming keratinocytes collected from one plucked hair. A hair follicle with an external root sheath was cultured on a plate with MEF-conditioned medium. Cells that growed from the bulge area were isolated and cultured, and the expression of Keratin 14 was confirmed by immunostaining. Isolated cells were transfected with episomal vectors carrying the OCT3/4, SOX2, LIN28, KLF4, L-MYC and mouse p53DD genes by electroporation. The medium was gradually switched from EpiLife ™ to Essential 8™ Flex Medium. Cells expressed iPSC markers and could differentiate into all three germ layers. Karyotyping analysis confirmed that these iPSCs cells had no chromosomal abnormalities. MSCs were obtained by direct isolation and cultivation from one plucked hair. A pulled hair follicle was cultured on a plate with DMEM medium containing bFGF, EGF, FBS and Pen/Strep. Cells from the papilla were grown and subjected to a flow cytometric analysis, which revealed the expression of CD73, CD90 and CD105. These cells differentiated into adipocytes, osteoblasts, and chondrocytes. Based on these results, cells isolated from one plucked hair follicle were identified as MSCs. The present study provides an approach to obtain iPSCs and MSCs from only one plucked hair follicle with minimal invasiveness and ease. This method will provide novel insights into and contribute to the expansion and development of regenerative medicine.

Keywords: Induced pluripotent stem cells (iPSCs), Mesenchymal stem cells (MSCs), Plucked hair

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RETROSPECTIVE ANALYSIS OF ENHANCER ACTIVITY AND TRANSCRIPTOME HISTORY

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Cell state changes in development and disease are controlled by gene regulatory networks, the dynamics of which are difficult to track in real-time. Therefore, we developed the DCM time machine (DCM-TM), a system that enables the tracing of genome-wide gene transcription and enhancer activity during stem cell differentiation. The DCM-TM is based on a fusion between the bacterial methyltransferase DCM and subunit b of the RNA polymerase II machinery. We generated mice with a doxycycline-inducible DCM-Polr2b fusion gene which, upon induction, methylates the CCWGG sequence in active genes and enhancers. DCM methylation labels can then be detected using the methylation-dependent restriction enzyme LpnPI (MeD-seq), which recognizes all methylated DCM sites and 50% of the methylated CpG sites.

MeD-seq analysis of DCM-TM data shows enrichment of DCM methylation labels at the gene bodies of active genes and active enhancer regions. These labels do not affect gene expression and are propagated to daughter cells during S-phase, enabling lineage tracing. We apply the DCM-TM technology to study intestinal homeostasis, resulting in gene and enhancer activity maps that trace the intestinal stem cell (ISC) state to the enterocyte state. These maps allow us to predict transcription factor activity and study the changes in chromatin landscape during ISC differentiation. To conclude, DCM-TM provides a powerful technology for tracing genome-wide gene transcription and enhancer activity back in time without relying on predictions or the requirement to isolate stem cells. DCM-TM has wide applicability in tracking cell states, providing new insights into the regulatory networks underlying cell state changes.

Keywords: Stem cell differentiation, Lineage tracing, Intestinal homeostasis

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UNDERSTANDING THE INTERPLAY BETWEEN PHYSICS AND BIOLOGY THANKS TO A FINITE ELEMENT METHOD-IMPLEMENTED VERTEX ORGANOID MODEL

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Understanding the interplay between biology and physics involved in tissue architecture is a challenging issue, especially when it comes to 3D tissue organization. This requires both an appropriate biological model, allowing multiscale observations from cellular resolution to the overall tissue architecture, and relevant theoretical and computational approaches to generate a synthetic model that is most relevant to the biological model and allows access to the physical constraints experienced by the tissue. Here, using the monolayer organoid of the human colon epithelium as a biological model, and combining a vertex model and the finite element method (FEM), we generated a finite element model of the human colon organoid. Briefly, using an approach combining real-time fluorescence imaging and 2D and 3D image analysis, we extracted biological parameters characterizing the organoid biological model during the establishment of its architecture. Then, the implementation of an 'organoid' vertex model allowed us to observe the limits of this vertex method by comparing the data obtained on the biological organoid and the in silico vertex model. Finally, in order to obtain a more relevant organoid model and to understand the impact of the physics involved in the dynamic morphological changes contributing to the establishment of the colorectal organoid architecture, we generated a new specific FEM model. The analysis of the results obtained then demonstrates how the flexibility of the new FEM model can provide a basis for linking cell shape, tissue deformation and stresses at the cellular level due to imposed constraints. In conclusion, we have demonstrated that the combination of vertex and FEM approaches allows for better modeling of organoid morphology alteration over time and better assessment of the mechanical cues involved in establishing the architecture of the human colon epithelium.

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Keywords: In silico model, Organoid, Finite Element Method

Clinical Trial ID number: NCT 02874365

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3-DIMENSIONAL CELLULAR AND SUBCELLULAR MAP OF IPSC-RPE DURING MATURATION

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The retinal pigment epithelium (RPE) is a monolayer of cells supporting photoreceptors apically and the choroid basally. RPE performs specific functions on each side. These functions require polarization of cellular structures (apical processes, basal infoldings) and organelles (melanosomes, mitochondria etc). Failure to acquire a polarized phenotype leads to retinal degeneration, as seen in some ciliopathies. In this study, we generated a three-dimensional reference map of RPE organelles and cytoskeletal structures during the maturation process using high-content imaging and object segmentation. We used induced pluripotent stem cells (iPSC) reporter lines to derive RPE cells using our tri-phasic differentiation protocol. Each iPSC line is fluorescently tagged to a protein expressed in a specific intra-cellular structure. Over 10,000 images of iPSC-RPE were taken during the five-week maturation phase, with pictures taken every week. A machine-learning algorithm was trained to identify and segment cells and nuclei borders. These were used as a reference to calculate organelle location. Each organelle or structure was segmented using a combination of classic segmentation algorithms, and an imaging expert verified the quality of the segmentation. Three rounds of segmentation with the "human in the loop" were carried out to ensure the accuracy of the results. We calculated the average organelle location and its phenotypical properties. iPSC-RPE cell became more compact horizontally and elongated on the vertical axis during maturation. Nuclei in maturing iPSC-RPE monolayer were more rounded, and nuclear envelop developed invaginations. Matured iPSC-RPE acquired more ER volume while the volume of Golgi bodies remained unchanged. Similarly, lysosomes increased in volume and localized toward the center of the cell, while endosomes didn't appear to change volume or location. In conclusion, we developed a three-dimensional reference map of organelle morphology and distribution during normal RPE development. It can be used to compare the phenotype of degenerating or abnormal RPE in different retinal degeneration to understand the underlying mechanisms of action.

Keywords: iPSC-RPE, 3D cellular structure, apical basal polarisation

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TOWARD OPTOGENETIC CONTROL OF GUT STEM CELL NECROPTOSIS

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Necroptosis is an immunogenic cell death implicated in numerous inflammatory diseases, such as inflammatory bowel diseases

(IBDs). The immunogenicity arises from plasma membrane permeabilization and the production and secretion of proinflammatory cytokines. Recent work reports that necroptosis in gut stem cells induces disruption of epithelial barrier homeostasis and promotes bowel inflammation in mice. We speculate that gut stem cell-niche communication could facilitate the spreading of necroptosis in IBDs. However, the current pharmacological approach cannot precisely control necroptosis with spatiotemporal accuracy. To address this issue, we propose to use our recently developed Light-activatable RIPK3 (La-RIPK3) system to study gut stem cell necroptosis. Receptor Interacting Protein Kinase 3 (RIPK3) has been characterized as an essential protein to induce different types of necroptosis via the formation of an oligomeric protein complex called necrosome. We observed that the blue-light activation of La-RIPK3 induces necroptotic cell death and inflammatory gene production in HT-29 cell, a colonic epithelial cell. Upon successful isolation of crypts and culture of mouse intestinal organoids, we will deliver La-RIPK3 genes using lentivirus with multiple gut stem cell-specific promoters, including LGR5 and VIL1. We aim to understand how the necroptosis of gut stem cell can induce the disruption of epithelial barrier. To ask this question, I will perform real-time imaging of intercellular spreading of necroptosis in intestinal organoid, initiated by precise photo-activation of necroptosis in gut stem cell using La-RIPK3 system. We expect necroptosis of gut stem cells may trigger cell death signaling to neighboring epithelial cells which can degenerate epithelial barrier. Spatiotemporal control of necroptosis using La-RIPK3 promises to offer new insights into the etiology of IBD by controlling necroptosis at the level of individual cells.

Funding Source: Cancer center at Illinois and NIH.

Keywords: stem cell necroptosis, Optogenetics, Inflammatory bowel diseases

TOPIC: GERMLINE AND EARLY EMBRYO

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MICA: A MULTI-OMICS METHOD TO PREDICT GENE REGULATORY NETWORKS IN EARLY HUMAN EMBRYOS

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Recent advances in single cell omics have been transformative in the characterisation of challenging to study biological contexts, including when the source material is precious, such as the early human embryo. Single cell datasets are technically challenging to infer transcription factor-gene regulatory interactions, due to low read-depth leading to zero inflated data. Here we have systematically assessed the application of four different machine learning linear or non-linear gene regulatory network prediction strategies to single cell simulated and human embryo transcriptome datasets. We have also compared how the method of gene expression normalisation impacts on regulatory network predictions. Integrating chromatin accessibility together with transcript expression datasets improved the reproducibility of the predicted gene regulatory networks. We found that the application of a non-linear network prediction method based on mutual information (MI) to single cell transcriptome datasets refined with chromatin accessibility (CA) (called MICA), exhibited higher reproducibility compared to the alternative network interference methods tested. Moreover, MICA was used to make predictions about GRNs in the preimplantation human embryo, which were supported by previous findings in other developmental and stem cell contexts. Based on the gene regulatory networks predicted by MICA, we discovered co-localisation of the AP-1 transcription factor subunit proto-oncogene JUND and the TFAP2C transcription factor AP-2g in human preimplantation embryos. Overall, our comparative analysis of gene regulatory network prediction methods defines a pipeline that can be applied to single-cell multi-omics datasets to infer interactions between transcription factor expression and target gene regulation, which can then be functionally tested.

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Keywords: Gene regulatory network inference, Multi-omics, Human Pre-implantation Embryo

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PARP INHIBITION OF HUMAN NAÏVE STEM CELLS AWAKENS A DUX4-ACTIVATED EXPRESSION OF 8-CELL-SPECIFIC PIONEER FACTORS THAT REGULATE ZYGOTIC GENOME ACTIVATION

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Culture of primed human pluripotent stem cells (hPSCs) with LIF-2i supplemented with the promiscuous poly-ADP-ribose-polymerase (PARP)/tankyrase (TNKS) inhibitor XAV939 generates TNKS/PARP inhibitor-regulated naïve (TIRN) human stem cells

with significantly improved functional pluripotency. Here, we show that TIRN reversion of primed hPSC awakens a DUX4-mediated functional zygotic genome activation (ZGA)/cleavage stage program. We performed multi-omic (bulk/single-cell RNA-seq), epigenomic (ChIP-seq; PARP1, NANOG, SOX2, POU5F1, phospho-STAT3, H3K4me3, H3K27me3, H3K27ac, BRD4), whole proteome (Tandem Mass Tag mass spectrometry) and ubiquitinome studies on isogenic primed vs TIRN-reverted hPSC. TIRN-reverted hPSCs stably expressed protein for the ZGA-activating pioneer factor DUX4. Non-specific TNKS/PARP1 inhibition stabilized both transcript and protein expressions of pioneer factors that are known to regulate not only naïve pluripotent stem cell self-renewal (e.g., NANOG, TFAP2C), but also ZGA-associated pioneer factors that are normally expressed exclusively during the human 8-cell and cleavage embryonic stages (e.g., DUX4, TPRXL, HOX/GATA factors). To determine the effects of graded protein expression levels of DUX4, we analyzed proteomic and transcriptomic profiles of primed vs TIRN hPSCs with inducible transgenic DUX4. This multi-omic design revealed that non-specific TNKS/PARP1 inhibition reprogrammed the transcriptome, epigenome, and PAR-dependent proteome of human TIRN stem cells, including epigenetic reprogramming of enhancer regions with PARP1/NANOG/OCT4/SOX2 binding at pioneer factor loci (e.g., HOX/FOX/GATA/PRD-like). Single-cell RNA-seq expression profiles of DUX4-expressing TIRN-hPSCs were mapped against published human embryo reference datasets and revealed cleavage and totipotent 8C stage-like TIRN subpopulations that co-expressed NANOG along with ZGA-specific pioneer factors (e.g., DUXA, TPRXL, GATA6). Finally, TIRN-hPSCs efficiently contributed human embryonic and extra-embryonic lineages following injection into murine embryos. Further chemical reprogramming of the PARylome and post-translational proteome of naïve hPSC may facilitate derivation of improved human stem cells for regenerative medicine.

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Keywords: totipotency, embryonic development, PARP

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KNOCK-IN GENOME ENGINEERING AT NOVEL GENOMIC SAFE HARBOR SITES IN PLURIPOTENT STEM CELL AND ANIMAL MODELS

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Knock-in genome engineering refers to the process of precise and targeted insertion of DNA fragments or payloads and is a powerful reverse genetics tool for manipulating the genomes of human pluripotent stem cells and biomedical animal models. A handful of genomic safe harbor sites are widely used for the integration of large DNA fragments, often several to tens of kilobases in size, that may contain regulatory elements such as enhancers, promoters or synthetic transgene circuits, or may contain genetic modifications that recreate a disease phenotype. Overall, the choice of safe harbor site for generating cellular and animal models will depend on the experimental criteria, but to qualify as an authentic safe harbor, the integration site should yield minimal disruption to the neighboring and endogenous gene expression patterns; and importantly, any disruption should not lead to genotoxic stress, abnormal cellular functions, developmental abnormalities, and disease. The most common safe harbors include: ROSA26, AAVS1, COL1A1, H11 and CCR5. Recently, Aznauryan et al, 2022 discovered two novel regions of gene insertion, eponymously named Rogi1 and Rogi2, that are safe harbors on human chromosomes 1 and 3, respectively. Using bioinformatics, we discover and map new Rogi1 orthologous safe harbor sites in both mice and swine. Additionally, we develop genome engineering reagents such as CRISPR-Cas9 high-performing sgRNAs and PASTEv3 editing with integrases Bxb1 and Bacillus cereus (BceINTa) suitable for the insertion of the large DNA payloads at Rogi1. Using pluripotent stem cells from mice including embryonic stem (ES) and iPS cells, and porcine expanded potential stem cells (EPSCs) we demonstrate high-level and ubiquitous expression at Rogi1 safe harbor using a CAG promoter driven StayGold, a recently discovered highly photostable and bright green fluorescent protein (GFP) derived from the jellyfish Cytaeis uchidae. We have microinjected the StayGold genetic reporter into both mouse and pig zygotes to monitor transgene expression in whole tissues and validate Rogi1 as a safe harbor in both small and large biomedical animal models. Knock-in genome engineering at Rogi1 has broad applications in fields such as biotechnology, agriculture, and medicine.

Keywords: genome engineering, genomic safe harbor, biomedical swine model

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GENERATION OF HEAD-AND-TRUNK EMBRYO-LIKE STRUCTURES USING MICROFLUIDIC DROPLETS

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The formation of organoids recapitulates the processes of self-organization, symmetry breaking and tissue patterning that occur during the embryonic development. These morphogenetic events are driven by the creation of specific niches promoting the spatially controlled differentiation into specialized cell types. The biochemical confinement of differentiating PSCs into oil-isolated droplets has the potential to regulate the autocrine signaling that triggers tissue patterning during differentiation, which has not been demonstrated so far. Here, I will first present a new droplet microfluidic platform that sustains the long-term culture of mouse embryonic stem cells (mESCs) at the undifferentiated state using reduced culture volumes. In addition, the derivation of embryoid bodies in droplets biased their fate toward the ectoderm, while it preserves their capacity for primitive streak formation. Moreover, I found that the culture of mESCs into anchored microfluidic droplets enables the maturation of gastruloids and embryonic-like structures (ELSs), a type of organoids that recapitulate the early steps of the embryonic development. I show that ELSs display a unique head-trunk structure, which demonstrates high degree of similarity with the stage E8.5 of the mouse embryonic development (i.e. gene expression and structural organization, such as patterned somites and brain-like structures). The process of generation of ELSs using droplet microfluidics proved high degree of reproducibility, with more than 75% of generated structures displaying a head-and-trunck structures. As such, the differentiation of PSCs into microfluidics droplets provides a novel approach

towards the derivation of more functional organoids, in view of tissue engineering and disease modeling applications.

Keywords: Embryo-like structures, Gastruloids, Droplet micro fluidics

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HIGHLY-COOPERATIVE CHIMERIC SUPER-SOX ENABLES COMPLETE DEVELOPMENTAL RESET ACROSS SPECIES

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The discovery of induced pluripotent stem cell (iPSC) technology by Shinya Yamanaka has truly enabled the stem cell field. However, 17 years later our understanding of pluripotency remains limited: iPSC technology is inaccessible for many valuable species, iPSC lines exhibit variable developmental potential, and germline transmission has only been demonstrated for mouse and rat. Here, we found that Sox2 could be enhanced by key structural elements of the endoderm-specific Sox factor Sox17. By swapping multiple beneficial elements of Sox17 into Sox2, we built a chimeric super-SOX factor—Sox2-17—that improved iPSC generation for five species: mouse, human, cynomolgus macaque, cow, and pig. Sox2-17 enhanced five-, four, and three-factor reprogramming efficiency up to hundreds of times and enabled two-factor human iPSC production. We confirmed the superior performance of super-SOX using viral, episomal, and RNA delivery methods. A single residue swap in the DNA binding domain of Sox2 led to the most significant gain-of-function: A61→V, which stabilizes Sox/Oct heterodimer on DNA through hydrophobic interactions between the Sox and Oct molecules. Sox2AV alone enabled induction of pluripotency by functionally deficient Oct4 mutants and even by tissue-specific Oct factors, such as Oct2 and Oct6. Importantly, Sox2AV boosted the developmental potential of OSKM iPSCs, as measured by the ability to support normal development of all-iPSC mice in tetraploid complementation assay. We concluded that stabilizing Sox2/Oct4 dimerization leads to a more complete developmental reset. Correspondingly, mouse naïve pluripotent stem cells, which represent the pre-implantation epiblast and have exceptionally high developmental potential, exhibit high levels of Sox2/Oct4 dimerization, whereas heterodimerization in primed cells declines; along wanes the ability of primed cells to contribute to animal development. A drop in Sox2 levels along the naïve-to-primed continuum results in low Sox2/Oct4 co-binding in primed cells, while Oct4 levels remain the same. Transient overexpression of super-SOX and KLF4 could restore the dimerization and induce naïveté, providing a powerful technology to reset pluripotent cells in different species.

Keywords: Sox2/Oct4 heterodimer model of pluripotency, enhanced generation of human, mouse, NHP, bovine, porcine iPSCs, primed to naïve reset

TOPIC: HEMATOPOIETIC, IMMUNE, AND ENDOTHELIAL

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OFF-THE-SHELF THIRD-PARTY HSC-ENGINEERED INKT CELLS FOR AMELIORATING GVHD WHILE PRESERVING GVL EFFECT IN THE TREATMENT OF BLOOD CANCERS

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Allo-HSCT is a curative therapy for hematologic malignancies owing to GvL effect mediated by alloreactive T cells; however, the same T cells also mediate GvHD, a severe side effect limiting the widespread application of allo-HSCT in clinics. Invariant natural killer T (iNKT) cells can ameliorate GvHD while preserving GvL effect, but the clinical application of these cells is restricted by their scarcity. Here, we report the successful generation of third-party HSC-engineered human iNKT (3rdHSC-iNKT) cells using a method combining HSC gene engineering and in vitro HSC differentiation. The 3rdHSC-iNKT cells closely resembled the CD4 CD8 /+ subsets of endogenous human iNKT cells in phenotype and function- ality. These cells displayed potent anti-GvHD functions by eliminating antigen- presenting myeloid cells in vitro and in xenograft models without negatively impacting tumor eradication by allogeneic T cells in preclinical models of lym- phoma and leukemia, supporting 3rdHSC-iNKT cells as a promising off-the-shelf cell therapy candidate for GvHD prophylaxis.

Keywords: Invariant nature killer T (iNKT) cells, Allogeneic hematopoietic stem cell transplantation (allo-HSCT), Graft-versus-host disease (GvHD)

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IN VIVO TIME-COURSE AND TISSUE-WIDE GENE PROFILING REVEALS DISTINCT SIGNATURES OF CAR-T AND CAR-INKT CELL RESPONSES TO SOLID TUMORS

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CAR-T cell therapy has shown success in treating blood cancers but only limited efficacy against solid tumors. Lack of knowledge of in vivo behaviors of CAR-T cells, especially in the solid tumor microenvironments, is considered as a major hinder hampering the application of CAR-T cell therapy for treating solid tumors. Invariant natural killer T (iNKT) cells, a type of unconventional T cells, are gaining interest as an alternative to traditional CAR-T cell therapy due to their tumor killing capacity, solid tumor homing ability, as well as their potential for developing “off-the-shelf” cell therapy. iNKT cells exist in low numbers in health donor blood, that limits their clinical applications. To overcome this limitation, our lab has developed a technology to produce therapeutical levels of CAR-iNKT cells through a stem-cell engineering and iNKT cell culture strategy. Here, we compare the in vivo behaviors of conventional CAR-T cells with the CAR-iNKT cells against solid tumors. Mesothelin targeting CAR-T and CAR-iNKT cells were administered into NSG mice bearing OVCAR3 human ovarian tumor xenograft. Overtimes, tissues were collected from tumor site and other sites (peripheral blood, spleen and liver); single cells were isolated and subjected to single-cell RNA sequencing analysis. Our results revealed several findings. 1) Across all tissues examined, both CAR-T and CAR-iNKT cells exhibited dynamic gene profiling changes, starting by acquiring an effector/proliferating signature, and gradually transition to progenitor/terminally exhausted gene profiles. 2) In contrast to CAR-T cells, CAR-iNKT cells displayed enhanced gene profiles that correlated with their stronger in vivo anti-tumor efficacy. 3) CAR-T cells induced immune evasion in tumor site—loss of antigens, while CAR-iNKT cells did not; likely due to their multi-targeting mechanism. 4) CAR-T cells triggered an immunosuppressive and cytokine release syndrome (CRS)-prone TEM while CAR-iNKT cells actively altered TME and reduced CRS gene signatures by eliminating tumor associated macrophages. Our result provides the first-time insight into the in vivo spatial and temporal behaviors of CAR-T and CAR-iNKT cells. Such knowledge will be valuable for selecting proper cell therapy products, and further improving these cell therapies for solid tumors.

Keywords: gene profiles, CAR-T cell therapy, in vivo behaviors

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DISSECTING THE HETEROGENEITY OF EX VIVO EXPANDED MOUSE HEMATOPOIETIC STEM CELLS WITH MIRNA SWITCHES

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The rarity and scarcity of hematopoietic stem cells (HSC) hinder progress in HSC research. Despite recent progress in expansion and long-term maintenance of HSCs ex vivo, the expanded cells remain functionally heterogeneous even after sorting for immunophenotypically-defined stem cell subtypes. We have previously developed a miRNA-based cell type identification method, called miRNA switch, and achieved higher enrichment of target cells than traditional surface marker-based approaches. Here, we illustrate the identification of functional HSCs using synthetic miRNA switches. We first introduced a miRNA switch that responds to miR-126a-3p, a highly-expressed miRNA in HSCs, into ex vivo expanded mouse HSCs. We observed heterogeneity in miR-126a-3p activity in these ex vivo expanded cells and enrichment of immunophenotypic c-Kit+Sca-1+Lineage- (KSL) cells in the miR-126a-3p+ population. The miR-126a-3p activity was heterogeneous even when we analyzed the surface marker-defined CD201+KSL fraction, hinting at the possibility that this fraction can be further divided into subfractions based on miR-126a-3p activity. To compare the in vivo repopulation potential of miR-126a-3p-defined subsets, we competitively transplanted miR-126a-3p+ and miR-126a-3p- cells. The miR-126a-3p+ cells showed higher donor chimerism in peripheral blood than the miR-126a-3p- cells, indicating that the miR-126a-3p+ fraction was enriched with engraftable cells. Furthermore, miR-126a-3p activity correlated with in vivo repopulation potential. Finally, to gain further insights into the miRNA signature that may mark functional HSCs, we performed small RNA sequencing and identified differentially regulated miRNAs in immunophenotypic HSCs. Several miRNAs showed heterogeneity in the miR-126a-3p-defined fraction, suggesting the possibility of further subclassification of HSCs based on multiple miRNA activities. Altogether, we have demonstrated that miRNA switches can be used to dissect the heterogeneity of hematopoietic stem cells. miRNA switches will enable high-resolution identification of functional HSCs after ex vivo culture.

Funding Source: This work was supported by the Japan Society for Promotion of Science (JSPS) KAKENHI (grant numbers JP19J21199 to H.O. and JP22K20628 to H.O.), Nippon Boehringer Ingelheim Co., Ltd., and the iPS Cell Research Fund.

Keywords: Hematopoietic Stem Cell, miRNA, Synthetic Biology

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A NOVEL, NON-VIRAL, TARGETED GENOME ENGINEERING APPROACH FOR IPSC-DERIVED CAR-NK CELL IMMUNOTHERAPY

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Advances in CAR-NK cell therapy presents a promising next generation immune-cell therapy, due to its allogeneic nature, potent anti-tumor activity, and reduced cytokine-release syndrome and neurotoxicity. Yet, obstacles such as expansion and inadequate solid tumor penetration confine the applications of CAR-modified blood-derived primary NK therapy. Developments in iPSC engineering have facilitated iPSC-derived CAR-NK cell production, presenting a solution for challenges yet to be overcome in CAR-NK therapy, such as improved gene modification, scalable production, thus providing a safer, more effective “off the shelf” therapy for cancer patients. Despite this, current viral and non-viral genome engineering approaches for iPSCs are limited by payload size, scalability, and relatively low genome integration efficiency and increased cytotoxicity, thus restricting their therapeutic applications. Here, we developed a novel, non-viral technology comprised of circular single stranded DNA (cssDNA) as a precise gene writing tool to engineer CAR-expressing iPSC-derived functional CAR-NK cells. With our proprietary gene writing platform, we engineered iPSCs having specific integration of our in-house CD19 CAR construct into a genomic safe harbor, which were further clonally selected and expanded before differentiation. Through an optimized differentiation and expansion process, we phenotypically characterized the iPSC-derived CAR-NK cells by surface marker detection (defined as CD45+CD56+CD3-). Cell killing assays confirmed the functionality of these iPSC-derived CAR-NK cells, validating that our cssDNA-mediated genome editing can effectively engineer iPSCs which can be differentiated into functional CAR-NK cells showing similar anti-tumor activity with primary NK cells. As a result, our method to differentiate CAR-NK cells from engineered iPSCs offers great potential to satisfy a therapeutic need in cancer cell therapy. With the increased ease of scalability, lower cost, and targeted integration, our cssDNA-mediated gene writing tool showcases the ability to pave the way for enhanced iPSC engineering and CAR-NK cell therapy.

Keywords: genome engineering, cell gene therapy, iPSC derived NK

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TYPE 1 INTERFERON PERTURBATES CLONAL COMPETITION BY RESHAPING HUMAN BLOOD DEVELOPMENT

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Inflammation perturbs the evolutionary dynamics of hematopoietic stem cell (HSC) clones in clonal hematopoiesis and myeloid neoplasms. To decipher the downstream effects of inflammatory cytokines on clonal stem cell fitness, we studied HSCs and progenitors from patients with CALR-mutated myeloproliferative neoplasm (MPN), at baseline and following interferon-alpha (IFNa) treatment, the only MPN therapy to deplete clonal stem cells. As the mutated cells are admixed with the wildtype without cell sur-

face markers to isolate them, we leveraged single-cell multi-omics methods that integrate somatic genotyping with whole transcriptomes, immunophenotyping and chromatin accessibility across thousands of CD34+ cells. IFNa simultaneously activated HSCs into two polarized states, a lymphoid progenitor expansion associated with an anti-inflammatory state and an IFNa-specific inflammatory granulocytic progenitor (IGP) state derived directly from HSCs. The augmented lymphoid differentiation balanced the typical MPN-induced myeloid bias, thereby normalizing blood counts. The lymphoid differentiation was constrained in the mutated HSCs due to the CALR-mutation induced myeloid bias. Clonal fitness upon IFNa exposure was due to resistance of clonal stem cells to differentiate into IGPs. These results indicate that IFNa remodels the hematopoietic differentiation landscape to effect hematologic remission. The data also support a paradigm wherein inflammation perturbs clonal dynamics by HSC induction into an alternate IFNa-specific IGP differentiation program.

Funding Source: National Institutes of Health Director's Early Independence Award (DP5 OD029619-01) Starr Cancer Consortium Burroughs Wellcome Fund Career Award for Medical Scientists

Keywords: Type 1 interferon, Myeloproliferative neoplasm, Clonal evolution

Clinical Trial ID number: NCT01259817, NCT01258856

TOPIC: KIDNEY

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MATURATION OF KIDNEY ORGANOIDS IN THE MICROWELL-MESH FOR NEPHROTOXICITY SCREENING

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Organoids are three-dimensional (3D) cell aggregates, and these 3D tissues have become preferred cell culture tools, relative to monolayer cell culture, for numerous applications. Cell organization in organoids can be sophisticated, often yielding structures and function reminiscent of native tissue. Organoids can be used to study tissue physiology, in drug discovery and screening, or in some instances they may be suitable building blocks for use in tissue repair. Because the complex structures that evolve within organoids are guided by morphogenetic fields, consistent organoid size and shape contribute to a more reproducible organoid product. The goal of this work was to generate kidney organoids for in vitro functional assays and potentially for organ repair. Most kidney organoid generation protocols utilize transwells, bioreactors, or spinner flasks, but these techniques can fail to reproducibly produce uniformly sized organoids. Herein we used a microwell platform, the Microwell-mesh, to facilitate mass production of uniformly sized kidney organoids. The Microwell-mesh consists of a polydimethylsiloxane (PDMS) insert that fits into a cell culture well. This insert has patterned microwell surface, with a nylon mesh bonded over the microwell openings. The mesh pore size, 45 mm, is large enough to allow single cells to be centrifuged through the mesh and into the microwells. However, once the cells aggregate into an organoid, they become too large to escape back through the mesh. This feature retains organoids in microwells over the complex culture period required for kidney organoid differentiation. Kidney organoids cultured in the Microwell-mesh showed higher expression of markers related to proximal and distal tubule (GATA3 and LRP2), collecting duct (CALB1), and epithelial cells (ECAD and CDH1) compared to organoids cultured in suspension. Organoids were treated with well-known nephrotoxin, cisplatin, in 3 different concentrations. Then, the viability of kidney organoids was measured through a metabolism assay, Live/Dead staining, and LDH assay. The expression levels of kidney injury markers (KIM-1, IL-18, and caspase-3) were also compared against referenced in vivo results. These data demonstrate the utility of this culture approach for generating kidney organoids.

Keywords: kidney organoid, microwell, drug screening

TOPIC: MESENCHYMAL STROMAL CELLS, ADIPOSE, AND CONNECTIVE TISSUE

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OCULAR INSTILLATION OF THE CONDITIONED MEDIUM DERIVED FROM HUMAN MESENCHYMAL STEM CELLS IS EFFECTIVE FOR DRY EYE SYNDROME VIA IMPROVEMENT OF CORNEAL BARRIER FUNCTION

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Dry eye syndrome (DES) is induced epithelial damage to the cornea by decreased tear production or tear quality. In particular, severe DES such as Sjogren's syndrome has not been established adequate treatments due to complicated pathological conditions. To solve this problem, we focused on the conditioned medium of

human adipose-derived mesenchymal stem cells (hAdMSCs-CM), which had multiple therapeutic properties. In this study, we evaluated the effects of hAdMSCs-CM for complex pathologies of DES such as cytotoxicity, inflammation, and barrier dysfunction of human corneal epithelial cells (hCECs) and we investigated the efficacy of ocular instillation of hAdMSCs-CM in vivo DES model rats with exorbital and intraorbital lacrimal gland excision (LGs-Ex rats). Firstly, hAdMSCs were expanded to 80% confluent, then the medium was changed to the culture medium for hCECs. After 72 h culture, hAdMSCs-CM were collected, and which improved Benzalkonium Chloride (BAC)-induced cytotoxicity, inflammation, and barrier dysfunction of hCECs. Also, RNA-seq analysis indicated that the effects of hAdMSCs-CM were associated with Transforming growth factor beta (TGFβ) and Janus kinase/signal transducer and activator of transcription (JAK-STAT) signaling pathway. Moreover, ocular instillation of hAdMSCs-CM suppressed epithelial damage to the cornea by improved barrier dysfunction of the cornea in LGs-Ex Rats. Thus, we demonstrated that hAdMSCs-CM had multiple therapeutic properties which were associated with TGFβ and JAK-STAT signaling pathway, and ocular instillation of hAdMSCs-CM could be an innovative therapeutic agent for DES via improvement of corneal barrier function.

Funding Source: The project for the Osaka City Innovation Support Grant.

Keywords: Mesenchymal stem cells, Dry eye syndrome, Conditioned medium

897

SIMPLICON SELF-REPLICATIVE RNAS FOR REVERSIBLE IMMORTALIZATION OF PRIMARY CELLS WITH KEEPING THE PRIMARY CELL INTEGRITY, MOUSE IPSC GENERATION AND GENOME EDITING

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We previously developed a self-replicative RNA system for generating safer human induced pluripotent stem cells (iPSCs). Simplicon is a positive sense, synthetic, polycistronic, self-replicating, single-stranded RNA that mimics cellular mRNA. Here we show significant improvements on the Simplicon system as well as exciting, new applications in footprint-free cell engineering. Simplicon is an RNA-based expression system, so there is no risk of genomic integration or damage to the host cell genome. Continued expression of Simplicon RNA requires interferon suppressor(s) because the introduction and replication of Simplicon RNA induce the type I interferon response. The improved Simplicon system uses vaccinia virus proteins, B18R and E3L, and a JAK kinase inhibitor, Ruxolitinib. Combining these reagents strongly suppressed the interferon response and significantly improved the robustness of the Simplicon system. These improvements allowed us to use the Simplicon RNA in a variety of novel applications such as reversible primary cell immortalization, mouse iPSC generation, human iPSC generation from biopsies, and Cas9 genome editing. The Simplicon immortalization RNA containing hTert and/or E6E7 can successfully expand human primary cells such as fibroblasts, mesenchymal stem cells (MSCs), adipose stem cells, cardiomyocytes and keratinocytes. Of note, Simplicon RNA is removable, so phenotypic change such as the decrease of markers of MSCs caused by oncogenic E6E7 can be reverted after the removal of Simplicon RNA. Moreover, we can generate iPSCs without losing the efficiency after 10 passaging of HFFs. These data strongly suggest that Simplicon immortalization RNAs can expand primary cells without losing the primary cell integrity. Lastly, the improvement of Simplicon system enabled to make mouse iPSCs and Cas9 genome editing as same as Lentivirus Cas9. The updated Simplicon platform enables immediate, robust, sustained, and yet tunable expression of multiple genes after a single transfection step without any risk of plasmid DNA/virus-induced toxicity and genomic integration or any need for viral clearance. These features make Simplicon a uniquely safe and versatile protein expression system with tremendous potential for use in cell engineering for regenerative medicine.

Keywords: Reversible Immortalization of primary cells, saRNA/srRNA, Genome editing/iPSC generation

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TUNING ADSC ADIPOGENIC DIFFERENTIATION BY REAC TECHNOLOGY: INVOLVEMENT OF CELLULAR ENDOGENOUS BIOELECTRICAL ACTIVITY

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Adipose-derived stem cells (ADSCs) represent an ideal stem cell population for regenerative medicine. ADSC adipogenic differentiation is controlled by the activation of a specific transcriptional program, which includes epigenetic factors and key adipogenic genes. Several chemical stimuli can be used to counteract lipid accumulation and regulate adipogenesis, pushing toward an intermediate phenotype, as beige adipocytes. The present work aimed at evaluating ADSC adipogenic differentiation in the presence of a specific adipogenic conditioned medium and manipulation of the cellular endogenous bioelectrical activity (EBA) through a metabolic optimization treatment (MOT) by the Radio Electric Asymmetric Conveyer (REAC) technology, in the attempt to modulate stem cell differentiation toward an intermediate phenotype between white and brown adipocytes, known as beige. In this study, the EBA manipulation by REAC MOT, in the presence of an adipogenic medium, induced significant changes in the expression of stemness-associated genes, Oct-4, Sox2 and Nanog, and specific adipogenic genes, as PPAR-γ, aP2, LPL and ACOT2. We also analyzed the gene expression of the thermogenic protein UCP1 and the expression of the main markers of white, brown, and beige phenotype, Asc-1, PAT2 and TMEM26 by confocal microscopy. Our results clearly demonstrate a role of REAC MOT in inducing the appearance of a beige phenotype, as inferred by the detection of the expression of specific genes as well as the main antigenic markers of beige phenotype. This in vitro experimental approach could represent a potential therapeu-

tic strategy to counteract the accumulation of adipose deposits, obesity, and related metabolic disorders.

Keywords: Adipogenic differentiation, REAC technology, Cellular mechanisms

TOPIC: MUSCULOSKELETAL

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GENE REGULATORY NETWORKS GOVERNING MULTIPOTENCY OF MURINE BONE MARROW STROMAL CELLS

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Bone marrow harbors a multitude of cell types, including stromal cells that can undergo clonal expansion in vitro and osteogenic, adipogenic, and chondrogenic differentiation in vitro and in vivo. However, we currently lack a detailed map of how some bone marrow stromal cells (BMSCs), which typically are expanded in vitro prior to their use, self-renew and maintain their multipotency. Recent scRNAseq atlases of murine BMSCs have highlighted their heterogeneity and the absence of a single, distinctive population that presents signatures of multipotency and self-renewal. We hypothesized that chromatin accessibility to trilineage transcriptional programs distinguishes multipotent BMSCs from other, less potent bone marrow cell populations. To test this, we first determined the chromatin and transcriptional circuits associated with in vitro trilineage differentiation of murine BMSCs using scMultiomics and computational inference of regulatory networks. Then, we subjected BMSCs from day 0 to day 3 of isolation to scMultiomics to see if a native BMSC population exhibited trilineage capacity defined at the chromatin accessibility level and how it evolved over the course of expansion in vitro. Day 0 BMSCs consisted mainly of Cxcl12 abundant reticular (CAR) cells and two distinct populations of osteoprogenitors (OSPs). In contrast to our hypothesis, over three days in culture, BMSCs collapsed into a single, OSP-fibroblast-like state predicted to have a greater lineage potential both at the transcriptional and the chromatin accessibility levels. Our network analyses predict that subsets of BMSCs in vivo are biased towards their respective lineages as determined by regulon priming of the lineage specifiers Runx2, Mef2c, and Pparg, and that self-renewal of BMSCs subsets is regulated by Foxs1, Smarcc1, and Nfic. Prospectively testing these and other predictions from our data will yield incisive glimpses into BMSC biology.

Funding Source: This work was supported by funds from NIH/NIGMS R35GM124725 and a Johns Hopkins Catalyst Award.

Keywords: Bone Marrow Stromal Cell, Single-cell Multiomics, Gene Regulatory Network

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3'-SIALYLLACTOSE PROMOTES CHONDROGENIC DIFFERENTIATION OF HUMAN BONE MARROW-DERIVED MESENCHYMAL STEM CELLS

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Cell-based therapies have been developed to restore cartilage damage and prevent cartilage degeneration. Especially, human bone marrow-derived mesenchymal stem cells (hBMSCs) have self-renewal capacity and the potential for multi-lineage differentiation. However, loss of self-renewal and multi-lineage differentiation potential occurs in long-term in vitro cultivation. Therefore, new techniques require to preserve hBMSCs multipotency after long-term expansion. 3′-Sialyllactose, a natural compound, is present in human milk and exhibits anti-inflammatory properties and modulates immune homeostasis. In this study, we elucidate the potential therapeutic effects of 3'-Sialyllactose on hBMSCs. Continuously treated with 3'-Siallylactose on hBMSCs, the stemness capacity was significantly increased and senescence was significantly decreased compared to those of untreated hBMSCs. Next, to induce chondrogenesis on hBMSCs, 3'-Siallylactose was treated continuously with chondrogenesis differentiation medium, and micromass were conducted. The expression levels of chondrogenic markers were significantly increased and the expression levels of hypertrophic markers were significantly decreased in 3'-Siallylactose treated hBMSCs. Taken together, these effects suggest that 3'-Siallylactose is considered as a natural therapeutic agent for clinical application.

Funding Source: This study was supported by the NRF-2022R1I1A1A01065666 and 2021R1F1A1062829, the Korea Health Industry Development Institute (HI21C1314 and HI22C1588), the Korean Fund for Regenerative Medicine grant (21A0202L1 and 21C0715L1).

Keywords: Human bone marrow-derived mesenchymal stem cells, 3'-Siallylactose, Chondrogenesis

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POOLED SCREENING TO IDENTIFY NEURONAL MITOCHONDRIAL PHENOTYPES IN IPSC-DERIVED MOTOR NEURONS

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While induced pluripotent stem cell (iPSC) research has been widely adopted, genome engineering studies on differentiated single cells are still challenging. Similarly, single cell morphological quantification on stem cell derived neurons is limited to low throughput experiments. Mitochondrial fusion abnormalities in neurons have been widely observed in neurodegenerative diseases. The distal ends of the neurons are highly damage-prone due to energy demanding cellular processes leading to axon degeneration and inflammation. Hence, it is vital to study distally localized mitochondria in neurons. Our aim is to perform pooled image-based screening with a gRNA library against genes which regulate mitochondria in iPSC- derived motor neurons using Raft-Seq technology to observe mitochondrial morphology in a high-throughput setting. The Raft-Seq platform enables visualizing individual single neurons and their mitochondria and then physically capturing and genotyping neurons of interest. For this, we differentiated numerous disease-derived iPSC cell lines into motor neurons. Quantification of neuronal markers through different stages of differentiation process was performed via immunostaining. We profiled mutant iPSC cell lines which were edited using CRISPR to knock out MFN2, OPTN, and FUS genes. Machine learning models were applied to differentiate between wild type and mutant cells. These models were evaluated to have AUCs greater than 0.8. Using this technology, we aim to discover pathogenic genetic mutations in iPSC-derived neurons that produce a mitochondrial phenotype observed in patients with neurodegenerative diseases.

Keywords: Mitochondria, Neurons, Neurodegeneration

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GENERATING A PERFUSABLE VASCULARIZED BRAIN ORGANOID MODEL USING MICROFLUIDICS AND PHOTODEGRADABLE POLYMER SCAFFOLDS

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Although brain organoids (BOs) have advanced our understanding of the human brain, their growth, cellular complexity, tissue organization, and functionality are limited by the absence of a functional vasculature. Current organoid vascularization efforts rely on either: (1) the in situ formation of neo-vessels in endothelial cell (EC) co-cultures, or (2) microfluidics channels that flow adjacent to organoids. We designed a system combining these two strategies by incorporating BOs with in situ generated neo-vessels into a microfluidic device containing tissue engineered blood vessels (TEBVs). Upon exposure to EC-activating shear stresses, angiogenic sprouts connect TEBVs to neo-vessels, generating a perfusable vascular network. To fabricate this system, we first developed a microfluidic device composed of a chamber that is gated by pillars to restrict BO motility. Physics calculations and computational modeling were used to determine the flow profile within the microfluidics channels and flow rates necessary to produce physiologically-relevant wall shear stresses. The device also contains posts onto which a biocompatible photodegradable fiber scaffold is woven in a square lattice resembling the cerebral vascular network and embedded into a fibrin hydrogel matrix with BOs. Photodegradable fibers are formed by extruding and crosslinking a solution of two multi-arm polyethylene glycol (PEG) components which contain allyl sulfide to facilitate degradation via radical addition. Within 5 minutes of low-intensity UV irradiation, the PEG fibers are solubilized, leaving a continuous, hollow framework to be seeded with ECs and form TEBVs in the hydrogel-BO matrix. We hypothesize that long-term perfusion of vascularized BOs will reduce vessel permeability, reduce neural cell death, promote the formation of more complex and organized cerebral structures, and increase neuronal maturity. This vascularized BO model will generate opportunities to study neurovascular development, brain cancers, or other neurological conditions affecting vasculature using a completely human and in vitro system.

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Keywords: tissue engineering, neurovascular modeling, microfluidics

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FUNCTIONAL CHARACTERIZATION OF A HUMAN PLURIPOTENT STEM CELL-DERIVED CEREBELLAR ORGANOID MODEL OF RNA EXOSOME-LINKED PONTOCEREBELLAR HYPOPLASIA TYPE 1B

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Converging evidence indicates that altered RNA processing is a key pathophysiological component causing several neurological disorders. Recessive missense mutations in the EXOSC3 gene, which encodes a subunit of a key RNA processing factor, the RNA exosome, cause Pontocerebellar Hypoplasia Type 1b (PCH1b). PCH1b is a neurodevelopmental disorder characterized by atrophy of cerebellum and brainstem structures. The goal of this study is to provide insight into how EXOSC3 variants cause cerebellar pathology in PCH1b. To achieve this goal, we developed a 3D hiPSC-derived cerebellar organoid model of PCH1b. Thus, we edited hiPSCs via CRISPR technology to model PCH1b variants. Here, we provide functional characterization of hiPSC-derived cerebellar organoids modeling PCH1b by performing electrophysiological and functional imaging studies. We employed Multi-Electrode Array (MEA) to understand the function of neuronal networks. In addition, we employed live functional imaging, Fluorescent Lifetime Imaging Microscopy (FLIM), to provide insight into metabolic function during maturation of organoids. Preliminary data from MEA functional network recordings on organoids modeling PCH1b reveal increased neuronal firing and burst frequency and duration as compared to wildtype. Next, we employed FLIM to detect changes in metabolic activity in PCH1b-edited organoids compared to wildtype. Phasor analysis of FLIM images showed regions with higher bound/free NADH ratios in wildtype organoids, which correlates with greater glycolytic activity, as compared to organoids modeling PCH1b. Inversely, organoids modeling PCH1b show regions with lower bound/free NADH ratios, which correlates with greater oxidative phosphorylation, as compared to wildtype. Therefore, we show both increased neuronal activity and oxidative phosphorylation, an indicator of differentiation, in organoids modeling PCH1b as compared to wildtype. Our results suggest an accelerated differentiation of PCH1b-edited hiPSCs into cerebellar lineages as compared to wildtype cerebellar organoids. Taken together, these data provide evidence that the cerebellar organoid platform can be used to provide insight into how RNA exosome-linked PCH1b variants affect both neuronal activity and metabolic homeostasis during cerebellar development.

Keywords: Live Imaging, Organoid, Neuronal Development

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PARAMETERIZATION OF NEURONAL ACTION POTENTIAL WAVEFORM IN HIGH-DENSITY MEA AND PATCH CLAMP RECORDINGS OF CONTROL AND AUTISTIC-PATIENT DERIVED HUMAN CORTICAL ORGANOIDS

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Neuronal action potentials (APs) are typically thought of as binary -"all-or-nothing"- events, with analyses of neural data commonly focusing on the number and timing of spike events. However, a considerable body of evidence suggests that AP waveforms recorded intracellularly and extracellularly exhibit systematic, within-neuron variation that may contribute additional physiological information beyond the rate or timing of discharge. We argue that this within-neuron variance in AP waveform shape contains meaningful neural information that is often ignored in the interpretation of classical results in cognitive and systems neuroscience, and is consequently worth exploring. To this end, our lab has developed an AP shape parameterization approach that quantifies the fine-scale features of both extracellular and intracellular spike waveforms. Using this approach, our results show differences in spike waveform in relation to local-field potential (LFP) oscillatory states. We also observe that systematic within-neuron waveform variability artificially splits single neurons in typical spike sorting algorithms. To study this variability in spike waveform in human circuits, we use human cortical organoids. Although invasive electrophysiological data acquisition cannot be performed in humans, recently, cortical organoids generated from hiPSCs have emerged as a promising 3D model of human circuits. We applied our parameterization approach to high-density MEA and patch clamp recordings of control human cortical organoids that allows us to obtain electrophysiological activity with cutting-edge spatial resolution. In addition, we parameterize MEA and patch-clamp recordings of organoids and neural cultures derived from autistic patients to explore spike waveform variability in a model of ASD and how it may be altered by processes known to be affected in ASD, such as impaired axon guidance and synapse formation, as well as reduced alpha oscillations and reduced evoked gamma response. Further, we treat these cultures with CBD to study the effect of the endocannabinoid system and its perturbation on the developing physiological properties of ASD neuronal networks. Preliminary results indicate that CBD is able to decrease neuronal activity in a reversible fashion.

Keywords: electrophysiology, spike waveform parameterization, autism

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VASCULARIZATION OF CEREBRAL ORGANOIDS USING 3D-PRINTED PERFUSABLE SYNTHETIC STRUCTURES

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Cerebral organoids are in vitro 3D tissue models of the human brain grown from stem cells. These organoids lack a blood circulatory system (vasculature) which results in a lack of supply of oxygen and nutrients to the inner tissue regions. This leads to the formation of a growing dead tissue core (necrotic core). Only a few hundred microns (passive diffusion reach) of the tissue from the periphery survive. The presence of this necrotic core in cerebral organoids is found to affect cortical layer organization and neuronal development. We address this problem by 3D-printing high-resolution, porous, mechanically robust, cytocompatible, synthetic vasculature arrays using two-photon polymerization (2PP). 2PP is a scalable technology that can 3D-print any size of complex, free-standing, branching vasculature that mirrors in vivo capillary networks. The vasculature array printed using this technology is connected to a perfusion platform. The perfusion platform includes a peristaltic pump to continuously pump media through the vascular tubes and provide nutrients and oxygen to the whole tissue. Perfused vascularization of cerebral organoids prevented apoptosis and necrotic core formation (reduction in cleaved caspase3+ and TUNEL assay expressions). Perfusion preserved ventricular structures with proliferating (ki67+) neural progenitor (sox2+) cells throughout the tissue in 4-week-old cerebral organoids. sc-RNA sequencing of the perfused vascularized cerebral organoids showed downregulation of glycolysis, hypoxia-related pathways, and an upregulation of the oxidative phosphorylation (oxphos) pathway. The shift in metabolism from glycolysis to oxphos was noticed across all cell clusters. This technology platform can be used for high throughput long-term organoid cultures for drug screening and disease modeling. It can be directly translated to other organoids/ tissues that lack vasculature, especially to heterogeneous organoids like liver organoids where slicing cultures can be challenging. It can also be integrated with applications where shaking cultures are not ideal such as continuous live imaging for temporal analyses, and clamping organoids with electrodes for futuristic projects involving information transfer and biocomputing.

Funding Source: Picower Institute Innovation Fund (PIIF)

Keywords: Vascularization, 3D-printing, Cerebral Organoids

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EXPOSURE TO AN ENVIRONMENTAL ENRICHMENT MODULATES SYNAPTIC VESICLE CYCLE IN THE MICE MODEL WITH SPINAL CORD INJURY MODEL

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Spinal cord injury (SCI) results in motor and/or sensory impairment below the level of injury, for this reason, rehabilitation was necessary to for this reason. An environmental enrichment (EE) consists in a large cage to increase social interaction and locomotor activity in mice model. We aimed to investigate the therapeutic effect and mechanisms of EE in SCI mice model. CD-1 mice with spinal cord contusion and assigned to either standard cage (indicated as control) or EE condition (indicated as EE). Each group for Control and EE were housed for 2 or 8 weeks, separately. The mice received only a dorsal laminectomy was assigned as the sham. RNA-Sequencing was performed in SCI for two and eight weeks compared to the sham. qRT-PCR, western blot, IHC, and neurobehavioral tests were conducted for further study. The synaptic vesicle cycle (SVC) pathway and related genes were identified by significantly down-regulated after SCI for two and eight weeks compared to sham. According to these results, we identified whether SVC pathway and related genes were modulated by EE exposure after SCI compared to exposure to control. Exposure to EE for eight weeks compared to exposure to control had a greater significant reversal effect in the loss of SVC related gene expression and locomotor activity. Based on these results, this study confirms that prolonged EE exposure has led to increase presynaptic activity and functional improvement by modulating SVC in SCI mice model.

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Keywords: Environmental enrichment, Spinal cord injury, Synaptic vesicle cycle pathway

TOPIC: PANCREAS

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IN VITRO LINEAGE TRACING DURING HUMAN PANCREATIC ENDOCRINE FORMATION

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During human development, pancreatic hormone-producing cells are formed from endocrine progenitors in the pancreatic epithelium. It remains elusive, however, whether the distinct endocrine cell types, such as beta, alpha, delta, and epsilon cells are bifurcating from a common endocrine progenitor population. To date, there is no proper lineage tree of pancreatic development. Here we use human pluripotent stem cell (hPSC)-derived islet cells as a model system to study the lineage relationships between the different cell types. We present iScarTrace, a CRISPR/Cas9-based and Cre-inducible cellular recorder that accumulates heritable genetic "scars" in single cells. iScarTrace allows us to reconstruct lineage history during in vitro differentiation of hPSCs to pancreatic islets by single-cell sequencing of scars and transcriptome. In a first set of experiments, we randomly integrated the recorder in the genome of differentiating cells. Our data revealed that Cre-activation at the pluripotent stage resulted in complex scar accumulation until three days after induction in ~30% of the cells. However, this was followed by the immediate epigenetic silencing of iScarTrace before the cells reached the definitive endoderm stage. Next, we inserted the iScarTrace recorder in the ROSA26 genomic safe harbor, and found that fluorescence marker expression is maintained during differentiation, indicating that epigenetic silencing was prevented. This approach will help unravel the lineage segregation of endocrine cells in vitro, allowing researchers to systematically improve differentiation protocols for islet replacement therapy.

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Keywords: beta cells, lineage tracing, development

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SINGLE-NUCLEI MULTIOMIC ANALYSIS REVEALS DYNAMIC REGULATION OF ENDOCRINE SPECIFICATION IN STEM CELL-DERIVED ISLETS

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Human pluripotent stem cell-derived islets (SC-islets) have recently emerged as a viable product for cell replacement therapy to treat diabetes. However, inefficiencies in the generation of SC-islets has hindered the widespread application of SC-islets. Specifically, SC-islet differentiation protocols produce heterogeneous cell populations consisting of detrimental off-target cells. Epigenetics and chromatin accessibility have been shown to play an important role in guiding cellular differentiation, but very little is known of the epigenetic regulation of cell fate in during SC-islet differentiation. Here, we developed a bioinformatics toolbox, the Leary Docker, to analyze single-nuclei multiomic sequencing (snMultiSeq) data and generated a novel roadmap of gene expression and chromatin accessibility dynamics during SC-islet differentiation. Simultaneous characterization of gene expression and chromatin accessibility in 35,823 cells, across 6 time points, enabled quantitative analysis of the temporal relationship between epigenomic and transcriptomic changes using multiomic velocity analysis. This analysis revealed a relatively linear differentiation trajectory toward CHGA expressing pancreatic endocrine progenitor populations during early stages. During later differentiation stages, multiomic velocity analysis revealed divergent differentiation trajectories toward expected SC-islet cell types. Notably, beta-like and off-target enterochromaffin-like (EC-like) populations appear to emerge from a common progenitor population. Cell state-specific gene regulatory networks (GRNs) were computationally inferred based on accessibility of transcription factor (TF) binding motifs and expression of TF target genes. The GRN models were leveraged to perform in silico perturbations of novel TFs regulating beta-like and EC-like specification. Collectively, these analyses provide the most detailed characterization to date of the gene regulatory dynamics of endocrine specification during SC-islet differentiation. Further, in vitro validation of TFs identified through in silico analysis will provide novel targets to improve SC-islet differentiation protocols.

Funding Source: NIH Human Islet Research Network (HIRN) Catalyst Award and NIH NRSA Postdoctoral Fellowship (5 T32 DK007120)

Keywords: stem cell-derived islets, bioinformatics, epigenetics

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ESTABLISHMENT OF LENTIVIRAL INFECTIOUS TITER AND VECTOR COPY NUMBER ASSAYS FOR TRANSLATIONAL APPLICATIONS

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The translation of cell-based therapies from the research to clinical landscape requires robust analytical methods that integrate with current industry processes and regulatory guidelines. As lentivirus is an advantageous vehicle of exogenous gene delivery for viral-modified cell products, standardized characterization assays for potency and safety have gained momentum. Translational applications require assays that are universal which can be applied for lentivirus with various GOI. In this study, an assay for a universal method to determine lentivirus infectious titer and vector copy number (VCN) of lenti-modified cells using droplet digital PCR (ddPCR) was developed. Established methods rely on a lenti-specific target and housekeeping gene which demonstrate comparability among the gene-specific target. Standards and controls were used to determine assay limits of quantification, sensitivity, and an assay positive control. Using ddPCR, the LLOQ was determined with spiked gDNA to the standard plasmid containing the target genes, and results indicate sensitivity at 1.8 copies/uL and a linear range of 3 log orders while maintaining target copy recovery between 80-120%. Next, a suitable sample dilution buffer was identified for assay implementation based on established validity criteria: % recovery, linearity, and stability. ddPCR methods were generated to achieve clear separation between positive and negative populations for singleplex and duplex reactions, demonstrating comparability among target measurement. Orthogonal methods using PCR and flow cytometry were tested to further support ddPCR infectious titer assay results which indicate alignment regardless of flow cytometry target or antibody such as ScFv, V5 tag, EGFRt, and FMC63. Further, VCN assay of CAR-T cells was tested against a validated standard with a known copy number, and deviation from the expected value was shown to be consistently < 15% which satisfies the current industry limit and demonstrates accuracy. VCN of the validated standards show assay consistency with CV <7% for intra-assay variability and CV <5% for inter-assay variability. The development and early implementation of these universal assays with necessary standards and controls eases translation towards further qualifiable assays for GMP applications.

Keywords: Infectious Titer, Vector Copy Number, ddPCR

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GENOME INTEGRITY ASSESSMENT AND VERIFICATION BY OPTICAL GENOME MAPPING FOR CELL MANUFACTURING/BIOPROCESSING APPLICATIONS

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Cell lines are used for multiple applications such as bioprocessing, therapy, and research. To ensure quality of cells and downstream applications, appropriate quality control (QC) methods are critical. However, historically, karyotyping has been employed, but it is limited by its very low resolution and tedious laboratory workflow. Other methods such as, PCR and targeted-sequencing can characterize only small genomic variants at specific loci. Whole genome sequencing can detect small variants genome-wide, but it has limited sensitivity in detecting structural variants (SVs). Optical genome mapping (OGM) is a novel genome analysis technique that can fill many of the gaps in current capabilities in genome integrity analysis. To find clonal variants, 400 Gbp of data is collected from the parental and test samples. For both samples, a de novo assembly is constructed, and homozygous or heterozygous SVs are assessed. Subsequently, Bionano Access software's dual variant annotation pipeline (VAP) identifies unique SVs in the test sample compared to the parental sample. To discover subclonal SVs down to 5% variant allele fraction (VAF), the workflow leverages the generation of 1.5 Tbp of data (simply requiring a longer data collection time). Comparison of test sample to parental sample by VAP easily enables the determination of acquired variants. Finally, generation of ~5 Tbp of data, generally requiring 2-3 flow cells to be run, enables the detection of SVs at ultra-low VAF (down to 1%). In this study, 17 serial dilution experiments were performed to examine OGM's limit of detection on 45+ SV loci. Targeting a coverage of 5 Tbp and analysis using the somatic SV-analysis workflow revealed that OGM has the sensitivity to detect deletions >50kbp, insertions >20kbp, duplications >150 kbp, and translocations at ~1% VAF. We have applied the clonal and somatic workflows to verify genomes' integrity after cell immortalization, induced pluripotency, transgene-integration and gene-editing. The data using the robust and sensitive workflows demonstrate that the OGM platform is a cost-effective solution for cell manufacturing/bioprocessing QC applications.

Keywords: Quality assessment for cell bioprocessing, Off-target genome rearrangements, optical genome mapping

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A MOUSE MODEL WITH HIGH CLONAL BARCODE DIVERSITY FOR JOINT LINEAGE, TRANSCRIPTOMIC, AND EPIGENOMIC PROFILING IN SINGLE CELLS

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Cellular lineage histories along with their molecular states encode fundamental principles of tissue development and homeostasis. Current lineage-recording mouse models have limited barcode diversity and poor single-cell lineage coverage, thus precluding

their use in tissues composed of millions of cells. Here, we developed DARLIN, an improved Cas9 barcoding mouse line that utilizes terminal deoxynucleotidyl transferase (TdT) to enhance insertion events over 30 CRISPR target sites, stably integrated into 3 distinct genomic loci. DARLIN is inducible, has an estimated ~1018 lineage barcodes across tissues, and enables detection of usable barcodes in ~60% of profiled single cells. Using DARLIN, we examined fate priming within developing hematopoietic stem cells (HSCs) and revealed unique features of HSC migration. Additionally, we adapted a method to jointly profile DNA methylation, chromatin accessibility, gene expression, and lineage information in single cells. Applying it to study clonal memory of HSCs over time, we found that cells within a clone have more similar genome-wide DNA methylation than either gene expression or chromatin accessibility. We anticipate that DARLIN will enable high-resolution study of lineage relationships and their molecular signatures in diverse tissues and physiological contexts.

Keywords: lineage tracing, single-cell multiomic profiling, hematopoiesis

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EFFICIENT DERIVATION AND BANKING OF CLINICAL-GRADE HUMAN EMBRYONIC STEM CELL LINES IN ACCORDANCE WITH JAPANESE REGULATIONS

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We recently reported a clinical-grade human embryonic stem cell (hESC) line KthES11 in accordance with current good manufacturing practice standards in Japan. We used xeno-free chemically defined medium StemFit AK03N with the E8 fragments of Laminin-511 instead of feeder cells. Despite this success, the establishment efficiency was very low at 7.1% in the first period. We optimized our protocol to establish clinical-grade hESC lines efficiently, especially in the early culture phase. We established five hESC lines (KthES12, KthES13, KthES14, KthES15, and KthES16) with 45.5% efficiency. All five hESC lines showed typical hESC-like morphology, a normal karyotype, pluripotent state, and differentiation potential for all three germ layers. Furthermore, we developed efficient procedures to prepare master cell stocks for clinical-grade hESC lines and an efficient strategy for quality control testing. Our master cell stocks of hESC lines may contribute to therapeutic applications using human pluripotent stem cells in Japan and other countries.

Keywords: human pluripotent stem cells, Clinical grade, Cell banking

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CHEMICALLY-DEFINED HYDROPHOBIC POLYMER SCAFFOLD TO PROMOTE AUTOMATION OF IPSC CULTURE

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For regenerative medicine to become widely used, cell culture operations require automation, continuous control and the use of safe and stable scaffold materials. Conventional scaffold materials such as proteins pose several problems in the cell manufacturing process. Commonly used extracellular matrix (ECM) proteins such as Matrigel and Vitronectin exhibit high variability and have a narrow process margin due to manual handling involved in the coating process. Also, these ECM proteins raise the culture cost and increase the risk of viral contamination due to animal-derived components. To address these issues, we have previously developed a chemically-defined synthetic (CDS) scaffold for human pluripotent stem cell (iPSC) attachment and maintenance. This scaffold comprises a hydrophobic polymer with ECM-mimetic structure that enables easy and uniform coating of typical cell culture ware such as 6 well plate. This CDS-coated plate exhibited heat resistance, elution resistance and high stability at room temperature storage (25 ℃, 1 month). The CDS scaffold showed cell growth similar to that of commercial ECM proteins. It supported the long-term expansion of examined iPSC lines with normal karyotypes during their undifferentiated state. It also allowed the directed differentiation of three germ layers. In this study, we demonstrate the automation of iPSC culture by combining our CDS-coated plate and a commercially available automatic culture device. After 6 days of automated culture with daily medium changes, the resulting cell doubling time was 23.7±0.4 hours. Furthermore, in order to fully automate the cell manufacturing process, it is preferable to easily detach the cells without requiring incubation for a long time. So, we tested and verified the applicability of several commercially available detachment reagents in this process. Our results demonstrate a practical chemically-defined scaffold that accelerates the automated and scalable production of iPSC and has potential to promote the use of iPSC-derived functional cells in desired clinical application.

Keywords: scaffold, iPSC, automation

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A NEW CLASS OF KILL SWITCHES FOR CELL THERAPIES THROUGH CONTROLLED DEGRADATION OF AN ESSENTIAL GENE

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Many cell therapies are designed to be permanently transplanted into a patient. Due to the nature and complexity of these "living drugs", it may be desirable to have the ability to remove the cell product after introduction into a patient in case of an adverse event, or as a safety measure after the cell product has performed its function. One avenue to ablate transplanted cell therapies is to engineer an inducible apoptosis mechanism that can be activated by a clinically-approved drug. Here, we describe a new class of synthetic kill switches that can be activated with immunomodulatory imide drugs (IMiDs). Previous kill switch failure modes often occur because the engineered kill switch construct is either transcriptionally silenced or the switch itself is mutated. To mitigate these design concerns, we developed a kill switch that relies on an endogenous essential component of the cell itself, rather than introducing an ectopic construct, to synthetically activate cell death. We reasoned that the inducible degradation of an engineered cell's essential gene could improve upon previous classes of kill switches, because: 1) an essential gene cannot be transcriptionally silenced without cell death, and 2) the kill switch itself cannot be mutated without killing the cell. To test our kill switch design, we fused inducible degrons onto native GAPDH, a gene essential for cell survival. Cells engineered with the degron grew with normal kinetics in the absence of the IMiD kill switch inducer, but underwent cell death after the application of the IMiD, as a consequence of GAPDH protein degradation. We believe that many inducible degrons could be combined with any essential gene to expand on this new class of kill switches for engineered cell therapies.

Keywords: Kill Switch, Genome Engineering, Cell Therapy

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ELECTROCHEMICAL SIGNALS FROM LIVING CELLS ORIGINATE FROM CELLULAR METABOLISM

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As an novel method for analyzing living cells, the direct detection of cellular redox signals has shown immense potential. However, the origin of such signals remains unknown, which hinders the widespread use of electrochemical methods for cellular research. By isolating a number of cellular components and inhibiting several enzymatic and biomolecular activities, we found that intracellular metabolic pathways and ATP levels are the main contributors to extracellularly detectable electrochemical signals. By supplementing metabolic intermediates, which we referred to as metabolic activator cocktails (MAC), the cellular signals detected by differential pulse voltammetry (DPV) can be amplified within 90 minutes of incubation. This DPV approach combined with MAC treatment showed a remarkable performance to detect the effects of the anticancer drug CPI-613 on cervical cancer, both at a low drug concentration (2 μM) and an extremely short treatment time (1 hour). Furthermore, senescence of mesenchymal stem cells, which is a major issue for long-term in vitro culture, could also be sensitively quantified using the proposed DPV+MAC method even at a low passage number (P6). Collectively, our findings unveiled the origin of redox signals in living cells, which has important implications for the characterisation of various cellular functions and behaviours using electrochemical approaches.

Funding Source: National Research Foundation of Korea (NRF) grant funded (Grant Nos. NRF-2022R1A4aaA2000776, NRF-2019M3A9H2031820, NRF-2022R1A2C4002217) and Korean Fund for Regenerative Medicine (Grant number RS-2022-00070316).

Keywords: Live cell sensing, Metabolic reaction, Electrochemical detection

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AUTOMATED TISSUE CULTURE WORKFLOW FOR CRYOVIAL BANKING AND SAMPLE PREPERATION OF DIFFERENTIATED AND NON-DIFFERENTIATED HIPSCS FOR 3D LIVE CELL MICRSCOPY

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The Allen Institute for Cell Science aims to understand the principles by which human induced pluripotent stem cells (hiPSCs) establish and maintain robust dynamic localization of cellular structures, and how they transition between states during differentiation and disease. We do this by visualizing hiPSC lines expressing fluorescently tagged proteins that represent a specific cellular structure or organelle using 3D live cell microscopy. These gene-edited hiPSCs and our other openly available scientific resources can be found at allencell.org. To produce a large number of standardized cell images for analysis, we have developed a fully automated workflow on Hamilton Star, a robotic liquid-handling platform that can perform hiPSC culture and cryovial banking seamlessly from single or multiple sources. We have also automated the seeding, maintenance, passage, and differentiation of edited hiPSCs at timed intervals, on 6-well plastic plates for propagation, and 96-well glass bottom plates for high-resolution 3D microscopy. Here, we describe this automated workflow that has been instrumental in the successful scale-up and optimization of our work, along with aspiration and dispense speeds, movements of plates, and seeding strategies. Upon comparing quality control results with manual modes, we found that automation considerably improved our pipeline by providing more uniformity, reproducibility, and overall cell quality while eliminating operator-to-operator variability and the need for weekend main-

tenance of cell lines. We have also developed an automated image-based colony segmentation and feature extraction pipeline to streamline the process of predicting cell count and selecting wells with consistent morphology for high-resolution 3D microscopy. Overall, automation not only increased consistency and reproducibility, but also ensured standardization and scalability. Our method considerably decreased error and variability introduced by repetitive handling of cultures and reduced the need for stringent training standards. Our intention is for any research labs working with hiPSCs to easily reproduce some or all parts of this platform using our strategies and settings that can easily be transferred to other automated platforms as a starting point to their development.

Keywords: Automation, Tissue Culture, Biobanking

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TOWARD THE DISCOVERY OF NOVEL MIRNAS THAT PROMOTE IPSC CELL GENERATION

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RNA-based methods for producing human iPS cell have focused attention due to the low risk of insertional mutagenesis and malignant transformation compared to other methods using DNA or viral vectors. Also, miRNAs are well known to promote cellular reprogramming. In order to find new miRNAs that enhance iPS cell production, we have developed a method that can visualize the activity of miRNAs in cells. The method utilizes messenger RNAs(mRNAs) that contain target sites of miRNA on the 5'-UTR and fluorescent protein in the ORF. In the presence of miRNA, the mRNA is cleaved and further digested. We can thus quantify the activity of the miRNAs in different cell types. Based on this system, we found that the miRNA activity and expression levels of miRNA analyzed by small RNA-seq did not correlate well. This result suggests that the activity profile may help to discover new miRNAs with biological functions, which could not be found from the miRNA expression profile. We constructed a miRNA-responsive mRNA library and compared miRNA activity between iPS cells and human dermal fibroblasts (HDFs) to select new miRNA candidates that could contribute to iPS cell production. When selected miRNAs were introduced into HDF with a Sendai virus vector carrying initialization factors, several candidate miRNAs enhanced reprogramming efficiencies. Thus, our miRNA switch-based method could find new miRNAs that enhance human iPSC generation.

Keywords: miRNA, iPS cell, reprogramming

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GSH DYNAMICS OF MESENCHYMAL STEM CELL IN BLOCK COPOLYMER NANOPATTERNS

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Existing stem cell therapies produced by simple cell culture have technical difficulties such as low therapeutic efficacy, engraftment rate, and lack of understanding of treatment mechanisms. Various cells, including stem cells, are exposed to complex nano- and micro-scale structural environments, and it is known that cell functions respond sensitively to these structures. Focusing on the environmental structure of cells, it is necessary to develop a nanopatterned surface structure that optimizes cell culture conditions and improves the function for stem cell therapy. Here, technology to enhance the antioxidant capacity of mesenchymal stem cells was developed by the surface modification of cell culture substrate with block copolymer nanopattern. It was confirmed that the cells cultured on block copolymer nanopattern have high GSH level compared with the cells cultured on general cover glasses, showing elongated spindle like shape. The GSH level was measured in a different BCP nanopatterns. Through this, our goal is to upgrade stem cell culture conditions by screening the effects of various BCP nanopatterns on stem cell GSH, and further to increase the clinical application potential of stem cells.

Keywords: GSH, stem cell, nanopattern

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NEXT GENERATION EXPANSION OF INDUCED PLURIPOTENT STEM CELLS USING TERUMO BCT QUANTUM® FLEX PROTOTYPE

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Pluripotent Stem cell (PSC)-derived therapies target large patient populations and require high doses making them unsuited to traditional 2D expansion. The Quantum® Hollow Fibre Bioreactor from Terumo BCT offers an alternative to traditional T-flask seed train culture with a functionally closed, and automated adherent perfusion system. The Cell and Gene Therapy Catapult has developed methodologies and protocols for the expansion of PSC in 1.7 m2 Quantum® hollow-fibre bioreactor producing above one billion undifferentiated cells, able to differentiate into the three, embryonic germ layers. Here, we demonstrate the use of the 0.2 m2 bioreactor kit in Quantum® Flex hollow-fibre bioreactor as

scale-down model tool for process development, achieving comparable fold expansion (10x), doubling time (50 hrs) and harvest viability (>90%) compared to established process using 1.7m2 bioreactor kit over 6 days of expansion. Additionally, the use of high-density cell bank to fully-close the seed train is assessed using a 0.2m2 bioreactor kit, achieving an elevated 12-fold expansion and maintaining high harvest viability (>90%) over 8 days of expansion. Following expansion in both initial scale-down experiments and direct thaw strategies, harvested PSCs exhibit high levels of pluripotency (FC >80% detection: SSEA3, TRA-1-60, Nanog, Oct3/4).

Keywords: bioreactor, manufacturing, iPSC

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COLOR: AN APPROACH FOR MULTIPLEX SPATIAL DETECTION OF NUCLEAR INTERACTIONS AND TRANSCRIPTION

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Control over pluripotency and cell fate involves tight regulation of gene expression, where localization within the nuclear space dictates its expression pattern. For an in-depth study of gene regulatory networks, it is necessary to develop high-resolution techniques for the multiplexed assessment of DNA localization and RNA transcript level in single cells while maintaining tissue structure. Conventional in situ approaches allow for the preservation of tissue organization, however they necessitate a denaturation step for DNA detection which incurs in RNA degradation thus limiting their simultaneous imaging. Cas9-based approaches allow for fluorophore recruitment to DNA without chemical denaturation but lack signal amplification, reducing resolution and increasing experimental complexity. To overcome these obstacles, we developed Cas9 Observation of Loci via Oligomerization Reaction (COLOR): an imaging-based approach combining the natural ability of dCas9 to stably bind and locally denature DNA with the signal amplification and multiplexing capabilities of Multimodal Universal Signal Enhancement (MUSE) in situ probes. Initial experiments show that COLOR is capable of mediating local DNA denaturation, allowing for MUSE probe binding and subsequent signal amplification under conditions identical to those conducive to quantitative RNA detection. Due to the strong signal amplification provided by MUSE, COLOR is compatible with signal-intensive high-resolution approaches such as Structured Illumination Microscopy, highlighting its potential application for the study of nanometer-range DNA-DNA interactions. Leveraging local DNA denaturation and modular signal amplification, COLOR represents a fast and powerful method for the high-resolution, single-cell, multiplexed study of chromatin organization and gene transcription dynamics in their natural spatial context. The application of COLOR will allow for a better understanding of gene regulatory network heterogeneity at the population level and its effect in cell homeostasis, fate, aging, and disease.

Funding Source: USC NIH T32 PhD Fellowship in Developmental Biology, Stem Cells, and Regeneration NIH R01HL140472

Keywords: Spatial Genomics, In Situ Hybridization, Multiplex Imaging

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GENERATION OF ASIAN ELEPHANT IPSCS FOR CONSERVATION AND DE-EXTINCTION OF THE WOOLLY MAMMOTH

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The de-extinction of the woolly mammoth is a large-scale effort to rehabilitate the steppes and restore balance to these ecological environments. One of the most important branches of this project is to produce induced pluripotent stem cells (iPSCs) from the mammoth's closest living ancestor – the Asian elephant. These generation of these cells accomplishes many goals – they can be used to differentiate into different cell types for in vitro testing of gene-edited cell types, can be driven to totipotency for embryogenesis, and can be differentiated into gametes for population diversity. Furthermore, many of these cellular developmental biology tasks overlap with needs for restoring endangered Asian elephant populations via gametogenesis and IVF. Towards this end, we lay the groundwork for mutliplex reprogramming techniques to transform somatic Asian elephant cells into naïve iPSCs

Keywords: De-extinction, Elephant iPSCs, De-differentiation

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A SET-THEORETIC DEFINITION OF CELL TYPES WITH AN ALGEBRAIC STRUCTURE ON GENE REGULATORY NETWORKS AND APPLICATION IN ANNOTATION OF RNA-SEQ DATA

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The emergence of single-cell RNA sequencing (RNA-seq) has radically changed the observation of cellular diversity. Although annotations of RNA-seq data require preserved properties among cells of an identity, annotations using conventional methods have not been able to capture universal characters of a cell type. Analysis of expression levels cannot be accurately annotated for cells because differences in transcription do not necessarily explain biological characteristics in terms of cellular functions and because the data themselves do not inform about the correct mapping between cell types and genes. Hence, in this study, we developed a new representation of cellular identities that can be compared

over different datasets while preserving nontrivial biological semantics. To generalize the notion of cell types, we developed a new framework to manage cellular identities in terms of set theory. We provided further insights into cells by installing mathematical descriptions of cell biology. We also performed experiments that could correspond to practical applications in annotations of RNA-seq data.

Funding Source: The Keio University Medical Science Fund, the General Insurance Association of Japan, the Takeda Science Foundation, JSPS KAKENHI, the Next-Generation Research Project Promotion Program, and the Yagami Data Security Lab Project.

Keywords: annotation, cell type, scRNA-seq

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DISSECTING THE ROLE OF ADARS IN HUMAN DEVELOPMENT VIA SINGLE-CELL MULTI-LINEAGE CRISPR SCREENS AND RNA EDITING ANALYSES

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Adenosine to inosine (A-to-I) conversion mediated by the adenosine deaminases acting on RNA (ADAR) family of enzymes can have myriad biological implications, such as alterations of amino acids and regulation of innate immunity. Correspondingly, ADAR deregulation is implicated in diverse disorders such as neurological diseases and tumorigenesis. The comprehensive profiling of ADAR expression and bulk RNA editing in adult human tissues has illuminated regulators of RNA editing and identified genetic variants that lead to its deregulation; however, there is a gap in our understanding of how ADARs impact human development. Here, we leverage multi-lineage, time-course differentiation of human pluripotent stem cells to teratomas, coupled with next-generation sequencing technologies to characterize cell-fate specification and RNA editing at single-cell resolution. Assaying across weeks 4, 6, 8 and 10 of teratoma development, we observed that global RNA editing levels broadly correlated with ADAR expression in teratoma cell-types, and there was a 2-fold increase in global editing levels from weeks 4 to 10 of teratoma development. Interestingly, we observed RNA editing levels in muscle cells were significantly lower when compared to all other cell-types. Next, we disrupted the three ADAR family members (ADAR1, ADAR2 and ADAR3) in teratomas, via pooled CRISPR-Cas9 knockout screens, and assayed resulting impact on cell-fate specification and RNA editing across all three germ-layers. We observed that knocking out the ADAR1 gene led to a global decrease in RNA editing levels across all cell-types, while ADAR2 and ADAR3 knockouts had no overt effect. Ongoing studies are examining the impacts of these knockouts on gene modules and cell-fitness across tissue types. Collectively, our work demonstrates the utility of assaying transcriptomes and RNA editing at the single-cell level in multi-lineage differentiation models to uncover salient cell-type specific epitranscriptomic patterns and ADAR functions.

Keywords: Single-Cell RNA editing Analysis, Multi-Lineage Differentiation, CRISPR-Cas9 KO Screen

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FULL-TIME ENVIRONMENTAL CONTROLS REDUCE MICROBIAL RISKS TO STEM CELL CULTURES, EVEN WITH FLAWED DISINFECTION

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The most expensive experiment or production run is one that gets contaminated. Next Generation Isolators (NGIs) close the cell handling process and make the atmospheric variables in traditional cell processing controllable. Temperature, relative humidity (RH), oxygen, and carbon dioxide levels are maintained at all times. We showed previously that NGIs actively reduce microbial risks by controlling the RH and temp. Here we present new data on the ability of this ISO 5-compliant environment to reduce the viability of common problem contaminants, even if disinfection routines fail. Five different problem microbes were inoculated on coupons and placed on walls, ceiling, and floor of the cell handling chamber. Every week for 8 weeks, a new set of contaminated coupons was introduced as if massively contaminated items were imported without disinfection. Routine cell handling was immediately performed with color-changing microbial broth (Day 0) and Days 3, 7, and 10. An adherent stem cell process was mimicked in triplicate with routine culture set-up, medium changes, and cell harvesting activities, all with contaminants in the same chamber. Waste media and the “cell harvest” triplicate samples were incubated for 14 days for microbial growth. Mimicking flawed disinfection, at the end of each handling, high-touch surfaces were disinfected with wipes dampened with a quaternary ammonium disinfectant, avoiding coupon contact. Microbe-loaded coupons were allowed to accumulate over time, maximizing at 120 in the chamber. We performed 21 media runs over 37 weeks in this chamber and 0/369 media samples were contaminated. Viable B. subtilis spores were still found on coupons at the conclusion of the study, although 2-3 logs reduced in numbers. Zero other microbes were viable on the coupons. This suggests that even if disinfection is not thorough, the controlled environment of the NGI can dramatically reduce microbial risks to highly valuable stem cell cultures.

Keywords: Regulatory, Cleanroom, Disinfection

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PLURIPOTENT STEM CELL EXPANSION IN THE QUANTUM CELL EXPANSION SYSTEM - PROTOCOL OPTIMIZATION

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Manufacturing of induced pluripotent stem cells for clinical applications requires robust and highly scalable cell expansion protocols. At the scale of billions of cells traditional cell culture systems demand extensive time and resources. Utilization of a close culture system offers an efficient alternative for the large-scale expansion of pluripotent stem cells under clinically applicable conditions. Quantum Cell Expansion System (Terumo BCT) is an automated platform design to culture cells in the disposable, functionally closed cell expansion set, consisting of hollow-fiber bioreactor and fluidics system with several inlet and outlet lines, separate intra- and extracapillary circulation loops, and gas transfer module. It is operated by a flexible control software that supports wide range of customized protocols. Here we report our progress in the development of large-scale pluripotent stem cell expansion protocol in the iMatrix-511/StemFit AK03N system. Monitoring of the cell growth in the bioreactor is achieved by sampling the extracapillary circulation loop and measuring lactate and glucose levels, which combined with medium supply rate allows for the calculation of glucose consumption and lactate generation in the bioreactor. Additionally, we are evaluating usefulness of constant monitoring of the acid base equilibrium and gas saturation of the medium in the extracapillary loop. At present, we can achieve exponential cell growth of the cells and with high yield and viability at harvest. Our results suggest that pluripotent stem cells are growing in the bioreactor in the form of 3D aggregates, and our current efforts are aimed at optimization of the feeding strategy, that will allow for the cell expansion up to several billion cells. Another avenue of the protocol optimization is application of customized culture medium. The Quantum system with separate intra- and extracapillary circulation loops makes possible to use costly medium containing recombinant proteins and growth factors to be supplied only to the intracapillary compartment, with the extracapillary compartment feeding realized with basal medium supplemented with low molecular weight components. This approach will further reduce the cost of pluripotent stem cells manufacturing at a large scale.

Funding Source: Japan Agency for Medical Research and Development, grant 2022_JP22bm0104001

Keywords: closed culture system, large-scale manufacturing, pluripotent stem cells

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INVESTIGATING HPSC GENETIC INTEGRITY USING NGS TESTING: TURNING A DEEP ANALYSIS INTO A STRAIGHTFORWARD INTERPRETATION

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Ensuring the genetic integrity of human pluripotent stem cells (hPSCs) is of utmost importance for the reliability of disease modeling and the safety of subsequent clinical applications. Genetic abnormalities can accumulate at critical stages in the hPSC workflow, including hPSC generation, amplification, and genome editing, underscoring the need for frequent monitoring of hPSC genomes. It was recently shown that in addition to recurrent chromosomal abnormalities, hPSCs acquire point mutations in cancer-related genes such as TP53 or BCOR. However, current techniques to evaluate genomic integrity in hPSCs do not provide the sufficient resolution to detect single nucleotide variations (SNVs). To overcome this limitation, a novel protocol was developed based on the next-generation sequencing (NGS) strategy for targeted sequencing of the coding region of 361 genes. Specific genes involved in the development and differentiation of hPSCs were added to a comprehensive set of genes linked to cancer. Two different mixtures of DNA derived from hPSC lines were examined to detect mosaicism at different prevalence levels (10%, 5%, 2%, and 1%). The protocol implemented allowed for uniform library preparation and deeper sequencing, which facilitated the identification of variants, with particularly low frequencies (variant allele frequency, VAF=1%). Additionally, the bioinformatics pipeline was assessed for its capacity to detect a range of variants, including single nucleotide variants (SNV), splice variants, and insertions/deletions (indels). The results were subsequently validated using a commercial DNA set to establish appropriate thresholds. Our newly established protocol has enabled us to gain a more comprehensive understanding of the genomic integrity of hPSCs, which is a significant milestone in the field.

Keywords: genetic integrity, hEPSC, NGS investigations, mosaicism, Single nucleotide variation (SNV), variant pathogenecity interpretation, targeting genes,

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UNLOCKING THE POTENTIAL FOR SINGLE-CELL SEQUENCING AT SCALE WITH COMBINATORIAL INDEXING

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Single-cell sequencing technologies have allowed for in-depth analyses of neuronal cell types and demonstrated the vast heterogeneity within these tissues. However, these studies are often limited in cell and sample throughput due to prohibitive costs. In

this study we show that combinatorial indexing can be used to drastically increase cell output from a single experiment while simplifying sample multiplexing, significantly reducing bench time and cost per cell. Here we show two workflows harnessing combinatorial barcoding technology, using kits from ScaleBio to profile chromatin accessibility and transcriptome data. In the first workflow, used for scATAC profiling, barcodes are added upstream of an on-market system to increase throughput while decreasing cost. Briefly, nuclei from up to 24 samples are distributed across 24 wells of a provided plate for barcoded tagmentation, which introduces a cell-based barcode. Nuclei are then pooled and can be super-loaded onto existing single-cell capture systems. Despite the loading of 100,000 nuclei per channel, the additional tagmentation barcode creates a low effective doublet rate (< 5%). In the second workflow, used for scRNA profiling, cells are taken through three rounds of sequential barcoding in an instrument-free, plate-based workflow. With streamlined reagents, and the use of smart plastics for pooling, both hands-on time and total workflow time are reduced when compared to previously published combinatorial indexing methods, allowing for the recovery of up to 125,000 cells per experiment. For both workflows, this technology was first evaluated using mixed species experiments, in which human and mouse nuclei (scATAC) or cells (scRNA) are mixed prior to beginning the workflow. Despite the loading of 100k nuclei (scATAC) or 125k cells (scRNA), effective doublet rates remain low (< 5%), with effective recovery of nuclei/cells across multiple sites and users (~55-60% from the final barcoding step). Data analysis shows a clear distinction between human and mouse populations with good sensitivity and low background. Analysis of PBMCs with similar loading rates shows high barcode and mapping rates, good recovery of nuclei/cells, and successful identification of major PBMC subsets.

Keywords: Single-cell combinatorial indexing, single-cell sequencing, Single-cell phenotyping

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ELECTROPORATION BUFFERLESS MULTIPLEXED GENE DELIVERY USING CELLSHOT PARTITIONED FLOW TRANSFECTION SYSTEM

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CellShot, an electroporation device, challenges the difficulties of viral transduction via Partitioned Flow Electroporation (PFEP). PFEP does not require an electroporation buffer, hence eliminates the need for washing or mixing. Reducing cell loss and damage, it increases cell viability and transfection efficiency. It also enables multiplexed cell engineering that is crucial for stem-cell-based therapies including reprogramming and differentiation. In this study, we evaluated the mRNA delivery efficiency of CellShot by delivering eGFP-mRNA to various types of cells using CellShot PFEP and assessed cell viability and transfection efficiency. 24 hours after delivery, the percentage of eGFP-positive cells was 92.2% (±1.84) in HepG2 human liver cancer cells, 84.76% (±1.85) in BJ1 human fibroblast cells, 94.77% (±2.06) in Jurkat human T cells, and 99.39% (±0.74) in NK92 human natural killer cells. The cell viability at this time point, confirmed by ETHD-1 staining, ranged from a minimum of 79.16 ±1.2% (BJ1 cells) to a maximum of 98.95±0.66% (HepG2 cells). These results suggest that CellShot has efficient mRNA delivery capabilities in various types of cells without significant cytotoxicity. In addition, we evaluated the delivery performance of eGFP-mRNAs to HepG2 cells via multiplexed (sequential) transfection. After 24 hours of delivery, cells maintained a viability of over 95.36% (±0.06) compared to the control group up to the third transfection. Only at the 4th transfection, the viability decreased by 19% to 81.39% (±0.24). In all delivery groups except for the control, the proportion of eGFP-positive cells was over 98.16% (±1.15), with no significant difference in transfection efficiency between groups. Also, the eGFP MFI fold value showed an increasing trend with each round of transfection. The study demonstrates CellShot can offer both technical and cost benefits in the development of nonviral therapy through simplified procedures and improved yield (productivity). CellShot leads to improved cell function and transplantation in fields such as CAR-NK/T, iPSC, and MSC therapies, proving to be a valuable tool in advancing cell therapy.

Keywords: Electroporation Bufferless Transfection, Nonviral Gene Delivery, Multiplexed Cell Engineering

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OPTIMIZED HIDEF-B8 MEDIUM SUPPLEMENT REDUCES COSTS AND IMPROVES ACCESSIBILITY ASSOCIATED WITH PLURIPOTENT STEM CELL RESEARCH

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Inconsistency of cell culture media and variation in animal-derived products is a significant problem in both commercial and academic sectors that affects research laboratories and manufacturing. Additionally, accessibility to ingredients for pluripotent stem cell (PSC) culture is limited where shipping conditions and timelines remain unreliable. We commercialized liquid HiDef-B8 supplement with hyperstable FGF2-G3 and extensively assessed PSC culture performance using data from a semi-empirical 3-factor rotatable central composite DOE for complete media variants around our product formulation. Fit to an (RSM) model, the data verified that HiDef-B8 performance is optimized for cost. Here, we follow-up on our previous report with additional verification data from a separate lab and highlight an advancement to further drive down associated shipping costs for added savings and increase global accessibility to ingredients for stem cell culture: freeze-dried HiDef-B8-FD stem cell maintenance supplement. In our previous study, the NRG1*FGF2-G3 interaction demonstrated highest impact resulting in significant pValue of 0.013 combined across two cell lines. Insulin had an independent significant PValue of 0.018 combining both cell lines. However, during these experiments we discovered solubility inconsistencies during supplement thawing that were attributed to insulin instability, local-

ized pH and also affected downstream detectability of FGF2-G3. The commercial result was a manufacturing change reducing supplement concentration by 20% to resolve performance inconsistencies. Here, we repeated testing of the 15-variant media matrix from 400x supplements compared to previous 500x, modifying B8 componentry for three key factors - NRG1, insulin and FGF2-G3, across two new model iPSC lines, in a second academic lab. We included two additional repeat variants for controls across three assay plates per iPSC line, per technical repeat, and an 18th variant control with substantial historic baseline data across several iPSC and ESC lines. Insulin and FGF2-G3 again demonstrated highest significant impact to formulation performance in our post 5-passage PrestoBlue™ outgrowth assay in two new iPSC lines, but the interactions with highest impact differed substantially. The insulin*FGF2-G3 combinatorial interaction demonstrated highest impact resulting in a PValue of 0.003 and FGF2-G3 independently registered a PValue of 0.006. These revelations facilitated a new freeze-dry stable HiDef-B8-FD supplement formulation, verified active through the sublimation process followed by reconstitution to an equivalent 400x supplement with pure water before adding to basal medium to make complete HiDef-B8. This new B8 iteration demonstrated no difference in performance compared to the current HiDef-B8-cGMP product in multi-passage iPSC assays across two cell lines.

Funding Source: NIH R44GM140750

Keywords: DOE, PSC maintenance, Formulation development

TOPIC: NEURAL

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SPATIAL MULTI-OMICS MAPPING OF THE HUMAN HIPPOCAMPUS

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Cellular functions and processes are quite dependent on their location and proximity to other cells which led to the development of the spatial omics field with efforts to address the loss of this information in the widely-used single-cell omics technologies. As the brain is a highly complex organ and system, there is growing interest in building a spatial atlas to map the omics and look at regional expression differences of cells based where they are and which cells they interact with. Current approaches are able to interrogate the transcriptomic landscape in tissue histology sections, but so far there is not a comprehensive human hippocampus atlas. Here we use the technology platform developed in our lab which utilizes a microfluidic approach to spatial omics to not only map the transcriptome in healthy post-mortem human hippocampus but simultaneously include the chromatin accessibility. Adjacent sections are further used for multiplexed protein identification to give precise cell-type information resulting in a triple modality spatial omic view in the human hippocampus. Focusing on the dentate gyrus region of the hippocampus, we have mapped 10 human samples. At a spot size of 50 microns, we are able to identify all the cell types using the transcriptome data including rare cell populations such as inhibitory interneurons falling in the CA3/CA4 regions and adjacent to the subgranular zone. Unsupervised clustering of transcriptome and chromatin accessibility (which uses the ATACseq chemistry) separately are both able to separate the regions due to major cell types. For example, the granular cell layer is highly enriched in PROX1, a marker for granule cells, which is distinct in the clustering analysis and is able to reconstruct the layer in the data. Adding the spatial proteome via CODEX provides highly reliable cell identification using a multiplexed antibody panel allowing us to connect exact cells and cell types with their spatial transcriptome and chromatin accessibility. By mapping the transcriptome, epigenome, and proteome in the human hippocampus of healthy individuals, we aim to provide a framework for the spatial atlas for the field in this region of the brain and further extend it to clinical samples to compare and investigate differences in disease groups such as major depressive disorder.

Funding Source: BRAIN Initiative Cell Atlas Network

Keywords: Spatial, Omics, Neural

TRACK: NEW TECHNOLOGIES (NT)

Session 4: Even

10:15 AM – 11:00 AM

TOPIC: CARDIAC

850

EFFICIENT AND REPRODUCIBLE GENERATION OF HUMAN IPSC-DERIVED CARDIOMYOCYTES USING A STIRRED BIOREACTOR

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In the last decade human iPSC-derived cardiomyocytes (hiPSC-CMs) proved to be valuable for cardiac disease modeling and cardiac regeneration, yet challenges with scale, quality, inter-batch consistency, and cryopreservation remain, reducing experimental reproducibility and limiting clinical translation. Here, we report a robust cardiac differentiation protocol that uses Wnt modulation and a stirred suspension bioreactor to produce on average 124 million hiPSC-CMs with >90% purity using a variety of hiPSC lines (19 differentiations; 10 iPSC lines). After controlled freeze and thaw, bioreactor-derived CMs (bCMs) showed high viability (>90%), interbatch reproducibility in cellular morphology, function, drug response and ventricular identity, which was further supported by single cell transcriptomes. bCMs on microcontact printed substrates revealed a higher degree of sarcomere maturation and viability during long-term culture compared to monolayer-derived CMs (mCMs). Moreover, functional investigation of bCMs in 3D engineered heart tissues showed earlier and stronger force production during long-term culture, and robust pacing capture up to 4 Hz when compared to mCMs. bCMs derived from this differentiation protocol will expand the applications of hiPSC-CMs by providing a reproducible, scalable, and resource efficient method to generate cardiac cells with well-characterized structural and functional properties superior to standard mCMs.

Funding Source: WTP and KKP were supported by the NCATS Tissue Chips Consortium (UH3 HL141798 and UH3 TR003279) and by charitable support from the Boston Children's Heart Foundation. WTP and MP were supported by funding from Additional Ventures.

Keywords: Cardiac differentiation in a bioreactor, Monolayer vs suspension cultures, in vitro maturation of iPSC-cardiomyocytes

852

A 96-WELL PLATE MICRO-TISSUE STRIP ASSAY FOR AUTOMATED HIGH THROUGHPUT SCREENING OF CARDIAC CONTRACTILITY

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With increased availability of human pluripotent-stem-cell derived cardiomyocytes, and recent passing of the FDA Modernization Act 2.0, interest in generating engineered cardiac tissues for cytotoxicity testing, disease modelling, and drug discovery applications has expanded rapidly. However, most engineered tissue assays are limited in throughput and consistency, hampering progress in such screening efforts. Based on Novoheart's established human ventricular cardiac tissue strip assay (hvCTS), we developed a 96-well micro-hvCTS (µCTS) that is 1/3 of the length (~3 vs. 9mm) and requires only 15% of the cardiomyocytes per tissue (150k vs. 1M) versus the standard hvCTS. The standard 96-well plate format is compatible with automated high throughput liquid handling and imaging systems. Each µCTS is suspended between two micro-posts, with anchors for consistent tissue height across all wells, and fluorescent beads to track post tip deflections and analyze µCTS contractile force using custom software. In designing this system, the mold and post geometry, as well as cell density and seeding volume, were optimized to ensure a tissue aspect ratio that maximizes ease of use, twitch force sensitivity, and tissue retention over extended culture periods. In conjunction with the plate, an array of biocompatible electrodes was designed and validated for field stimulation of tissues during long-term culture, enabling tissue maturation by electrical conditioning as well as control of beating frequency during data acquisition. To validate the plate technology, µCTS were used for dose escalation testing with the following compounds: isoproterenol, milrinone, nifedipine, flecainide, and aspirin, demonstrating expected inotropic responses. µCTS were also successfully matured by combined electrical stimulation and maturation media similar to the hvCTS

counterpart. Hence, Novoheart's µCTS 96-well plate offers an assay with increased sample number, reduced variability across tissues, decreased overall measurement time, and compatibility with automated plate handling systems, advancing cardiac contractility screening to the next level with improved accuracy and throughput.

Keywords: cardiac tissue strip, high throughput contractility measurement, 96 well plate assay

854

ENGINEERED DEVELOPMENT: DIRECTED MORPHOGENESIS OF AN EMBRYONIC HEART TISSUE MODEL

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Directed morphogenesis of an engineered tissue could be useful as an embryonic model. This model would provide unique insights into development and generate complex structures that have not been previously engineered. We have generated such a model system through the directed morphogenesis of an engineered tissue mimic of the embryonic heart. The heart is one of the first organs developed and undergoes a complex morphogenesis, also known as looping, transforming it from a tubular structure into the four-chambered organ. This phenomenon properly aligns the heart that leads to further development of more specialized forms of the cardiac tissue. Disruptions in the looping process are indicative for the onset of congenital heart defects. Embryonic heart tubes (EHT) were engineered using a novel casting approach, resulting in an inner acellular layer surrounded by a thin iPS derived cardiomyocyte layer, mimicking the in vivo anatomy. In the embryo, cardiac looping is driven through both internal and external forces, leading to elongation of the tube resulting in a rightward rotation at the cranial end of the embryo and a dorsal deflection at the caudal end. To direct the morphogenesis of the EHTs, we created a novel bioreactor that mimics the external forces that are exhibited upon the heart within the embryo. Our bioreactor allows for rotation on the cranial end, dorsal deflection on the caudal end, and tissue shortening on the entire tube. EHTs were able to be looped using the bioreactor system and were found to recapitulate specific anatomical shapes seen during the looping process. Looped EHTs were maintained for seven days via perfusion culture. After the culture period, ends of the looped EHTs were found to have fused together. Histological investigation discovered the generation of different complex anatomical structures. Namely, the generation of an intraventricular septum, cellular orientations that match measured angles within mammalian hearts, and ventricular shapes that closely resemble that of the native heart. In conclusion, through our approach of directed morphogenesis, we were able to generate a model of the embryonic heart that matches native anatomical structures. Directed morphogenesis could be translated to other organ types to create a systemic three-dimensional model of embryogenesis.

Funding Source: Funding for this project was provided through the Harvard Stem Cell Institute, Department of Defense, and National Institutes of Health.

Keywords: Cardiac Morphogenesis, Embryonic Tissue Model, Tissue Engineering

856

HUMAN ENGINEERED HEART-ON-CHIP MODEL FOR RISK ASSESSMENT OF CARDIAC ARRHYTHMIA

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Cardiovascular diseases, including abnormal heart beats (arrhythmias), are number one cause of mortality and morbidity worldwide and form a huge impact on society and economy. Until know therapy for arrhythmia mainly helps to reduce symptoms, but is not able to solve underlying causes. Mainly because current models are not able to recapitulate all aspects of cardiac disease and have not proven effective in extrapolating experimental findings to the patient. Therefore, there is an urgent necessity to develop improved, more predictable disease models of arrhythmia. A human engineered 3D in vitro model using human pluripotent stem cell (hPSC)-derived cardiac cells is a great alternative to existing models as it can mimic different facets of arrhythmia with the goal to understand the pathogenic mechanisms of the disease and develop new therapies. To more closely recapitulate onset and progression of arrhythmia in patients, a spatial organization in a specific geometry is required as it predisposes to arrhythmia. We were able to develop such a 3D cardiac tissue model by engineering a bone-shaped cardiac tissue by utilizing PMMA molding in combination with tissue embedding into Xanthan gum and imitating the native extracellular cardiac matrix build from collagen I and III. This model mimics the source-to-sink mismatch and thus creates a vulnerable substrate for cardiac arrhythmia. This tissue could be maintained in shape for more than 40 days. By comparison of 2D and 3D culture, we generated proof-of-concept that the shape can only be maintained in 3D, but not as monolayer highlighting the importance of 3D culture. We developed an optical mapping technique to visualize the conduction of calcium or voltage wave propagation through the tissue. Arrhythmia-like events were triggered through subjecting the cardiac tissues to Epinephrine. Amiodarone and Quinidine, which are prescribed to lower arrhythmia incidents in patients, were successful in counteracting the effects of Epinephrine. Our human-based in vitro model mimics several aspects of the diseased human heart and will not only shed light on underlying mechanisms of arrhythmia, but will be further developed into a multidisciplinary platform for risk assessment of cardiac arrhythmia.

Funding Source: Dutch Heart Foundation

Keywords: Engineered cardiac model, Disease model, Arrhythmia

858

HIGHLY DURABLE, STRETCHABLE AND MULTI-FUNCTIONAL ENGINEERING SYSTEM FOR ENHANCED MATURATION AND MONITORING OF CARDIAC CONSTRUCTS

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The use of reprogrammed human-induced pluripotent stem cells (hiPSCs) for personalized treatment strategies is gaining attention due to their accessibility and compatibility with the human body. These hiPSCs can be differentiated into various cell types, including cardiomyocytes (CMs). However, current approaches have been unable to fully replicate the electro-mechanical functions of natural CMs. While several methods have been employed to stimulate hiPSC-CM maturation, the ability to integrate multiple stimuli that influence myocardial cell function has been limited. To address this, we have designed a multi-functional CM engineering system that is durable, stretchable, and can independently control electrical and mechanical co-stimulation parameters. Additionally, the system can simultaneously monitor the status of hiPSCs and hiPSC-CMs. Our design utilizes a stretchable multielectrode array (SMEA) on a 3D micro-patterned elastomeric substrate that enables co-stimulation and impedance spectroscopy measurements of hiPSCs. Housed in a mini-incubator, our system employs various combinations of electrical and mechanical stimulation conditions to adjust hiPSC-CM maturity and monitor the cells throughout their proliferation, differentiation, and stimulation. Our innovative system holds great promise as a tool for enhancing the culture and engineering of CMs with enhanced maturation.

Keywords: human-induced pluripotent stem cells(hiPSCs), human-induced pluripotent stem cell derived cardiomyocyte(hiPSC-CM) maturation, Cell engineering system, Stretchable multi electrode array, electrical and mechanical co-stimulation

TOPIC: EPITHELIAL INCLUDES SKIN GUT LUNG

860

AUTOMATED INTESTINAL ORGANOIDS SCREENING ASSAYS: AUTOMATED CULTURE, IMAGING AND ANALYSIS OF ORGANOID MORPHOLOGY

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Three-dimensional (3D) cell models representing various tissues are being successfully used for modeling complex biological effects and tissue architecture. However, the complexity of 3D models remains a hurdle for their wider adoption in research and drug screening. We describe the system that allows automation of the complex organoid workflow that includes monitoring, maintenance, characterization of organoids, and testing for the effects of various compounds. This integrated solution includes an automated incubator, liquid handler, imaging system, as well as scheduling and decision-making software. We developed methods for automated seeding, media exchange, passaging of organoids, as well as monitoring organoids development. In addition, these methods allow to automate compound testing and evaluation of toxicity effects. 3D intestinal organoids were derived from mouse intestinal cells or from human iPSC cells. Organoids were cultured in Matrigel domes. The use of an automated liquid handling allowed automated cell seeding in Matrigel droplets followed by automated media addition and media exchanges every 2 days. Organoids were autonomously monitored every 2 days using imaging in transmitted light. Then, machine learning-based image analysis allowed finding organoids and characterization of their size, shape, and density. Reaching phenotypic maturity triggered the organoid harvest step followed up by re-passaging organoids into additional 24well or 96well plates. Organoid cultures were treated with selected compounds allowing detection of toxicity effects on development of intestinal organoids. For endpoint measurements organoids were stained with viability dyes or fluorescently labeled antibodies and imaged using the imaging system. Advanced image analysis was done by 3D reconstitution and phenotypic evaluation of organoid structures, including characterization of organoid complexity, cell morphology, differentiation markers, and cell viability. We demonstrated concentration-dependent toxicity effects of several anti-cancer drugs. We demonstrate the automation methods allowing automation of organoid culture and assays, and also described analysis approaches and descriptors that allow to gain information about phenotypic changes and morphology of 3D organoids.

Funding Source: none

Keywords: assay automation, high content imaging, machine learning

862

FUNCTIONAL RECOVERY OF A NOVEL GUT TISSUE MODEL OF HIRSCHSPRUNG'S DISEASE BY TREATMENT WITH HUMAN PLURIPOTENT STEM CELL-DERIVED ENTERIC NEURAL PROGENITORS

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Dysfunction of the enteric nervous system leads to motility disorders of the gastrointestinal tract including severe and rare disorders such as Hirschsprung's disease (HSCR). For HSCR patient treatment, no options exist other than surgical removal of the dysfunctional bowel. Here, we show the development of human induced pluripotent stem cell (iPSC)-derived artificial gut tissue (HSCR-iGUT) that models a HSCR phenotype by harboring a HSCR-related point mutation. Furthermore, motility dysfunction of HSCR-iGUT was restored by treatment with human iPSC-derived enteric neural progenitors (iENPs). To create HSCR-iGUT of sufficient size for injection of iENPs and functional assessment of motility, we intraperitoneally implanted human intestinal organoid derived from iPSCs in immunocompromised mice. Ten weeks after implantation, iENPs were injected into the muscle layer of HSCR-iGUT that had grown to 5-30 mm in diameter. Eight weeks after iENP injection, we harvested the HSCR-iGUT and assessed smooth muscle motility by organ bath assay. HSCR-iGUT that had not been treated with iENPs showed a lack of nitric oxide-dependent relaxation response to electrical field stimulation. In contrast, iENP-injected HSCR-iGUT showed clear relaxation responses, the intensity of which was equivalent to the maximal relaxation induced by the nitric oxide donor, sodium nitroprusside. In conclusion, HSCR-iGUT is a novel HSCR-relevant model of dysmotility, where the therapeutic potential of iENPs was demonstrated. These results pave the way for using regenerative cell therapy for the treatment of gut dysmotility disorders.

Keywords: a gut tissue model of Hirschsprung's disease, induced pluripotent stem cells (iPSCs), iPSC-derived enteric neural progenitors

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RESTORATION OF CTRP5 EXPRESSION IN VITRO USING LENTIVIRUS-MEDIATED GENE THERAPY AMELIORATES RPE DYSFUNCTION IN LATE-ONSET RETINAL DEGENERATION

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Late-onset retinal degeneration (L-ORD) is an autosomal dominant disorder in which a missense mutation in the CTRP5 gene leads to retinal pigment epithelium (RPE) atrophy and choroidal neovascularization. CTRP5 is a paralogue of Adiponectin family proteins which regulate cell metabolism. We showed that in L-ORD RPE, mutant CTRP5 interferes with its apical secretion and reduces binding affinity toward AdipoR1, the receptor for Adiponectin, resulting in AMPK dysregulation inside the cell. Here we provide evidence that CTRP5 heterooligomers are entrapped in endolysosomal compartments and likely targeted for degradation. Overexpression of WT CTRP5 in patient cells overcame lower CTRP5 levels and rescued mis-polarized VEGF secretion. RPE were differentiated from induced pluripotent stem cells (iRPE) derived from four siblings: two clinically and genotypically confirmed L-ORD patients and two unaffected siblings. iRPE were cultured with and without bafilomycin A1 (50nm) to determine if mutant CTRP5 was degraded by lysosomes. Immunostaining revealed strong co-labeling of CTRP5 with LAMP1/2 and ATG5 in L-ORD iRPE especially when lysosomal degradation was blocked using bafilomycin A1. For overexpression study, coding sequences of the human CTRP5 WT or mutant variant were cloned into a tagged lentiviral vector and transduced in L-ORD iRPE. With increasing MOI of the WT-CTRP5 expressing construct, 6-7x higher expression of CTRP5 on the apical side and 3-4x higher expression on the basal side of cells could be detected [MOI 1.5: Ap ($p< 0.01$), Ba ($p< 0.001$); MOI 3.0: Ap ($p< 0.001$), Ba ($p< 0.05$]. Consistent with increased secretion of CTRP5, pAMPK levels were reduced ~23% in L-ORD-iRPE with increasing concentration of WT-CTRP5 (MOI 0.5 and 1.5: p=ns; MOI 3.0: $p< 0.05$). Again, consistent with the increased CTRP5 secretion and reduced pAMPK levels, mispolarized VEGF secretion was also corrected in WT-CTRP5 overexpressed L-ORD-iRPE [MOI 1.5: Ap ($p< 0.01$), Ba ($p< 0.001$); MOI 3.0: Ap ($p< 0.001$), Ba ($p< 0.05$)]. Together this data further re-enforces our hypothesis that the dominant behavior of S163R mutation is through decreased secretion of CTRP5 in RPE cells and suggests that gene augmentation provides a potential treatment option for L-ORD patients.

Keywords: CTRP5 protein, lentivirus- mediated gene therapy, iPSC-RPE disease modelling

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INTERROGATING IMMUNOSUPPRESSIVE NICHES OF COLON CANCER STEM CELLS USING SPATIAL TRANSCRIPTOMICS

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In recent years, immunotherapy has revolutionized cancer treatment in patients with certain types of cancers; however, colon cancer responds to Immunotherapy poorly. Failure to response to immune checkpoint blockade is in part due to the phenotypic plasticity of tumors/cancers, a feature which tumor-initiating stem cells (TSCs or CSCs) and their associated microenvironment (TME) play critical roles in establishing. Utilized single-cell RNA-sequencing to identify therapy resistant TSCs (TrTSCs) in mouse models of CRT-challenged intestinal adenoma, followed by bioinformatics analysis to reveal the bidirectional crosstalk that occurs between TSCs and the tumor microenvironment (TME). The results of the analysis indicate that TSCs shape the TME into an immunosuppressive barrier, in part via the signaling module RPS19-C5AR1 between TSCs and myeloid derived suppression cells (MDSCs). To investigate the underlying mechanism in this regard we applied spatial transcriptomics on MC38 colon cancer mouse model. Our result showed that while both innate and adaptive immune cells are normally enriched in the boundary region of tumor, the treatment of MC38 colon cancer animals using neutralizing anti-C5AR1 antibody significantly increase the infiltration of macrophages, nature killer cells, and CD8 +T cells into the tumor, accompanying with ~50% of reduction in tumor mass. Using bioinformatics analysis, we are interrogating the signaling cascade downstream of RPS19-C5AR1 that mediate the immunosuppression. Furthermore, we are testing whether the combinatorial treatment of overcoming the extrinsic immunosuppressive barrier using anti-C5AR1 antibody and anti-PD1 antibody, an immune-checkpoint inhibitor, can increase the therapeutic efficacy in colon cancer.

Funding Source: Stowers institute for Medical Research NIDDH

Keywords: therapy resistant tumor stem cell, tumor microenvironment, spatial transcriptomics

868

TISSUE-DERIVED EXTRACELLULAR MATRIX HYDROGELS AS CULTURE PLATFORM FOR GASTROINTESTINAL ORGANOIDS

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Organoids, three-dimensional (3D) structures that include stem cells and differentiated cells, have been in the spotlight because they have similarities with native tissues that go beyond existing models. Until now, Matrigel, one of the extracellular matrix (ECM) hydrogels, has been widely used as culture platform for organoids. Matrigel provides a suitable 3D environment for cells to proliferate, but it has safety issue due to its tumor origin and cannot provide a tissue-specific ECM environment. In this study, we developed gastrointestinal (GI) tissue-derived ECM hydrogels as culture platform for GI organoids. Developed matrices contained tissue-specific proteins present in native GI tissue, which make suitable ECM environment for GI organoids. In addition, these matrices also had low batch variation and adequate biocompatibility. We found that GI organoids expressing diverse cell types and functional phenotypes could be generated in our tissue-derived ECM hydrogels. Moreover, the tissue-specific effect and age-related effects of our hydrogels were confirmed. Finally, applicability of tissue-derived ECM hydrogels was demonstrated such as long-term storage and organoid transplantation to mouse injury model. Tissue-derived ECM hydrogel platforms developed in this study could be produced from other tissue types and utilized for other organoid types.

Funding Source: This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation of Korea(NRF) funded by the Korean government, the Ministry of Science and ICT(MSIT) (No.2022M3A9B6082675)

Keywords: Gastrointestinal organoid, Extracellular matrix, hydrogel

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ESTABLISHMENT OF A CULTIVATION METHOD FOR HUMAN INTESTINAL STEM CELLS DERIVED FROM HUMAN PLURIPOTENT STEM CELL-DERIVED STEM CELL-DERIVED INTESTINAL ORGANOIDS

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Three-dimensional human intestinal organoids (hIO) are widely used as a platform for biological and biomedical research. However, reproducibility and challenges for large-scale expansion limit their applicability. Here, we established a human intestinal stem cell (ISC) culture method expanded under feeder-free and fully defined conditions through selective enrichment of ISC populations (ISC3D-hIO) within hIO derived from human pluripotent stem cells. The intrinsic self-organisation property of ISC3D-hIO, combined with air-liquid interface culture in a minimally defined medium, forces ISC3D-hIO to differentiate into the intestinal epithelium with cellular diversity, villus-like structure, and barrier integrity. Notably, ISC3D-hIO is an ideal cell source for gene editing to study ISC biology and transplantation for intestinal diseases. We demonstrated the intestinal epithelium differentiated from ISC3D-hIO as a model system to study severe acute respiratory syndrome coronavirus 2 viral infection. ISC3D-hIO culture technology provides a new tool for use in regenerative medicine and disease modelling.

Funding Source: The National Research Foundation (NRF-2018M3A9H3023077, 2021M3A9H3016046) The Korean Fund for Regenerative Medicine (21A0404L1) The Technology Innovation Program (No. 20008777) KRIBB Research Initiative Program (KGM4722223)

Keywords: Intestinal stem cells derived from 3D intestinal organoids, Differentiation of 2.5D intestinal epithelium via air-liquid interface culture, gene editing and tissue regeneration

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SPATIOTEMPORAL CHARTING OF THE HUMAN FETAL ESOPHAGEAL DEVELOPMENT FOR THE DIRECTED DIFFERENTIATION TO ESOPHAGEAL BASAL CELLS

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Stem cell therapy holds promise for severe esophageal epithelial defects, such as Epidermolysis Bullosa and caustic injuries. Such a therapy demands scalable clinical manufacturing of esophageal basal cells (eBCs) from human pluripotent stem cells (hPSCs). Deriving eBCs is challenging due to lack of understanding in human esophageal development. To close this knowledge gap, we present a spatiotemporal single cell multi-omics atlas for human esophageal development, with a special focus on eBC specification. Using single cell RNA sequencing, we delineate the cellular heterogeneity and lineage dynamics of both epithelium and stroma. Coupling with Visium spatial transcriptomics and CODEX spatial proteomics, we define the proximal eBC niche and identify mesenchymal paracrine signals that drive eBC specification. We further use these in vivo identified signals to drive eBC specification in vitro. To prioritize signal combinations for efficient and effective eBC derivation, we develop Manatee, a deep learning algorithm to predict gene expression alterations caused by signaling pathway perturbations. We apply Manatee to systemati-

cally screen all the combinations, and validate that the prioritized perturbation strategy can generate functional eBCs in culture. We benchmark that the in vitro derived eBCs express key esophageal markers and they can stratify properly in Calcium-induced assay, 3D organoid and organotypic culture. In summary, our work provides a holistic survey of human esophageal development in vivo, establishes a chemical-defined hPSC-to-eBC differentiation system for future clinical application, and proposes a potential paradigm shift in the rational design of hPSC directed differentiation.

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Keywords: single cell multi-omics atlas, hPSC differentiation, in silico perturbation

Abstract Withdrawn

TOPIC: GERMLINE AND EARLY EMBRYO

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XAV939 IS A NON-SPECIFIC PARP INHIBITOR THAT GLOBALLY REPROGRAMS THE PARYLOME OF HUMAN NAÏVE STEM CELLS

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The small molecule tankyrase (TNKS1/2) inhibitor XAV939 is frequently utilized to modulate WNT pathway signaling in human pluripotent stem cell (hPSC) naïve reversion and differentiation protocols. XAV939 stabilizes the protein levels of the TNKS1/2 substrate Axin, which mediates β-catenin degradation. However, XAV939 is a promiscuous poly-ADP polymerase (PARP) inhibitor of multiple PARP proteins (e.g., TNKS1/2, PARP1, PARP2, PARP3). Notably, TNKS1/2 and PARP1 independently regulate cellular proteostasis by catalyzing the PARylation of hundreds of validated protein substrates. For example, TNKS-mediated PARylation regulates proteosomal degradation of protein targets by controlling RNF146-mediated ubiquitination. Here, we hypothesized that non-specific PARP inhibition by XAV939 mediates broad direct and indirect effects on both TNKS1/2 and PARP1 substrates, and on the whole proteome of conventional hPSC; when used in naïve reversion protocols. To elucidate the role of XAV939 in regulating both TNKS1/2 and PARP1 substrates, we performed Tandem Mass Tag mass spectrometry to differentially evaluate the whole proteome and PARylome (i.e., the subset of protein targets covalently-modified by poly-ribosylation), on triplicate isogenic primed vs. Tankyrase/PARP inhibitor-regulated naïve (TIRN) hPSC. Differential protein expression was analyzed by proteomic gene ontology (GO) and pathway analysis. Select TNKS targets were validated by western blotting using PAR-affinity resins. Proteomic GO analysis suggested that continuous TNKS/PARP inhibition by XAV939 in the context of LIF-2i globally reprogrammed the proteome and PARylome of human naïve-epiblast-like TIRN stem cells in broad cellular processes. Specifically, inhibition of PARylation by TNKS and PARP1 rewired the pluripotency-associated proteomic circuits of TIRN stem cells. XAV939 supplementation of the classical LIF-2i cocktail (i.e., LIF-3i) resulted in broad differential protein expressions of both TNKS and PARP1 targets between primed conventional and TIRN stem cells. These effects were context-dependent and reached far beyond impact on WNT modulation. A deeper understanding of the PARylome and proteomic reprogramming may be a gateway for deriving improved human stem cells for regenerative medicine.

Funding Source: NIH/NEI (R01EY032113; R01EY023962), NIH/NICHD (R01HD082098), Research to Prevent Blindness (Stein Innovation Award), The Maryland Stem Cell Research Fund (2020-MSCRFD-5374), and The Lisa Dean Moseley Foundation.

Keywords: Tankyrase, PARP1, Naïve Pluripotency, Proteome

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DISSECTING SIGNALING RULES OF THE FIRST LINEAGE BIFURCATION OF EMBRYONIC DEVELOPMENT USING DIFFERENTIATION SLINGSHOT

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Shortly after zygotic genome activation (ZGA), the first lineage bifurcation occurs where the embryo transitions from a uniform cluster of totipotent cells into two specified lineages, one being the compact inner cell mass (future fetus) and the other being the trophectoderm shell (future placenta). Although extensive studies have identified key transcriptional networks that govern early embryonic development, there is limited knowledge of the molecular mechanisms that govern the first fate separation event in humans. To fill this fundamental gap in our knowledge, we develop a two-step approach termed the differentiation slingshot, where we first revert pluripotent stem cells into a transient totipotent state and then push them to mimic trophectoderm and inner cell mass specification. Transcriptomic and immunofluorescent analysis shows this approach generates cells with the appropriate expression of ZGA and trophectoderm-related markers. Our novel transient reprogramming strategy, paired with single-cell RNA sequencing and genome-wide CRISPR screens, provides an in vitro platform to unveil the signaling rules that control the first lineage bifurcation and helps uncover more molecular details of human embryo development.

Keywords: In vitro model of embryo development, Pluripotent stem cells, Reprogramming

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INTEGRATING SINGLE CELL AND SPATIAL GENE EXPRESSION PROFILING OF MOUSE ORGANOGENESIS TO IDENTIFY AND LOCALIZE NOVEL CELL TYPES

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Mammalian organogenesis is a remarkable process, whereby cells rapidly proliferate and differentiate into diverse cell types. Single cell RNA-sequencing of whole embryos yields unprecedented views of development, revealing hundreds of unique cell types defined by gene expression precisely regulated in time and space. Although many methods exist to identify cell types defined by scRNAseq, annotating cells remains a challenging process. In this work, we leverage both scRNAseq and spatial gene expression profiling to identify novel cell populations during organogenesis, and discover changes in cell specification and localization in a developmental mutant. We used high-resolution single nucleus transcriptional profiling of millions of cells done by 3-level combinatorial indexing on staged mouse embryos in 2-6 hour increments from gastrulation to birth. Although an initial round of manual annotation based on marker genes and earlier atlases was fruitful, many ambiguities remained. To address these in part, we integrated matched timepoints with spatial whole transcriptome profiles of precise anatomical regions generated using the GeoMx® Digital Spatial Profiler (DSP). We used a cell type deconvolution algorithm to estimate the abundance of each cell type in each region and validated that known populations such as tissue-specific epithelial cell subtypes were localized with high accuracy. We then used this method to map trajectories derived from the lateral plate mesoderm, populations with limited research that are challenging to annotate. Next, we applied this method to understand how dysregulated cell lineage contributes to organ malformation in a developmental mutant. Absence of embryonic macrophages due to CSFR1 deficiency causes bone and brain deformities and perinatal lethality in mouse and humans. We performed massively scalable single-cell transcriptomics and GeoMx DSP on E18.5 wildtype and CSF1R-deficient mutant littermates. We find differential cell type abundance in both the scRNAseq and between matched spatial regions across many tissues, suggesting that organs beyond bone and brain are impacted by macrophage loss. In conclusion, this work provides a framework for integrating spatial data with scRNAseq in an automated pipeline to map cell populations in normal and pathological samples.

Keywords: scRNAseq, spatial transcriptomics, development

TOPIC: HEMATOPOIETIC, IMMUNE, AND ENDOTHELIAL

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DEVELOPMENT OF ALLOGENEIC HSC-ENGINEERED INKT CELLS FOR OFF-THE-SHELF CANCER IMMUNOTHERAPY

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Cell-based immunotherapy has become the new-generation cancer medicine, and ‘‘off-the-shelf’’ cell prod- ucts that can be manufactured at large scale and distributed readily to treat patients are necessary. Invariant natural killer T (iNKT) cells are ideal cell carriers for developing allogeneic cell therapy because they are power- ful immune cells targeting cancers without graft-versus-host disease (GvHD) risk. However, healthy donor blood contains extremely low numbers of endogenous iNKT cells. Here, by combining hematopoietic stem cell (HSC) gene engineering and in vitro differentiation, we generate human allogeneic HSC-engineered iNKT (AlloHSC-iNKT) cells at high yield and purity; these cells closely resemble endogenous iNKT cells, effec- tively target tumor cells using multiple mechanisms, and exhibit high safety and low immunogenicity. These cells can be further engineered with chimeric antigen receptor (CAR) to enhance tumor targeting or/and gene edited to ablate surface human leukocyte antigen (HLA) molecules and further reduce immunogenicity. Collectively, these preclinical studies demonstrate the feasibility and cancer therapy potential of AlloHSC-iNKT cell products and lay a foundation for their translational and clinical development.

Keywords: Hematopoietic stem cell, invariant natural killer T (iNKT) cell, cancer immunotherapy

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YTHDF2 INHIBITOR AND 3D-NCADHERIN PTPTIDES EX VIVO EXPAND HUMAN HEMATOPOIETIC STEM CELLS BY MEDIATING GENE EXPRESSION AND CHROMATINE ACTIVITY

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The limited availability of human hematopoietic stem cells (HSCs) is a bottleneck of life-saving HSC transplantations in the clinic. Thus, the ex vivo expansion of human HSCs has been considered the holy grail of hematology for decades. Current methods only expand short-term HSCs and compromise the self-renewal and multi-lineage potential of long-term (LT) HSCs, which is indispensable for life-long graft success upon transplantation. The mechanisms of such failure are poorly understood, especially at the epigenetic level. Given that adult HSCs reside in bone marrow niches where they are simultaneously fine-tuned by both intrinsic programs and extrinsic niche signals, we hypothesized that culture conditions that recapitulate these intrinsic and extrinsic signals may expand HSCs ex vivo. Here we showed that Y13-27, a small molecule inhibitor of YTHDF2, and three-dimensional peptide hydrogels conjugated with N-cadherin peptides (3D-PG-Ncad) substantially expand both human umbilical cord blood and bone marrow derived LT-HSCs with strong multi-lineage potential. m6A and single-cell RNA sequencing revealed that Y13-27 and 3D-PG-Ncad rewire the metabolism, respiration, and cell cycle of HSCs, while also increasing the gene expression of multiple hematopoietic transcriptional factors essential for HSC maintenance. We next used single-cell multi-omics sequencing to simultaneously examine gene expression and chromatin accessibility in the same cells. We observed high epigenetic heterogeneity in the transcriptionally homogeneous HSCs, indicating that epigenetic changes precede transcriptional changes. Although lineage related genes were expressed at very low levels in HSCs, the corresponding chromatin regions were wide open. Primed HSCs exhibited greater openness in the chromatin regions corresponding to one lineage related genes and turned off the other lineage related genes along differentiation trajectory. In summary, we demonstrate that Y13-27 and 3D-PG-Ncad enable the ex vivo expansion of human LT-HSCs and preserve their balanced multi-lineage potential – a finding that has massive potential for future clinical applications. This is the first time to use chromatin accessibility as a measurement for multi-lineage plasticity of HSCs that could be mediated by intrinsic and extrinsic signals.

Keywords: Human hematopoietic stem cell expansion, Single-cell multi-omics sequencing, self-renewal and multi-lineage plasticity

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NOTCH ACTIVATION WITH ENGINEERED SOLUBLE LIGANDS FOR THE GENERATION OF HEMATOPOIETIC STEM AND PROGENITOR CELLS AND T-CELLS FROM HUMAN IPSC

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During development, Hematopoietic Stem and Progenitor Cells (HSPC) that arise in the aorta gonad mesonephros (AGM) as well as T cells that arise in the thymus require Notch signaling as a key fate specifier. Due to the requirement for force-mediated activation of Notch receptors, canonical Notch signaling requires presentation of cell, stroma or matrix-associated Notch ligand. Human induced Pluripotent Stem Cells (iPSCs) can be differentiated in vitro into HSPC and further down blood cell lineages into T and NK cells, which hold great promise for the treatment of immune deficiency, viral infection, autoimmunity, and cancer through adoptive immunotherapies. One bottleneck in producing these therapeutic cells for clinical use is the need to present Notch ligand either in a plate-bound format or from an adherent stromal cell, which complicates cell manufacture. A soluble Notch ligand would enable bioreactor scale-up and facilitate T cell manufacture for clinical studies. Moreover, engineered forms of Notch ligand can be highly informative tools for probing mechanisms of Notch

receptor function. Using protein engineering, we have designed a variety of soluble multivalent Delta-like ligand 4 (DLL4) proteins with distinct geometric and numerical valencies (3, 5, 6, 8, 60, and 120 copies), and have observed that distinct soluble oligomeric DLL4 proteins (i.e C3-DLL4, Icos60-DLL4, and Icos120-DLL4) induce differential activation of Notch despite equimolar equivalents of ligand. We found a soluble trimeric complex (C3-DLL4) that is comparable to plate-bound DLL4 as a Notch activator and shows a hook effect in its dose-response. Activation assays were performed both in an adherent reporter cell line (reporter U2OS), and in a suspension-based cell line (reporter K562). Soluble C3-DLL4 complexes induced cell-cell clustering in the engineered suspension cells due to formation of a tight "Notch junction". Titration of Notch signaling using the C3-DLL4 protein complexes enabled generation of CD4+/CD8+ T cells from cord blood (CB) derived T cell progenitors (proTs). Incorporation of soluble Notch ligands should enable manufacturing of T and NK cells in stirred suspension liquid bioreactors, thereby amplifying cell yields, reducing cost of goods, and enhancing off-the-shelf strategies for adoptive cell therapy.

Keywords: Hematopoiesis, HSPCs; iPSC; T cell, Notch signaling; Protein engineering

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DEVELOPMENT OF A DYNAMIC PLATFORM FOR HAEMATOPOIETIC DIFFERENTIATION OF HUMAN IPSCS

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Cell-based immunotherapies are proving to be very effective treatments for late-stage cancer patients. However, the current autologous model poses manufacturing and logistical challenges with regard to treating larger clinical populations. These personalised medicines are expensive, difficult to manufacture, have a large quality control burden, and exhibit donor variability. As such, the industry is looking to mitigate these issues by employing pluripotent stem cells (PSCs) as an alternative, allogenic source of immune cells for therapeutic use. It should be possible to manufacture large numbers of PSC-derived immune cells that provide multiple doses of consistent quality to treat many patients per batch. To manufacture at the required scale, it is necessary to improve the volumetric yield of existing haematopoietic differentiation processes. The most beneficial approach is to translate adherent, monolayer cultures into 3D suspension processes capable of being cultured at higher densities. Here we describe the adaptation of a small-scale, fully-defined, xeno-free, human iPSC-hematopoietic differentiation process into a dynamic, shaker flask system. PSCs are adapted to culture as spontaneously formed aggregates in suspension before being differentiated towards a CD34+ haematopoietic progenitor phenotype at day 12. This dynamic platform will provide key information about the translation of small-scale differentiation processes into scalable systems. This know-how can subsequently be leveraged to support the development of at-scale manufacturing processes required to meet the clinical need for PSC-derived immunotherapy products.

Keywords: hiPSC manufacturing, Dynamic differentiation, Allogeneic immunotherapy

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A SERUM- AND FEEDER-FREE DIFFERENTIATION CULTURE SYSTEM FOR THE GENERATION OF HUMAN CD19+ B CELLS AND ANTIBODY-SECRETING CELLS FROM STEM CELLS

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Long-lived plasma cells (LLPCs) are B cells that are generated from antibody secreting cells (ASCs) following an immune response and can persist for decades producing antibodies to protect against pathogen re-exposure. LLPCs do not proliferate and reside at low frequencies in hard-to-access areas, such as the bone marrow. These features, combined with the lack of efficient in vitro generation methods, are a major obstacle in the use of LLPCs as a cellular therapeutic tool. To address this, we have developed a serum- and feeder-free culture system for generating B cells from human hematopoietic stem and/or progenitor cells

(HSPCs) derived from umbilical cord blood (CB) or pluripotent stem cells (PSCs). HSPCs isolated from CB were cultured in serum-free StemSpan™ SFEM II medium and multi-stage differentiation supplements. After 5 weeks, CD19+ B cells were produced with a frequency of 67 ± 3% and a yield of 246 ± 58 cells per input CD34+ cell (mean ± SEM, n = 30). CD19+IgM+ cells were generated with a frequency of 37 ± 4% and a yield of 157 ± 41 cells per input CD34+ cell (n = 30). The sequencing of the B cell receptor loci of CB-derived CD19+ B cells was performed, displaying V(D)J gene rearrangement, diverse complementary-determining region 3 (CDR3) sequences with a normal length distribution, and diverse V and J segment usage and joining. Further analysis by ELISpot assay suggested that a subset of CB-derived B cells contained ASCs, with an average IgM ASC yield of 14 ± 3 cells per input CD34+ cell (n = 24). Culture in conditioned medium (Nguyen et al., Nat. Commun. 2018) for one additional week produced CD19+CD38highCD138+ cells (418 IgM+ and 22 IgG+ ASCs per 1 x 10^4 day 42 cells [n = 1]), suggesting differentiation to plasma cell-like cells. PSC-derived HSPCs (H9 and WLS-1C cell lines) were generated using the STEMdiff™ Hematopoietic - EB kit and further differentiated to CD10+CD19+ B cells with average frequency of 6 ± 2% (n = 18), in a feeder cell-free culture system similar to CB HSPC-to-B cell differentiation. This novel culture system is the first report of a serum- and feeder-free system for the generation of PSC-derived B cells, and can also be used to generate CD19+ B cells and ASCs from CB-derived HSPCs. This work enables further B lineage-specific research and provides a path to in vitro generation of LLPCs for clinical applications.

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Keywords: Hematopoiesis, B cells, Antibody secreting cells

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GENERATION OF NOVEL CANCER IMMUNOTHERAPY FUNCTION IN HUMAN PLURIPOTENT STEM CELL-DERIVED MACROPHAGES USING MULTIPLEXED GENETIC ENGINEERING

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Engineered immune cells such as chimeric antigen receptor T cells (CAR-T) offer a novel, potent, and specific immunotherapy strategy for neoplasms like CD19-positive leukemias. However, this approach is less effective for solid tumors, and standardization and engineering of primary human immune cell sources are persistent challenges. Macrophages are non-antigen-specific immune cells with immunotherapy potential, given their ability to phagocytose cancer cells. However, cancer cells can upregulate CD47, a checkpoint inhibitor that signals through SIRPα to prevent phagocytosis. CD47 targeting antibodies (aCD47) can override this signal and enable phagocytosis of solid tumors in mice, but primary sourcing of macrophages presents similar challenges as in CAR-T cells. Additionally, macrophages lack antigen specificity, but recent advances show that the transgenic CAR approach can help address this issue. Here, we show that human pluripotent stem cells (hPSC) can create a uniform, reproducible, and constant supply of macrophages that can enable phagocytosis of solid and liquid tumor cells and be scaled at a rate of ~5x10^6 cells per 10 cm dish per week. Moreover, we show that controlled multiplexed genetic engineering can confer novel immunotherapy functionality. Specifically, we demonstrated that wild-type (WT) hPSC-macrophages could reduce epithelial adenocarcinoma growth in vitro by > 90% when cultured with aCD47. Genetic knock-out (KO) of SIRPα in hPSCs resulted in macrophages with enhanced phagocytosis relative to WT. Further, these KO macrophages could be used in an antigen-specific and aCD47-independent manner when combined with an epithelial growth factor receptor (EGFR) opsonizing antibody and cultured with epithelial tumor cells. We also examined two safe harbor loci for multiplexing orthogonal knock-in (KI) gene function and inserting both previously established and novel phagocytosis-inducing CARs. Co-culture of CD19+ B-cell lymphoma with KI-engineered CAR-Macrophages in the AAVS1 safe harbor locus resulted in a 77% decrease in cancer cell growth rate without SIRPα inhibition. Together, these results present a strategy to create a potent, scalable, standardizable, and engineered source of macrophages with enhanced functionality as cell-based cancer immunotherapies from hPSCs.

Funding Source: Wellcome-LEAP

Keywords: Cancer immunotherapy, Macrophages, Genetic Engineering

TOPIC: EPITHELIAL INCLUDES SKIN GUT LUNG

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STEM CELL-BASED NMD-KNOCKDOWN APPROACH TO TREAT CYSTIC FIBROSIS

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Cystic Fibrosis (CF) is an autosomal recessive genetic disease caused by mutations in the cystic fibrosis transmembrane regulator (CFTR) gene. CF patients harboring nonsense mutations in the CFTR gene are currently untreatable. Nonsense mutations have two consequences. First, the nonsense mutation leads to expression of a truncated protein as a result of the premature termination codon (PTC) in the mRNA, often leading to complete lack of function. Second, the PTC is recognized by nonsense-mediated decay (NMD), an RNA turnover pathway that rapidly degrades PTC-bearing mRNAs. Thus, nonsense mutations in the CFTR gene lead to lower CFTR mRNA levels and consequent reduction in the amount of CFTR protein produced. Here, we report

our progress on devising practical approaches to inhibit NMD and thereby raise CFTR mRNA levels. First, we found that knockdown of several factors critical for the NMD pathway increases nonsense-mutant CFTR mRNA levels in bronchial cells in vitro without measurable toxicity. Second, NMD factor knockdown combined with G418, a PTC-readthrough drug, led to a dramatic induction in CFTR protein expression. Third, delivery of antisense oligonucleotides targeting NMD factors into the lungs of nonsense mutant CFTR mice suppressed NMD and increased nonsense-mutant CFTR mRNA levels in lung and trachea. Finally, to develop a viable NMD knockdown approach for use in CF patients, we developed short-hairpin (sh) RNA constructs that inhibit NMD in human embryonic stem cells (hESCs) and pluripotent stem cells (iPSCs). Experiments are in progress to differentiate these NMD-inhibited pluripotent stem cells into pulmonary epithelial cells to test function (given that CF patients typically die from complications of inadequate CFTR function in the lung). We propose that this NMD-suppression therapy in combination with sub-toxic levels of PTC-readthrough drugs can confer clinical benefit to CF patients who currently have no treatment options.

Keywords: Cystic Fibrosis, Non-sense Mediated Decay, Nonsense mutation mRNA

TOPIC: MESENCHYMAL STROMAL CELLS, ADIPOSE, AND CONNECTIVE TISSUE

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EVALUTION OF PRIMED ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELL EXOSOME FOR TREATMENT OF DRY EYE SYNDROME

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Exosomes are extracellular vesicles with a diameter of 50 to 150 nm, and the effects of which are different depending on cell types. Human adipose-derived mesenchymal stem cells (hAdMSCs) are relatively easy to isolate and have multiple therapeutic properties such as anti-inflammatory effect and anti-apoptotic activity. Similarly, exosomes secreted from hAdMSCs (hMSC-Exo) have various effects, therefore which are expected to provide new therapeutic approaches. On the other hand, for application to corneal diseases, it is necessary to reveal the effects and mechanism of hMSC-Exo, and to collect a sufficient amount of hMSC-Exo for the treatment. Hence, in this study, we evaluated the function of hMSC-Exo as therapeutic agents for dry eye syndrome (DES), and whether stimulation of hAdMSCs with TNFα enhanced the production and effects of hMSC-Exo. Firstly, hAdMSCs were expanded to 80% confluent, then medium was replaced to two types of medium with or without TNFα. After 72 hours of cultivation, the conditioned medium was collected, and from which hMSC-Exo were isolated by ultracentrifugation. Then, we examined the effects of hMSC-Exo on Benzalkonium Chloride (BAC)-induced cytotoxicity, inflammation and barrier dysfunction in human corneal epithelial cells (hCECs), and this experiment showed that hMSCs-Exo suppressed cytotoxicity and expression of inflammation-related genes such as IL1A, TNFα and MMP9. Moreover, immunostaining of hCECs indicated that expression of barrier function-related proteins such as TJP1 and CDH1 was recovered by hMSCs-Exo. In addition, stimulation of hAd-MSCs with TNFα increased production of hMSCs-Exo (Primed-Exo), and Primed-Exo showed similar effects to hMSCs-Exo. Thus, these results indicated that Primed-Exo would be beneficial for treatment of DES. Next, we plan to investigate the mechanism by RNA-seq, and the effects of ocular instillation with Primed-Exo in vivo DES model.

Funding Source: This work was supported in part by the project for the Osaka City Innovation Support Grant.

Keywords: Mesenchymal stem cells, Dry eye syndrome, Exosome

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QUANTIFICATION OF SOLUBLE FACTORS PRODUCED BY BONE MARROW-DERIVED MESENCHYMAL STEM CELLS USING LEGENDPLEXTM PANELS

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Mesenchymal stem cells (MSCs) are known for their ability to secrete various soluble factors such as growth factors, cytokines, chemokines, soluble receptors and extracellular matrix components. These factors play a crucial role in mediating the beneficial effects of MSCs on tissue regeneration, inflammation, and immune modulation. The composition and conditions of the culture medium can impact the proliferation, differentiation, and function of MSCs, as well as the secretion of soluble factors. Therefore, it is important to carefully control the culture condition and composition of the MSC culture medium in order to obtain consistent results. In addition, having a tool to simultaneously quantify the soluble factors secreted by the MSCs will be very important for evaluating the optimal culture condition and can serve as quality control for MSCs used for clinical research or therapeutical applications. Here bone marrow-derived MSCs were cultured under various conditions and supernatant collected after 3 days. A total of 8 different LEGENDplexTM panels were selected and used for screening of the soluble factors (>90 targets) secreted by the MSCs. The effect of different medium without or with various concentration of FBS on the secretion of soluble factors were then compared. The results showed that the presence of FBS from 2-12% dose dependently correlated with the levels of secretion of VEGF, IL-8, GROα, SDF-1, IL-6, IL-11, SCF, LIF, ALCAM and PD-L2,

with the α MEM medium supported a higher level of secretion than DMEM F12 medium for IL-8, GROα, SDF-1, LIF, TGF-β1, ALCAM and sPD-L2 and mostly comparable levels for VEGF, SCF, IL-6, and IL-11. ANGPT-2, Arginase, sNCAM, and TGF-β1 were detected from both cell culture supernatants and medium controls, and the levels seemed to correlate with FBS concentrations, indicating these were at least partially endogenous levels of bovine proteins present in the medium. Other targets detected with significant levels, but not showing a clear dose dependent response to FBS concentrations include MCP-1, sCD44, sVCAM-1, PAI-1, sTREM-1, sST2, sTNF-R1, sRAGE, and sCD130. Soluble factor profiling, and further studies using Multiplex immunoassay, LEGENDplex, may help design an optimal, consistent culture condition, including culture media for MSC expansion and differentiation.

Funding Source: BioLegend

Keywords: Mesenchymal stem cells, Multiplex quantification, cell culture medium

900

RESEARCH ON SUSPENSION CULTURE OF NON-CRYOPRESERVE OF SPHEROIDS USING A THREE-DIMENSIONAL CULTURE MEDIUM FCEM

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In recent years, spheroid and organoid research are being developed rapidly and it is expected to apply to cell therapy. However, it is extremely difficult with current technologies to cryopreserve cell aggregates maintaining their structure and function. Therefore innovative transport and storage technologies are required to realize transplantation therapy of cell aggregates. We have discovered a polymer FP003B (FCeM™ Advance-CR) that can be used to prepare a three-dimensional culture medium that enables suspension culture under static condition. In this presentation, we report the results of non-cryopreservation of adipose-derived mesenchymal stem cell (ADSC) spheroids using 3D medium supplemented with polymer FP003B. ADSC spheroids were suspended in FP003B supplemented 3D culture medium, and storage under airtight and static condition at room temperature. It was confirmed that the cell viability and the expression of MSC surface markers were maintained. In addition, ADSC spheroids were actively metabolized even at room temperature, suggesting that the depletion of nutrients and/or the decrease in pH might affect the maintenance of cell viability. Therefore, we performed the experiment using a gas permeable culture bag. The pH of culture medium was maintained during storage period and this enabled storage ADSC spheroids in higher density. From these results, it is considered that FP003B is useful for the transport and storage of spheroids would also be applicable for organoids.

Keywords: storage of Spheroids, transportation of Spheroids, storage of Organoids

TOPIC: MUSCULOSKELETAL

902

FUNCTIONAL INNERVATED SKELETAL MUSCLE TISSUE USING 3D CELL PATTERNING

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Skeletal muscle has an innate regeneration mechanism, but muscle loss beyond a certain magnitude of size cannot be recovered, requiring external intervention. Therefore, development of therapeutic strategies for muscle tissue regeneration is urgently required. Here, we generated a functional three-dimensional (3D) skeletal muscle tissue with a neuro-muscular junction that mimics the hierarchical structure of the muscle microenvironment by combining 3D cell patterning technology with direct cell reprogramming. 3D patterning allowed nerve cells and muscle cells to align closely with each other within the hydrogel, thus precisely mimicking the structure of actual muscle tissue. The alignment of nerve-muscle cells promoted muscle differentiation and maturation, which enhanced the contractile ability of engineered skeletal muscle tissue. When engineered skeletal muscle was transplanted into a mouse model of volumetric muscle loss, we confirmed that the pre-innervated, aligned engineered skeletal muscle tissue promotes neuromuscular regeneration and enhances functional recovery of limb muscles. 3D skeletal muscle tissue engineering based on direct reprogramming combined with 3D cell patterning recapitulates the actual structure and functionality of native skeletal muscle tissue for neuromuscular disease modeling and muscle regeneration.

Funding Source: This work was supported by the NRF grant (No. 2021R1A2C3004262) funded by the Korea government, MSIT and Samsung Research Funding & Incubation Center of Samsung Electronics under Project Number SRFC-TC2003-03.

Keywords: Tissue engineering, Direct reprogramming, Skeletal muscle tissue

TOPIC: NEURAL

904

SIMULTANEOUS PROFILING OF SPATIAL GENE EXPRESSION AND CHROMATIN ACCESSIBILITY FOR MOUSE BRAIN DEVELOPMENT

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Brain are complex biological tissues which function relies on coordinated anatomical and molecular structure comprised by a large number of specialized cells. The spatial architecture of brain

which is key to the understanding of its physiological and pathological significance is formed during embryo development. However, the molecular annotation of discrete spatial organization of the brain is inadequate. Here, we describe microfluidic indexing based spatial ATAC and RNA sequencing (MISAR-seq), a method for joint profiling of chromatin accessibility and gene expression with spatial information retained. By applying MISAR-seq to mouse developing brain, we identified the chromatin potential and transcriptional activity to define cell fate determination and tissue organization, and unraveled the spatiotemporal regulatory logics in mouse brain development.

Funding Source: National Key *Research and Development* Program of China (2018YFA0801402), "Strategic Priority Research Program" of the Chinese Academy of Sciences (XDA16020404), National Natural Science Foundation of China (32270854), et al.

Keywords: Spatial gene expression and chromatin accessibility, spatial gene expression, spatial chromatin accessibility

906

ENABLING NEXT GENERATION FUNCTIONAL CHARACTERIZATION OF HUMAN NEURAL ORGANOIDS

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Mammalian organs, such as the brain, are challenging to study being inaccessible to direct optical observation and experimental manipulation. However, recent advances in stem cell research allowed to develop novel three-dimensional (3D) culture techniques, such as neural organoids, resembling cell type diversity, developmental processes and function of human brain. Measuring the electrical activity of self-organizing in vitro cellular models in real time and label-free add valuable insights into the complexity of their functional structure. As such, high-density microelectrode arrays (HD-MEAs) provide unprecedented means for non-invasive in vitro electrophysiological recordings, and can be used to acquire live measurements from iPSC-derived neural organoids, contributing to study neuronal development and/or to model neurological disorders. Here, we used a HD-MEA platform featuring 26,400 electrodes per well (MaxWell Biosystems AG, Switzerland) to capture live the fast propagating extracellular action potentials in neural organoids at different scales, from network through single-neuron with high spatio-temporal resolution and low noise. Metrics, such as firing rate, spike amplitude, network burst profile and other network features, were extrapolated in a parallelized manner. Furthermore, at the subcellular level, we tracked the propagating action potentials across axonal branches to compute and characterize the conduction velocity across multiple neurons within the network of individual iPSC-derived neural organoids. Our single- and multi-well HD-MEA platforms, together with the extracted parameters highlighted in this study, provide an uniquely powerful user-friendly approach for identifying and isolating active areas of a 3D cellular model in both acute recordings and longitudinal studies. This not only allows long-term disease modelling but also efficient compound testing on stem cell-derived in-vitro models

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Keywords: In vitro electrophysiology, HD-MEA, Neural Organoids

908

MATRIX-FREE GENERATION OF HUMAN BRAIN ORGANOIDS AND IMPROVED CHARACTERIZATION BY 3D-IF AND FLOW CYTOMETRY ANALYSIS

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Due to its complexity, the processes of human brain development and its function are incompletely understood. Development of new in vitro disease models as well as implementation of state-of-the-art analytical workflows are urgently required to enable improved understanding of neurological diseases and to advance therapeutic development. To overcome these limitations we developed a simple and standardized workflow covering matrix-free generation of hPSC-derived brain organoids followed by detailed characterization of the cellular composition including lightsheet microscopy, automated dissociation and flow cytometry analysis. Using various hPSC lines, we established a Matrigel-free differentiation protocol for suspension culture. Our defined and xenofree media formulation allowed for highly reproducible differentiation of hPSCs into brain organoids within 30 days, recapitulating early brain development in 3D. For proper characterization of the cellular composition and 3D assembly we developed an automated dissociation procedure followed by flow cytometry analysis as well as an improved sample preparation workflow for 3D-IF and 3D imaging including organoid clearing and antibody conjugate optimization and validation. Our data indicate that we developed a simple differentiation protocol, allowing for standardized generation of hPSC-derived organoids with high efficiency. Specific whole mount labeling using recombinant antibody conjugates was optimized on brain organoids at different developmental stages and followed by a non-toxic tissue clearing procedure. Imaging of organoid samples by lightsheet microscopy revealed the formation of multiple ventricular zones and cortical plate-like regions, indicating recapitulation of early cortical development. Automated dissociation followed by flow cytometry analysis allowed for quantification of the cellular composition on a single cell level, adding further prove to the findings obtained by 3D imaging. Next, this workflow will be used for characterization of patient-derived organoid models and in vitro evaluation of cellular therapeutics.

Keywords: brain organoid, lightsheet microscopy, tissue clearing

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PROBING AND ANALYZING THE ACTIVITY OF BRAIN ORGANOIDS WITH ADVANCED HIGH-DENSITY MICROCHIP TECHNOLOGY

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The development of brain organoids opened a window for tapping into the richness and complexity of neural circuits observed in the human brain. Many prevalent neurological disorders are characterized by the functional disruption of neural circuits, therefore probing the activity of brain organoids in therapeutical development is highly desired. However, dissecting the functional organization of brain organoids is still a biological and technological challenge. Novel microchip-based biointerfaces offer new ways to record, visualize and assess functional activity and connectivity of electrogenic tissues in real-time and label-free. Here we present an approach based on high-density multi-electrode array (HD-MEA) to measure and analyze the electrogenic activity observed in brain organoids and other biological samples. We show the functional characterization of 5-month-old brain organoids generated from the isogenic control N6 line. HD-MEA allows for the simultaneous recording of thousands of electrodes in a high signal-to-noise regime. After, we extracted single spikes and isolated the contributions of individual putative neurons to the signal recorded on each electrode. To exemplify the potential of our system for drug screenings, we measure the activity of organoids immediately before, and after, treatment with 4-Aminopyridine (4AP) and Cyclothiazide (CTZ). We record and observe drastic changes in many functional metrics, as well as in the network connectivity during drug modulation. Finally, we validate the HD-MEA as a technology to fully exploit the brain organoids' capabilities.

Keywords: label-free functional imaging, neural biointerface, neuromodulator & biomarker discovery

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GENERATION OF HIGHLY PURE, CONSISTENT AND FUNCTIONAL INHIBITORY GABAERGIC NEURONS FROM HUMAN IPSCS USING OPTI-OX TECHNOLOGY

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Neuronal circuits in the cortex consist of two main neuronal types, glutamatergic excitatory neurons and GABAergic inhibitory neurons (IN). The inputs of IN provide cortical networks with the ability to balance spontaneous and evoked excitatory activities, preventing runaway excitation. Abnormal IN function is associated with a variety of neurological diseases such as autism, epilepsy and schizophrenia. It has proven to be challenging to develop drugs to treat neurological diseases as less than 10% of findings derived from conventional animal models can be translated to the clinic. Scalable approaches are needed to generate human in vitro models suitable for high-content drug screening that consist of well-defined and pure populations of specific neurons, such as GABAergic neurons. We have used our precision cellular reprogramming technology opti-ox™ (optimised inducible overexpression), to tightly control the expression of a unique combination of transcription factors to generate a highly pure (>95%) population of GABAergic neurons, named ioGABAergic Neurons, from human iPSCs, at scale, within 12 days post-revival. A deep molecular characterisation of these neurons by immunocytochemistry, RT-qPCR and single-cell RNA-sequencing revealed that the cultures consist of over 95% pure GABAergic neurons expressing the classical marker genes GAD1, GAD2, VGAT, DLX1, as well as DLX2 and are positive for GABA. Remarkably, SST was the only main GABAergic subtype specific marker that was detected in the transcriptomes of the single cells, further highlighting the purity of our ioGABAergic Neurons. Moreover, the transcriptomic profile of ioGABAergic Neurons was highly equivalent across three independently manufactured lots, showing that cells can be produced in a consistent manner, at scale. The ioGABAergic Neurons are functional as they display spontaneous neuronal activity and can easily be co-cultured with our ioGlutamatergic Neurons in the presence of astrocytes. In summary, opti-ox precision cellular reprogramming enables the manufacturing of highly pure (>95%), consistent, and functional GABAergic neurons that can serve as a high-quality human model to study both neurodevelopment and neurological disorders.

Keywords: reprogramming, opti-ox, GABAergic neurons

914

APPLICATIONS OF AN ISOGENIC HUMAN IPSC-DERIVED BLOOD-BRAIN BARRIER (BBB) MODEL

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The blood-brain barrier (BBB) is a specialized network of cells that function to maintain a tightly controlled microenvironment around the brain. A robust BBB model is needed to evaluate barrier function, test drug permeability, and study how different diseases can affect it. Harnessing the power of iPSC technology, we were able to generate specific cell types of the human brain required to assemble such a model, including astrocytes, pericytes, & brain microvascular endothelial cells (BMEC). Importantly, the same donor iPSC line (01279) was used to make each of these cell types, yielding a fully isogenic tri-culture system. Marker expression and other cellular characterization data has been presented previously; therefore, we focused on the functional performance of the BBB model in various assay platforms. The traditional trans-endothelial electrical resistance (TEER) assay using cell culture inserts resulted in robust & reproducible signal (>1500 ohms ▪ cm2) after 3 days. TEER measurements were further investigated using impedance-based instrumentation and barrier disruption with VEGF and mannitol was quantified. BBB permeability of fluorescent dextran molecules was assessed, and the apparent permeability of drug compounds (e.g., atenolol, caffeine, chlorpromazine, & propranolol) was quantified via LC-MS/MS. Generation of 3D spheres was accomplished using ULA plates and imaging revealed insightful structural features. Development of a receptor-mediated transcytosis assay was also initiated, beginning with characterization of transferrin receptor expression and evaluation of detection techniques. Finally, integration with emerging organ-on-a-chip technologies, such as MIMETAS OrganoPlate and Emulate Brain Chip, offers a unique way to further enhance biological complexity. Importantly, the keys to success here were consistency of supply made possible by differentiation at-scale resulting in large batches of cells, cryopreservation of all three cell types for subsequent on-demand use, and an optimized formulation of media/supplements to enable long-term survival. Taken together, this study highlights the modular and flexible nature of an isogenic human iPSC-derived BBB model as a new capability to advance the understanding of BBB function with respect to human health and disease.

Keywords: Blood-Brain Barrier (BBB), iPSC, TEER

916

ELECTRICAL STIMULATION ENHANCES PLURIPOTENT STEM CELL-DERIVED PHOTORECEPTOR CELL REPLACEMENT THERAPY TO RESTORE VISUAL FUNCTION

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Photoreceptor cell replacement therapy offers a universal approach to treat incurable blindness. Human PSC-derived retinal organoids provide an unlimited source of functional photoreceptor cells. We have pioneered subretinal transplantation of photoreceptors to rescue vision. However, poor survival and integration of cells remains a challenge in moving to the clinic. This project aims to improve cell therapy efficacy using electrical stimulation (EStim), an emerging technique implicated in developmental cellular processes. We have established EStim delivery in vitro to retinal organoids, and in vivo to the eye after transplantation. We hypothesise that EStim promotes neuroplasticity of donor and host cells, and increases transplanted cell integration- leading to robust restoration of visual function. We demonstrate EStim of retinal organoids regulates key photoreceptor signalling pathways including IGF-1 and Ca 2+, whilst increasing photoreceptor yield by 24.2%. Next, we show EStim in vivo enhances neuroplasticity in the advanced degenerated retina. Finally, we demonstrate that transplantation of photoreceptor cells can restore of blind mice long-term. Moreover, we show that combinatorial cell transplantation with in vivo EStim significantly improves transplantation outcome. Animals receiving EStim post-transplantation were significantly more likely to exhibit both light perception behaviour and improved visual acuity function. Morphologically, EStim-transplanted cells display enhanced expression of mature markers of visual function and synaptogenesis. We therefore demonstrate in vitro EStim may significantly enhance PSC-derived organoid development whilst in vivo EStim may prime host cells and promote donor cell integration. We provide first demonstration of EStim as an easily implementable technique both in vitro for PSC-derived differentiation and in vitro for cell therapies, with huge promise in accelerating photoreceptor cell therapy to the clinic.

Funding Source: This work is supported by Luminesce Alliance (PPM1 K5116/RD274), established by the Government of NSW to coordinate and integrate paediatric research. Further support was provided by the Australian Government RTP Scholarship.

Keywords: Cell therapy, Retinal organoids, Photoreceptor

TOPIC: PANCREAS

918

BROWN ADIPOSE TISSUE: A PROMISING SITE FOR ISLET ORGANOIDS TRANSPLANTATION

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Transplantation of human induced pluripotent stem cell (hiPSC) derived islet organoids is a promising cell replacement therapy for type 1 diabetes (T1D). It is important to improve the cell engraftment by investigating new transplantation sites with high vascularization. hiPSCs-L1 was generated constitutively expressing luciferase. Luciferase expression was verified using bioluminescence imaging (BLI) using the IVIS SpectrumCT (Perkin Elmer). Luciferase-expressing hiPSCs were differentiated into islet organoids according to the established protocols. We transplanted islet organoids (20 IEQ) into the brown adipose tissue (BAT) of NOD/scid mice (12-week-old, female, n=4, the Jackson Laboratory) as BAT group. The same amount of islet organoids were transplanted under the left kidney capsule (KC) of NOD/scid mice (12-week-old, female, n=4, the Jackson Laboratory) as a control group. These two groups were followed up by BLI at day 1, 7, 14, 28, 35, 42, 49, 56, and 63 post transplantation. Quantitative assessment of the BLI signal intensity of grafts was performed using the Living Image software. Islet organoids grafts were collected and processed for histological examinations at 14 days post-transplantation. Islet organoids were successfully transplanted into the BAT of NOD/scid mice and were detected by BLI. The BLI signals were detected from all recipients including both BAT and control group on the first day post transplantation after injection of luciferin at a dose of 150 mg/kg. During the study, the BLI signals gradually decreased, both in the BAT and the KC group. However, the BLI signal intensity of the grafts under the left KC decreased substantially faster than those from the BAT, on day 63 post transplantation, there was no detectable BLI signal from the KC mice, while the graft BLI signal of the BAT group remained. Immunofluorescence staining of islet organoid graft in the BAT displayed the presence of functional organoid grafts as confirmed by expressing of insulin and glucagon. Our results demonstrated that in comparison to the transplantation site of under kidney capsule, the BAT enhances islet organoid graft survival. The results strongly suggest that the BAT is a potential desirable site for islet organoid transplantation for T1D therapy.

Funding Source: RCSA Scialog collaborative award to P. W.

Keywords: induced pluripotent stem cell, islet organoid, brown adipose tissue

TOPIC: NO TISSUE SPECIFICITY

920

ENGINEERING 3D MICRO-COMPARTMENTS FOR HIGHLY EFFICIENT AND SCALE-INDEPENDENT EXPANSION OF HUMAN PLURIPOTENT STEM CELLS IN BIOREACTORS

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Human pluripotent stem cells (hPSCs) have emerged as the most promising cellular source for cell therapies. To overcome the scale-up limitations of classical 2D culture systems, suspension cultures have been developed to meet the need for large-scale culture in regenerative medicine. Despite constant improvements, current protocols that use microcarriers or generate cell aggregates only achieve moderate amplification performance. Here, guided by reports showing that hPSCs can self-organize in vitro into cysts reminiscent of the epiblast stage in embryo development, we developed a physio-mimetic approach for hPSC culture. We engineered stem cell niche microenvironments inside microfluidics-assisted core-shell microcapsules. We demonstrate that lumenized three-dimensional colonies significantly improve viability and expansion rates while maintaining pluripotency compared to standard hPSC culture platforms such as 2D cultures, microcarriers, and aggregates. By further tuning capsule size and culture conditions, we scale up this method to industrial-scale stirred tank bioreactors and achieve an unprecedented hPSC amplification rate of 277-fold in 6.5 days. In brief, our findings - recently published in Biomaterials (Feb. 2023)* - indicate that our 3D culture system offers a suitable strategy both for basic stem cell biology experiments and for clinical applications. * Cohen et al. Engineering 3D micro-compartments for highly efficient and scale-independent expansion of human pluripotent stem cells in bioreactors. Biomaterials. 2023 Feb 2;295:122033. doi: 10.1016/j.biomaterials.2023.122033. Epub ahead of print. PMID: 36764194.

Keywords: Human pluripotent stem cells, Scale-up in bioreactors, biomimetic stem cell culture

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AUTOMATING THE MAINTENANCE AND EXPANSION OF HIGH-QUALITY HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) enable the study of otherwise difficult-to-obtain cell types from a diverse set of genetic backgrounds. However, manual maintenance of hPSCs is repetitive, laborious, and subject to variability between different operators, making large studies requiring a variety of cell lines at high numbers challenging. As a result, there is an increased need to implement automation in hPSC workflows while preserving high hPSC quality. To address this need, we developed RoboCult™-PSC, an automated hPSC maintenance and expansion platform that delivers a desired quantity of 6-well plates, on a specific date, at a defined target culture confluency. RoboCult™-PSC is able to maintain cell lines completely unattended for up to 3 days at its full capacity of 105 plates. hPSCs are fed, imaged, and passaged as small clumps with minimal operator intervention through the use of an intuitive user interface, predictive cell growth algorithms, and integrated error detection and response. During an 18-week validation period operating at 50% capacity (~46 plates/week), RoboCult™-PSC performed 4295 feeds and 859 passages with a 2.5-fold lower contamination rate than manually maintained cultures. Four hPSC lines maintained for 10 passages had similar morphology, undifferentiated PSC marker expression (> 90% OCT4+ and TRA-1-60+), differentiation efficiency, and genetic stability (G-banding) when compared to manually maintained cultures. RoboCult™-PSC cultures also showed reduced variation in confluency passage-to-passage (17.4 ± 3.6% versus 33.5 ± 4.7% for manually maintained cultures; data represent mean confluency coefficient of variation ± SEM), as well as an enhanced ability to attain the defined target confluency (10.3 ± 1.0% versus 18.0 ± 1.7% for manually maintained cultures; data represent average deviation from target confluency ± SEM). These results demonstrate that RoboCult™-PSC significantly improves the reproducibility of hPSC cultures while maintaining cell quality and greatly reducing manual labor and contamination rates, enabling more robust, high-throughput hPSC research.

Keywords: Automation, hPSC, Maintenance

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RECONSTITUTION OF ORGANOIDS USING MINIMIZED NUMBER OF SECTIONED CONFOCAL IMAGES: UTILIZING DEEP LEARNING AND VIRTUAL ORGANOID GENERATION

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Organoid and 3-Dimensional (3D) imaging techniques are powerful method that enable researchers to study morphological and physiological aspects of human tissues which are in developing or damaged condition. For reconstruction of 3D structure of organoid, current applications should utilize the whole set of Z-stack confocal images, which spends a lot of working cost and time. Recently, we developed a deep-learning based organoid 3D rendering system which shows robust performance and reproducibility. To minimize the required number of focal plane confocal images, we developed a fully-convolutional-neural-network-based deep learning model which can construct 3D voxel data using 1 to 10 focal plane confocal images as input. Although, massive amount of image sets are required for training deep-neural network, there are few public datasets. To overcome this problem, we developed artificially synthesized 3D organoids named virtual organoid (V.O.) and established massive datasets (more than 39,000 synthesized V.O.) for training deep learning network. Our results demonstrated that newly developed 3D rendering system successfully reconstructed the 3D structure of organoid using minimal number of images with 0.82 IOU values. Interestingly, our system could predict the structure of organoid regions which are located deep focal plane, even though confocal microscope is not able to detect those regions. We expect that our V.O. data sets and 3D rendering system may contribute substantially to development of advanced 3D bioimaging technology.

Funding Source: The Technology Innovation Program (20009125, Establishment of a high-contents 3D organ on a chip system for studying pancreatic modeling in vitro) funded By the Ministry of Trade, Industry & Energy(MOTIE, Korea)

Keywords: Deep Learning, Organoid, Confocal microscopy

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USING SINGLE CELL CRISPR/DCAS9-BASED REGULATORY ELEMENT SCREENING TO DISSECT CIS-REGULATORY INTERACTIONS IN THE MAJOR HISTOCOMPATIBILITY LOCUS

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The Major Histocompatibility (MHC) Locus is the most SNP-dense region in the human genome and has been linked with >100 polygenic disorders. However, it remains unknown which variants affect gene expression. To dissect cis-regulation within the locus, we performed noncoding CRISPR/dCas9-based regulatory element screening with targeted single-cell RNA-sequencing readout and identified putative regulatory element (pRE)-gene pairs in human induced pluripotent stem cells (hiPSCs), hiPSC-derived neural progenitor cells (hNPCs), and K562 cells. We designed a sgRNA library targeting 581 pREs with TSS-targeting positive controls and nontargeting negative controls (12,723 sgRNAs). We engineered hiPSCs, hNPCs, and K562 cells to express the repressor dCas9-KRAB, and hiPSCs and hNPCs to express the activator dCas9-p300 and transduced each cell line with the sgRNA library at a multiplicity of infection greater than one. We profiled 1.2 million single cell transcriptomes seven to nine days post-transduction, and then performed differential expression testing between all cells in which a given sgRNA was observed versus all cells in which a different sgRNA was observed. Across all five screens, we identified a pRE for 76.3% (106/139) of genes detected within the locus and connected genes to 50.4% (293/581) of all perturbed regions, with 48.2%-66.7% of pRE-gene pairs spanning < 100kb and 16.4%-23.4% spanning >1Mb. For all cell types, the overlap of active histone marks (H3K4me1, H3K4me3, H3K27ac) increases as the pRE-gene link becomes more significant. Concordantly, 97% of functional pREs in K562s are also in accessible chromatin in that cell type. In contrast, >33% of functional pREs in hiPSCs and hNPCs are not in accessible regions in these cell types and may also overlap the repressive mark, H3K27me3. We next investigated the cell-type specificity of the interactions and observed dense regulatory networks spanning ~25kb proximal to the genes IER3 and HLA-A in hiPSCs that are not present in the other cell types. Taken together, we clarified cis-regulatory mechanisms for lowly and highly expressed genes across three diverse cell types, and identified shared and cell-type specific regulatory interactions that can be further separated into subclasses based on functional annotations and may be broadly applicable to other complex genomic regions.

Keywords: CRISPR/dCas9 regulatory element screen, Single cell RNA sequencing, Regulatory elements and noncoding regions

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RNA-TEMPLATED EDITING USING TYPE V CRISPR EFFECTORS

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The programmable nature of RNA-guided CRISPR nucleases stimulated a genome editing revolution, and RNA-templated systems promise to enable precise genomic repair. In this work, we present new systems for genome-primed Reverse Transcriptase-editing (RT-editing) that operate with a CRISPR Type V nuclease. Our implementation with the Type V system demonstrates precision editing within human cells mediated by optimized versions of Type V CRISPR RT-editing systems. The compact nature of the Type V effectors and their gRNAs confer advantages regarding delivery and manufacture relative to existing precision editing systems. RT-editing using a Type V nuclease has shown significant precision editing in therapeutically relevant cell types including T-cells and iPSCs, an editing profile that can be well suited to addressing specific classes of human disease. This work highlights an initial proof of concept in expanding RNA-templated editing to the broad biochemical and mechanistic diversity of

Type V CRISPR systems, potentially enabling additional translational potential of RT editors.

Keywords: CRISPR, RT-Editing, T-cells

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OPEN TOOLS AND RESOURCES FOR QUANTITATIVE IMAGE ANALYSIS OF CELLULAR ORGANIZATION

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The Allen Institute for Cell Science aims to understand the principles by which human induced pluripotent stem cells (hiPSCs) establish and maintain robust dynamic localization of cellular structures, and how they transition between states during differentiation and disease. To understand and predict cellular organization, we have created the Allen Cell Collection of fluorescently-tagged hiPSC lines and a suite of openly available image-based computational tools for quantitation and analyses. Each hiPSC line expresses a monoallelic fluorescently tagged protein that represents a major cellular structure or organelle. The Allen Cell Collection now contains 56 high quality lines that target 43 key cellular structures and substructures and are available to academic and commercial researchers. Following tagging, we perform live 3D imaging via our microscopy pipeline and generate hundreds of thousands of high-resolution images. To meet the challenge of segmenting, analyzing, and visualizing this massive volume of imaging data, we have developed several tools which we make available to the public for free. These include a python-based toolkit for cell segmentation which can also be accessed as a plug in for the napari app called the Allen Cell and Structure Segmenter, a volume viewer tool with path-trace rendering called AGAVE, a visual analysis tool to interrogate biological simulations called Simularium, and an online tool to visualize and plot features of segmented images called the Cell Feature Explorer, among others. Finally, we have educational resources, software, and analysis code developed at the Institute and our datasets are openly available to the community for access and download. These tools, resources, and data can be found at allencell.org.

Keywords: hiPSC, imaging, software

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EXPLORING THE COMPLEX SIGNALING NETWORK REGULATING STEM CELL DIFFERENTIATION USING A CAPILLARY IMMUNOASSAY PLATFORM

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Studying signaling networks is particularly important in the field of regenerative medicine since stem cell reprogramming and differentiation are regulated by multiple signaling events. However, methods conventionally used to study protein expression during stem cell differentiation, including immunofluorescence staining and western blotting, have been inadequate to gain a complete, quantitative understanding of these complex signaling networks. Here, we have investigated differentiation of human induced pluripotent stem cells (iPSCs) by applying a capillary immunoassay platform (Simple Western™, ProteinSimple) to better understand stem cell signaling. Using this approach, we analyzed core differentiation markers and compared multiple signaling pathways in iPSCs cultured under naïve conditions and when stimulated to differentiate into germ layers in multiplexed experiments. Together, our results validate the potential of capillary immunoassays for studying signaling pathways in stem cells for regenerative medicine.

Keywords: Differentiation, Signaling, Method

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ASSESSMENT OF MICROBIOLOGICAL CONTAMINATION IN A CELL PROCESSING CENTER FOR A HUMANOID ROBOT

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Industrial robots have been utilized for cell manufacturing process since they can support cell productions with unified specifications. To produce cell therapy products, entire cell manufacturing process must be compliant to the Good Gene, Cellular, and Tissue-based Products Manufacturing Practice (GCTP), which meats the good manufacturing practice standards. We installed LabDroid Maholo is a humanoid robot system, which is developed by Robotic Biology Institute Inc., for the clinical grade iPSCs-derived retina pigment epithelial cells (RPEs) production in cell processing center (CPC) in Kobe-City Eye Hospital. However, the operational actions of robots on the environmental quality in cleanrooms should be evaluated to identify risks of microbiological contamination. In this study, we assessed microbial contamination levels in a CPC unit for LabDroid Maholo being currently used for the clinical research of RPEs transplantation therapy. We setup LabDroid Maholo in an All-in-one cell processing (CP) unit (DAI-DAN Co., Ltd.) in Kobe Eye Center, Facility for Retinal Regeneration. First, we performed a RPEs production test with CPC operation training using the robot system for Sep. to Dec. in 2021. After the training, the pilot and clinical manufacturing for RPEs were conducted for Jan. to Jun. in 2022. During the 10 months period, we measured a degree of airborne and surface-colonized bacterial contamination at a set of GCTP-grade A, B, and C areas in the All-in-one CP unit. Throughout this investigation, we prohibited entering the All-in-one CP unit unless cleaning and cell culture preparations were necessary. As a result of multiple bacterial

monitoring tests in the entire testing period, no microorganisms are detected in the GCTP-grade A area. This study indicates that LabDroid Maholo could be a promising industrial robot that is suitable for cell therapy products manufacturing.

Funding Source: Japan Agency for Medical Research and Development Japan Science and Technology Agency

Keywords: CPC, Environmental monitoring, Cell manufacturing

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ILLUMINATING DYNAMIC STRUCTURAL STATES IN HIPSCS THROUGH ENDOGENOUS GENE TAGGING

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The Allen Institute for Cell Science aims to understand the principles by which human induced pluripotent stem cells (hiPSCs) establish and maintain robust dynamic localization of cellular structures, and how they transition between states during differentiation and disease. We have created a large collection of endogenously tagged hiPSC lines to illuminate cell organization and state transitions. The tagged proteins include notable membrane-bound and membrane-less cellular organelles, signaling complexes, phase transition markers, transcription factors, and structural markers specific to differentiated cells. To date, the Allen Cell Collection consists of 56 single- or compound-edited lines that have undergone extensive quality control testing to ensure genomic, cell biological, and stem cell integrity. Here, we highlight our gene-editing and quality control workflow for mono- and biallelic editing of expressed or silent genes that are either expressed in stem cells or expressed specifically during differentiation. We also describe the construction of genetically encoded reporter cell lines to reveal cell signaling status. Furthermore, we underscore our efforts to increase manipulation efficiency by utilizing Adeno-Associated Virus 6 (AAV6) to deliver donor DNAs and multiplexing transfection strategies for gene tagging at multiple loci. Our most recently released lines are CTCF-mEGFP and PCNA-mEGFP, for dynamic visualization of chromatin domains and replication foci, G3BP1-mEGFP to label stress granules, mono- and bi-allelic mEGFP-CDH1 to visualize adherens junctions and DCP1a-mEGFP for P-bodies. Currently, we are characterizing cell populations with tags on CDH2 (N- cadherin) and CDH5 (VE-cadherin) for analysis of cell adhesions in differentiated lineages, as well as CENPA for monitoring centromere assembly, with intent to release clones following quality control assays. Our continued overarching goal is to make these tools openly available to the cell biology community to accelerate biomedical research. In addition to our cell lines, the donor plasmids, segmented 3D images of about 200,000 cells from our lines, image analysis and visualization tools, integrated cell models, and biological findings are all openly available to the research community (www.allencell.org).

Keywords: hiPSCs, Gene editing, Gene tagging

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CONTROL OF CELLULAR BEHAVIORS BY SYNTHETIC GENETIC CIRCUITS TO ESTABLISH VASCULARIZED HUMAN ORGANOIDS

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Organoids are 3D micro-tissues or organs recapitulating micro-anatomy and certain functions of organs in vitro, playing an important role in drug screening and cell therapy. Establishment of human pluripotent stem cell (hPSC)-derived organoids are mainly based on the understanding of embryonic development and cellular aggregation capacity. Currently, many types of hPSC-derived organoids have been set up, such as liver, kidney, brain and so forth. However, hPSC-derived organoids still have some limitations, from which lack of blood vessel network is one of the key challenges needed to be overcome. Here, we reported a novel strategy to establish functional vascularized hPSC-derived organoids by using synthetic genetic circuits. SynNotch system is a well-established synthetic genetic circuit modulating the cellular communication and spatial arrangement in vitro, which has been used in Car-T cell therapy. In view of this, we engineered hPSC cells with synNotch system to manually control cellular communication between hPSC-derived endothelial progenitor cells and hPSC-derived nephron progenitor cells, aiming to obtain organoids with plenty of glomerulus with correct structure and functions. To achieve our goal, we first modified three plasmids of synNotch system according to our demands. We constituted one plasmid to express GFP-ligase by dox-induction, named as plasmid A. At the same time, we constructed other two plasmids to express other three genes related to nephron maturation in response to GFP-ligase, named as B package. Then A plasmid and B package were infected into both K562 cells and hPSC cells, respectively. For system test, direct interaction between K562-synNotch-A cells and K562-synNotch-B cells successfully activated synNotch system, leading to express target genes and regulate spatial arrangement, respectively. Additionally, we have successfully obtained hPSC-derived endothelial progenitor cells, hPSC-derived nephron progenitor cells, as well as kidney organoids with plenty of podocytes, verified by immunofluorescent staining. By integration of hPSC-derived organoid technique and synNotch genetic circuits, we proposed that we could obtained nephron organoids with correct glomerulus structures and functional glomerulus.

Keywords: human pluripotent stem cell, SynNotch genetic circuit, Organoids

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RETINOIC ACID AND RCAN2 REGULATION OF AN INTRACELLULAR K+ CIRCUIT THAT SCALES EMBRYONIC FIN BUDS VIA INTRACELLULAR CA2+ SIGNALING

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The generation and regeneration of any entire anatomical structure by stem and progenitor cells requires controlling the proportional growth of these cells in each of the contributing tissues. K+ channels have a significant role in scaling fish appendages; however, it remains unknown how intracellular K+ is integrated into mechanisms that scale these anatomical structures. Using the zebrafish pectoral fin bud as a model for early vertebrate fin/limb development, we observed coordinated decreases in endogenous intracellular K+ levels during bud outgrowth of the entire mesenchyme and ectoderm tissues, and overexpression of the K+-leak channels is sufficient to increased bud size of the entire anatomical structure and upregulate the several morphogens (Fgf10, Fgf8, Shh and Aldh1a2) that control bud growth. These results indicate that this electrophysiological scaling phenomenon is conserved among all vertebrates appendages from fish to humans. Retinoic acid (RA) is a hormone morphogen that can increase limb proportions, and we found that RA decreased intracellular K+ in all bud tissues. We also found that RA up-regulated regulator of calcineurin (rcan2) in the buds and that rcan2 overexpression was sufficient to decrease intracellular K+ as well as to enhance growth of the entire buds. Conversely, removal of rcan2 increased intracellular K+ and decreased scaling. These results together with our previously published findings provide molecular control of a specific K+-leak channel, Kcnk5b; that is, RA upregulates the transcription of rcan2 to inhibit calcineurin and thereby increase the activity of the K+-leak channel Kcnk5b, which we found is present in the embryonic buds. Furthermore, we now also show that K+-channel-induced growth requires IP3R-mediated Ca2+ release from the endoplasmic reticulum and CaMKK activity in vivo. Thus, we provide mechanisms for how this electrophysiological scaling signal can be regulated by RA and can subsequently scale a vertebrate anatomical structure via regulation of IP3R-mediated intracellular Ca2+ signaling.

Funding Source: National Science Foundation of China, ShanghaiTech University

Keywords: Proportional growth control, Intracellular K+, embryonic fin/limb bud

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OPTIMIZATION OF HIPSC CULTURE CONDITIONS IN AMBR® BIOREACTOR APPLYING A DOE APPROACH

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The seminal discovery of human induced pluripotent stem cells (hiPSC) contemporaneously in the Yamanaka and Thomson laboratories has tremendously advanced the fields of cell therapy and regenerative medicine. This is due to the inherent characteristics of hiPSC, including pluripotency, self-renewal capability, and low immunogenicity. However, hiPSC are difficult to propagate because they can spontaneously differentiate, display genetic instability, and require costly growth factors for propagation. The foremost process parameters affecting hiPSC aggregate growth in three-dimensional (3D) culture include, media and growth factors, feed strategy, bioreactor (BR) geometry, impeller speed, and seeding density. The Ambr15® is an automated multi-parallel BR system that provides 24 or 48 15 ml vessels to optimize culture parameters by implementing design of experiment (DOE) approaches with MODDE 13® software. Together with the small volume of each BR, this approach is an excellent solution for low cost, high throughput experiments. Evolving regulatory recommendations and desire to optimize consistency of cell expansion have prompted development of animal component-free (ACF) cell culture medium. To meet this demand, Sartorius developed NutriStem® hPSC 3D ACF media which supports iPSC growth both, in planar and suspension cultures. The impact of media type (Nutristem hPSC 3D ACF or xeno free (XF)), stir speed (600 vs 800rpm), and seeding density (2.5E5 to 5E5 cells/mL) on hiPSC growth was explored. Aggregate size, harvest density, and fold increase was measured over 4 days. Aggregates formed in all BRs with a >90% cell viability at harvest. At higher speed (800rpm), aggregates were 50% smaller than those formed at 600rpm. A 20-80% higher fold increase of hiPSC was achieved in NutriStem 3D ACF compared to XF media. Increased seeding density above 2E5 cells/mL decreased fold increase achieved in both media. The highest fold increase was observed when cells

were cultured using NutriStem ACF media at 800rpm and 1.25E5 cells/mL seeding density. The results demonstrate the power of utilizing the Ambr15 and a high throughput DOE-based approach to rapidly identify conditions that significantly influence hiPSC growth in 3D culture.

Keywords: Bioreactor, DOE, pluripotent stem cells

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OPTIMIZATION OF GROWTH FACTORS FOR HUMAN PLURIPOTENT STEM CELL CULTURE IN BASAL 8 MEDIA

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Commercial media can empower most laboratories that wish to culture human induced pluripotent stem cells (hiPSCs). Though, democratization of this technology is hindered by high costs, particularly for applications that require large scale cultures. Among homemade media, Basal 8 (B8) stands out as an attractive negligible-cost and weekend-free chemically defined formulation. However, B8 requires homebrewing three growth factors in E. coli: TGF-B3, NRG-1, and thermostable FGF2 (t-FGF2). This represents a bottleneck for research laboratories with limited expertise in recombinant protein purification. Furthermore, B8 requires a high concentration of t-FGF2 (40 ng/mL), leading to a substantial burn rate. In all, we set out to explore sourcing options to stimulate pluripotency signaling pathways in a reproducible yet cost-effective manner. In house production of growth factors, following published protocols, was successful yet plagued by batch-to-batch variations in endotoxin levels and t-FGF2 bioactivity. We thus pivoted to testing growth factors manufactured by various commercial suppliers. First, we compared various types of TGF-B signaling inducers, concluding that an animal-free E.coli expressed TGF-B1 has equivalent bioactivity yet higher purity and lower costs compared to mammalian cell-derived options. Animal-free TGF-B1 and TGF-B3 are both effective in B8, though at higher doses than previously reported, while Activin A is unsuitable. Then, we tested various types of FGF2, comparing wild type versus thermostable, and full length (154 aa) versus truncated (145 aa) variants. Tag-free FGF2-G3 (a t-FGF2 patented for commercial use by Enantis/Masaryk University) outperforms homemade his-purified t-FGF2, and its higher stability in media is essential for weekend-free feeding schedules. Additionally, the truncated FGF2-G3 is more bioactive than full-length, and therefore its dosage in B8 can be optimized. Characterization of hiPSCs maintained in optimized B8 versus commercial Essential 8 (E8) via single cell RNA sequencing, immunostaining, and trilineage differentiation confirmed pluripotency. Finally, culture of hiPSCs in optimized B8 supports robust cardiac differentiation. In all, these advances enable strong cost reduction while maintaining reproducibility of hiPSC culture.

Funding Source: This work was supported by an Armenise-Harvard Foundation Career Development Award (to A.B.).

Keywords: Basal 8 (B8) media, Human induced pluripotent stem cells, Growth factor optimisation

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VERIFICATION OF LINEAGE REPORTER HPSCS USING CRISPR-MEDIATED ACTIVATION

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Generation of lineage reporter human pluripotent stem cells (hPSCs) by genetic engineering provides powerful tools with broad applications, including studying biological function of gene of interest, isolating intended cell population, investigating stem cell fate evolution and specification, and performing high-throughput screening. Current approach to identify the knock-in of fluorescent reporter is using genomic PCR, sanger sequencing, and monitor the expression of reporter gene at specific differentiation stage. However, some genes only express in the terminal differentiated cells which require a complicated, expensive, and time-consuming differentiation process. In addition, the cell differentiation variation will affect reporter gene expression, which gives vague results for evaluation of the lineage reporter itself. Here, we comprehensively compared and optimized the current transcriptional activation systems in hPSCs and established a robust platform to identify lineage reporter hPSCs. With this platform, the reporter gene can be turned on efficiently in a transient or a sustained manner. The platform allows us to verify reporter gene expression rapidly and effectively without the complex cell differentiation steps.

Keywords: hPSC, lineage reporter verification, CRISPR-mediated transcriptional activation

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INDUCIBLE KRAB-DCAS9 IPS CELL LINES FOR CROSS-SPECIES CRISPR-BASED PERTURBATIONS

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CRISPR-based perturbation screens in stem cells have become important tools in developmental biology and can be an efficient method to infer gene regulatory networks (GRNs). A powerful variant are CRISPR interference (CRISPRi) screens, using a catalytically inactive (dead) dCas9 fused to a repressor domain like KRAB to knock down specific genes. Furthermore, to distinguish primary from secondary effects of the perturbations, an inducible, time-controlled expression of dCas9 is advantageous. While perturbation screens are often performed in individual species, cross-species CRISPRi systems will help to compare knockdown effects and study the evolution of GRNs. Here we present a system for the generation of doxycycline (Dox)-inducible KRAB-dCas9 iPSCs and its application to various primate species. We assembled a ZIM3-KRAB-dCas9-HA-P2A-mCherry construct flanked by species-specific, interchangeable homology arms, allowing flexible adaptation to different species. We utilized this system for stable integration into the AAVS1 safe harbor locus of iPSCs of four primate species: Human (Homo sapiens), Cynomolgus macaque (Macaca fascicularis), Gorilla (Gorilla gorilla) and Orangutan (Pongo abelii). The generated cell lines (dCas9-iPSCs) were confirmed by PCR, demonstrating integration into the AAVS1-sites. Furthermore, we validated Dox-induced KRAB-dCas9 expression by Western Blot, imaging and flow cytometry, while ensuring preserved pluripotency of the dCas9-iPSCs. To assess knockdown efficiency, we performed an inducible knockdown of SOX2 in the primate dCas9-iPSCs, which was subsequently quantified by qPCR. This analysis revealed increased KRAB-dCas9 levels and downregulation of SOX2 in Dox-induced dCas9-iPSCs compared to non-induced cells. Finally, we could also demonstrate KRAB-dCas9 expression and target-gene downregulation in a single-cell CRISPR-screen in human and cynomolgus dCas9-iPSCs. In conclusion, we present the successful integration of a KRAB-dCas9 encoding construct into the AAVS1-locus of different primate species and robust Dox-inducible knockdown in iPSCs. The generated dCas9-iPSCs will expand the possibilities for cross-species perturbation screens and be a valuable resource for comparative approaches in primates to investigate the evolution of GRNs.

Funding Source: This project is funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) - 458888224

Keywords: dCas9-iPSCs, CRISPRi, primates

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ROBUST WORKFLOWS FOR THE EXPANSION AND DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS AS AGGREGATES IN SUSPENSION CULTURE

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3-dimensional (3D) suspension culture of human pluripotent stem cells (hPSCs) has been challenging due to the extreme shear sensitivity of hPSCs, hindering efforts to reproducibly generate large volumes of high-quality cells. To address this, we have developed TeSR™ 3D-based media and protocols for the robust and scalable suspension culture of hPSCs as aggregates. hPSCs (n = 8 lines) passaged by dissociation with Gentle Cell Dissociation Reagent and filter-based trituration undergo 1.5- to 1.9-fold expansion per day (cell line dependent) with > 85% viable, > 90% OCT4+ and TRA-1-60+, and expected karyotype. To verify the utility of this workflow and assess the compatibility of STEMdiff™ hPSC differentiation media formulations designed for 2D cultures in 3D suspension culture, 2 hPSC lines were differentiated into polyploid megakaryocytes (MKs) or neural crest cells (NCCs) in 3D. The differentiation initiation day and length of differentiation stages were optimized in small-scale cultures using 6-well plates mixed at 70 RPM on an orbital shaker. For 3D hPSC-generated MKs, a 10-day endothelial-to-hematopoietic transition phase and a 4-day progenitor-to-mature MK stage maximized MK marker expression and yields, with 30 - 75% co-expressing CD41a and CD42b, and 15 - 60 CD41a+CD42b+ cells generated per seeded hPSC (n = 3). The hPSC-derived MKs from 3D cultures produced 3 - 8 platelet-like particles per MK (n = 2; gating based on blood platelets). Approximately 30% and 15% of CD41a+CD42b+ cells had 4N and 8N+ DNA ploidy, respectively (n = 2). For 3D hPSC-derived NCCs, maximum marker expression and yields were achieved by pre-forming aggregates in TeSR™-AOF 3D medium for 24 - 48 hours before 6 days of NCC differentiation resulting in > 60% of cells co-expressing CD271 and SOX10, and 3 - 15 CD271+SOX10+ cells per seeded hPSC (n = 2). When 3D hPSC-derived NCCs were dissociated and replated on Matrigel®-coated plates, cells differentiated and matured into sensory neurons expressing peripherin and BRN3A. 3D hPSC differentiation to NCCs was also validated at a larger scale in a PBS-MINI 0.1 L Bioreactor with similar results to the small-scale studies. These results demonstrate that the combination of TeSR™ 3D workflows and low-shear bio-

reactors provides a robust system for the expansion and differentiation of hPSC lines to MKs or NCCs.

Keywords: PSC Manufacturing and Scaleup, PSC Suspension Culture, PSC Differentiation in Suspension Culture

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MANIPULATION OF THE NUCLEOSCAFFOLD POTENTIATES CELLULAR REPROGRAMMING KINETICS

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Somatic cell fate is an outcome set by the activity of specific transcription factors and chromatin landscape and maintained by gene silencing of alternate cell fates through physical interactions with the nuclear scaffold. Here we evaluate the role of the nuclear scaffold as a guardian of cell fate in human fibroblasts by comparing the effects of transient loss (knockdown) and genetic modification (progeria) of functional Lamin A/C, a core component of the nuclear scaffold. We observed that Lamin A/C deficiency or modification disrupts nuclear morphology, heterochromatin levels and increases access to DNA in lamina-associated domains. Changes in Lamin A/C were also found to impact mechanical properties of the nucleus when measured by a new microfluidic cellular squeezing device. We last show that transient loss of Lamin A/C accelerates the kinetics of cellular reprogramming to pluripotency through opening of previously silenced heterochromatin domains while genetic modification of Lamin A/C into progerin induces a senescent phenotype that inhibits the induction of reprogramming genes. Our results highlight the physical role of the nuclear scaffold in safeguarding cellular fate.

Funding Source: NSF CAREER (2045977), NIGMS R01GM123517, CDMRP W81XWH2010336 and W81XWH2110491 (C.A.A.), Defense Advanced Research Projects Agency (DARPA) “BETR” award D20AC0002 (C.A.A.) NIBIB T32 EB005582 (BAY).

Keywords: Reprogramming, Lamina, Microfluidics

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A VERSATILE ANIMAL-COMPONENT FREE MEDIUM FOR PSC MONOLAYER AND MID-SCALE AGGREGATE SUSPENSION CULTURE

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In the last several years, the number of clinical trials with human pluripotent stem cells (hPSC)-based therapies is rapidly increasing. These advancements raise the need for safe and efficient system towards large scale expansion. Production of hPSC in high quantities for clinical applications using standard 2D adherent culture is inefficient and process scalability is difficult. A developing strategy to overcome these hurdles, is 3D suspension culture of pluripotent aggregates in environmentally control bioreactors. This approach enables reproducible production of a high numbers of pluripotent stem cells that can be used for differentiation into mature cells of various tissue types. Aiming for therapeutic applications, the quality of culture medium and its performance are particularly crucial, since hPSC properties can significantly be affected by medium components and culture conditions. More than that, with the stringent regulatory requirements the need for an Animal-Components Free (ACF) culture media is preferred to minimize the risks associated with infectious agents transmission and immune rejection of the transplanted cells. The present study describes development of a versatile ACF medium suitable for hPSC expansion using recombinant matrices (e.g. Laminin, Vitronectin) in 2D culture for multiple passages and as aggregates in dynamic small-scale suspension culture (e.g. shaker flasks, spinner flasks). This ACF medium was used in design of experiment (DOE) studies implementing MODDE® software to identify optimal design space for aggregate formation and growth in the Ambr® 250 modular bioreactor. Results show that ACF medium enables high proliferation rate of hPSC, while maintaining high pluripotency marker expression and stable karyotype. This advanced culture system would greatly facilitate the development of a robust, clinically acceptable culture process for generating quality-assured cells.

Keywords: Aggregate suspension culture, Animal-component free, Mid-scale expansion

958

THE PIN-POINT BASE EDITING PLATFORM STREAMLINES THE GENERATION OF HYPOIMMUNOGENIC IPSCS FOR ALLOGENEIC CELL THERAPY

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The Pin-pointTM platform enables the modular assembly of base editors composed of a DNA modifying deaminase and a DNA binding Cas component via an aptamer encoded in the sequence-targeting guide RNA (gRNA). We optimized conditions for the delivery of synthetic gRNAs and mRNAs encoding a Pin-point base editor composed of Rat APOBEC1 and SpCas9 nickase to iPSCs, and demonstrated that simultaneous multiplex editing with this editor configuration was significantly more efficient, and resulted in substantially enhanced cell viability and genome integrity compared to CRISPR-Cas9. To apply the Pin-point base editing technology to the engineering of hypoimmunogenic allogeneic iPSC lines we screened sgRNAs for efficient knock-out of class 1 and class 2 HLA antigens. Clonal engineered hypoimmunogenic iPSC lines retained pluripotency and could be differentiated to products with potential in a range of cell replacement therapies. To expand the utility of the Pin-point platform we optimised conditions for the co-delivery of reagents for targeted transgene integration and base editing. Combining aptamer containing Pin-point base editing gRNAs with aptamer-less gRNAs and a donor DNA template enabled simultaneous multigene knockout and transgene knock-in. The Pin-point platform therefore provides the capability to safely, efficiently, and precisely perform multiple genome engineering operations in a one-step process, offering the opportunity to dramatically streamline the development of allogeneic iPSC-derived cell therapies.

Keywords: base-editing, multigene, allogeneic

960

HIGH RESOLUTION SPATIAL TRANSCRIPTOMICS OF MAMMALIAN EMBRYONIC DEVELOPMENT

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Embryonic development in time and space is one of the most fascinating processes in biology. In recent years, single cell molecular profiling studies have greatly advanced our knowledge of mammalian embryogenesis. However, the loss of anatomical information precludes a deeper understanding of the spatio-temporal regulation of cell identity in the developing embryo. The emerging field of spatial transcriptomics closes this gap by enabling the profiling of both cell identity and positioning within complex tissues. Here, we analyzed the full section of a formalin-fixed, paraffin-embedded (FFPE) mouse embryo using the synergistic power of both in situ and spatial transcriptomics. In a first step, we interrogated the mouse embryo section using the 10x Genomics Xenium In Situ platform, a novel high throughput microscopy-based technology that allows the automated analysis of both pre-validated and custom gene panels in situ. The subcellular resolution of the Xenium In Situ platform enabled us to resolve fine morphological structures and zoom into complex anatomical areas of the early mouse embryo. To leverage the non-destructive nature of the Xenium workflow, we next performed H&E staining followed by downstream whole transcriptome capture using the 10x Genomics Visium CytAssist platform. This novel technology allows the transfer of target molecules from any standard tissue section onto Visium Spatial Gene Expression slide capture arrays. Both datasets are highly complementary and together offer a more complete view on the sample type. It enabled us to refine both gene clustering and cell typing and study the co-expression of marker genes across the entire embryo section. In summary, the integration of both high resolution in situ data with the unbiased whole transcriptome readout of Visium provide deep insights into the complex spatial organization of the early mouse embryo and offer a compelling snapshot of mammalian development.

Keywords: Spatial Transcriptomics, In Situ Technology, Embryonic Development

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CLASS-I HLA ENGINEERED HIPSCS AS "OFF THE SHELF" IMMUNE-MATCHED CELLS FOR CELL THERAPY AND REGENERATIVE MEDICINE

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Induced Pluripotent Stem Cells (iPSCs) can be differentiated into virtually all cell types and can therefore serve as a renewable source of cells for cell therapy and regenerative medicine. However, using allogeneic cells in clinical settings is limited by the risk of adverse immune responses, particularly in cases of HLA mismatch. Finding HLA-matched donors is a strategy used to prevent graft vs. host disease during transplantation, but the likelihood of finding a match is slim, especially for individuals with rare HLA types. Having banks of iPSCs available for use in cell therapy and tissue engineering would significantly increase the availability of treatments. Here, we present a strategy for genetically engineering the MHC I locus of iPSCs to create an on-demand library of immune-matched stem cells that can be readily available to treat disease in patients with any HLA type. We have deleted the three HLA class I loci in human iPSCs and will use this 'hiPSC blank" cell line as a platform to insert HLA types of interest back into the genome using our patented REWRITE system. Using yeast recombineering, we relocated the HLA-A locus within the 80 kb intergenic region between the HLA-B and HLA-C loci, thereby generating a 100 kb refactored locus called synHLA, which retains native transcriptional control We have also created a minimized version of the HLA locus that contains only the HLA-A, -B, and -C genes and their respective regulatory regions that are required for proper HLA gene expression. We built three HLA haplotypes, and integrated one of the 100 kb synHLAs into the hiPSC bank, thus generating "HLA personalized" iPSCs. Our method for creating HLA-engineered iPSCs enables the creation of off-the-shelf banks of iPSCs that can be "personalized" for use in any patient. Additional cellular reprogramming and gene editing can be performed on these iPSCs to engineer cells and tissues for a variety of uses in clinical settings.

Funding Source: NIH (NIAID) Director's New Innovator Award (DP2)

Keywords: Genome Engineering, iPSCs, Off-the-shelf

TOPIC: NO TISSUE SPECIFICITY

964

A SMART PIPETTING SYSTEM FOR TRACEABLE CLONING OF SINGLE STEM CELLS

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The aim of this study was to develop a simple and inexpensive method that enables the cloning of living single cells. Most importantly, the method shall provide an indisputable proof of clonality to be compliant with the guidelines published by the WHO. We have engineered an impedance-based sensing tip coupled to a custom made pipetting system: DISPENCELL. The pipetting robot allows the isolation of individual single cells while providing a record of the trace of impedance. A single peak is the mark of a single cell whereas multiple peaks indicate that several cells or doublets have been isolated. Impedance records serve as traceability and quality control of the single cell isolation. The capability of DISPENCELL to isolate single cells into wells was first tested. Of the culture wells that passed the impedance-based quality control, 80% of wells contained a single cell, whereas the other wells were empty or contained doublets. Next, the cloning pipetting system was tested for its capacity to clone human epidermal normal and cancer stem cells as well as rat hair follicle multipotent stem cells. The regenerative capacity of the progeny of each clone was then assessed in state-of-the-art in vitro and in vivo assays. These tests demonstrated that the cloning pipetting system does not impact stem cell properties. Our new impedance-based method to isolate single stem cells using DISPENCELL is reliable, intuitive and cell-friendly. Above all, it has the capacity to record the isolation of single cells, a key feature for regulatory affairs. Therefore, it fulfils an unmet need of many scientists in search of a single-cell cloning method for the development of biotherapies and personalised medicine.

Keywords: stem cell cloning, stem cell functionality, regenerative capacity

TOPIC: NEURAL

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NEAR-INSTANT DETECTION OF GLIOMA STEM CELLS IN LIVE HUMAN GBM-TISSUE

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Emerging research suggests that failure to target glioma stem cells (GSCs) rather than the inability to remove tumors through surgery, radi¬ation, or chemotherapy, explains the poor survival

of GBM patients. In this study, a luminescent conjugated oligothiophene (LCO), named GlioStem (p-HTMI), is used for non-invasive and non-amplified real-time detection of GSCs in live human GBM-tissue. More than 90 patient samples were stained, quantified, and analyzed by fluorescent microscopy for the presence of Gliostem-positive (GS+) cells. Approximately 30 of the samples were FACS-sorted for GS+ and GS- cells where quantification by FACS could verify microscopy results. In addition, GS+ cells were shown to express significantly higher levels of stem cell markers (CD271, CD133, PDGFRa, CD44) in FACS-experiments. Bulk RNA sequencing of 7 GBM patient samples with paired GS+ and GS- sorted cells revealed that GS+ patient samples clustered together, whereas the GS- populations did not cluster to¬gether neither with each other nor with the GS+ populations. These data suggest a distinct heterogeneity in the GS- samples and a certain level of homogeneity regarding the GS+ populations, independent of intra-patient or patient-to-patient heterogeneity. Moreover, the GS+ samples were found to express significantly higher levels of stem cell markers including SOX10, OLIG1/2, and ASCL1, compared to the GS- samples. More specifically, the GS+ samples exhibited significantly higher expression of 35 genes associ¬ated with stemness compared to the GS- samples, with most markers being associated with pre-oligodendrocyte precursor cells (pre-OPCs) and pro-neural subtypes. Our results suggest that GlioStem is a versatile tool for near-instant and selective detection of GSCs in live tumor tissue. Detecting and eliminating these cells during tumor resection may therefore be an im¬portant aim in efficiently preventing tumor regrowth and would mean a crucial step towards increased patient survival.

Funding Source: The Swedish Childhood Cancer Foundation (Barncancerfonden), The Swedish Cancer Society (Cancerfonden), Bio-X/Tillväxtverket

Keywords: Glioblastoma; Thiophene; Neurosurgery, malign

TRACK: CLINICAL APPLICATIONS (CA)

Session 5: Odd

1:15 PM – 2:00 PM

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DEFINED CGMP ANIMAL-FREE MEDIUM SUPPORTS PLURIPOTENT STEM CELLS DURING, AND PROMOTES SINGLE-CELL CLONAL EXPANSION AFTER, MICROFLUIDIC SORTING

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Pluripotent stem cell (PSC) fate is, by nature, influenced by environmental cues. Dissociation, followed by deposition into a single-PSC microenvironment to establish a unique clonal cell line challenges the status quo of aggregate propagation. Subsequent inefficiency of PSC clonal expansion is a barrier to gene editing workflows for basic and translational research. Further, successful translation of stem cell-based therapies requires tracking the cell product and the components in contact with the product throughout the manufacturing process. To facilitate this transition, new fully defined, animal-free, cGMP-compliant tools are in demand. HiDef-B8-cGMP is an optimized PSC maintenance medium manufactured under relevant cGMPs and was developed with traceable, animal-free raw materials. Here we describe a protocol for robust single-cell cloning using microfluidics-based cell sorting in combination with HiDef-B8-cGMP complete medium used as both sheath fluid, as well as pluripotency maintenance medium. A single-cell suspension of PSC was obtained using a cGMP-compliant enzymatic reagent. To prevent natural aggregation in suspension, complete HiDef-B8 was supplemented with 10% enzymatic reagent and used as “sorting sample buffer.” PSC suspensions were then sorted using the gentle WOLF®, a microfluidic cell sorter that facilitates using HiDef-B8 as sheath fluid at operating pressure <2 psia. PSC were sorted for SSEA-4 and TRA-1-60-R, surface markers of self-renewal and pluripotency. Prior to cell sorting and seeding, 70% of the cells were positive for SSEA-4 and TRA-1-60-R. After sorting, greater than 90% were positive for both markers and greater than 99% of the cells were viable via SYTOX Green®. Next, we compared 5-day adherent outgrowth in a quantitative multi-well PrestoBlue™ assay after seeding PSC from single-cell suspensions and found no difference in the performance of HiDef-B8-cGMP 400x supplemented into Defined Bioscience’s cGMP-compliant stable formulation of DMEM:F12 compared to another commercially available formulations of DMEM:F12 also manufactured under cGMP.

Keywords: clonal expansion, cGMP, Microfluidic cell sorting

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USING IPSC AND GENE EDITING TECHNOLOGIES TO GENERATE IMMUNE-CLOAKED OFF-THE-SHELF CELL THERAPIES

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Human induced pluripotent stem cells (iPSCs) can be renewed, genetically modified, scaled and differentiated into almost any cell type. These characteristics make iPSCs an excellent tool for CAR cell therapies, with several clinical trials recently initiated. However, the risk of immune-rejection caused by the mismatch of human leukocyte antigens (HLA) between the therapy and the patient poses a challenge to the continued growth of the clinical applications of iPSC-based cell therapies. Though solutions, such as HLA-banking or generation of iPSCs from each patient have been proposed, they are unrealistic given the costs and regulatory compliance required. Generation of immune-cloaked cells can be accomplished with gene editing technologies by preventing presentation of the HLA class I and II antigens on the cell surface, while at the same time overexpressing specific transmembrane proteins. This solution will greatly reduce time and expenses of cell therapies, with huge benefits for the patients. However, application of the CRISPR technology remains challenging for generating cells with multiple genetic alterations, especially in iPSCs which are sensitive to these procedures. Here we present a

streamlined pipeline to produce gene edited iPSCs. We first determine the editing efficiencies of CRISPR complexes at target sites by performing deconvolution of Sanger traces. Once the CRISPR components are validated, iPSCs are single sorted and using imaging software for systematic monitoring we can assure single cell clonality. Clone screening for the correct edits is performed, followed by NGS analysis for validation. We have achieved up to 90% editing efficiencies using our optimized conditions and >30% recovery from single iPSCs. Our bespoke gene editing platform offers state-of-the-art facilities and fits in seamlessly with our good manufacturing practice (GMP)-compliant master cell banking and proprietary cell-specific differentiation pipelines.

Keywords: Allogeneic cell therapy, iPSC based cell therapy, Gene editing technology

TOPIC: CARDIAC

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IMMUNE SHIELDED AND SAFE IPSC-DERIVED CARDIOMYOCYTES FOR HEART REPAIR

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Heart failure (HF) is a global health burden aggravated by the marginal regenerative capacity of cardiomyocytes. A single myocardial infarction can cause irreversible loss of a billion cardiomyocytes, leading to HF with reduced ejection fraction (HFrEF). Despite optimal medical treatment, HF worsens over time, eventually leaving end stage HF patients with ventricular assist devices or rare heart transplantation as last treatment options. Regenerative cell therapy holds the potential for a novel treatment of HFrEF with curative character, and active investigations are ongoing to re-muscularize the failing heart with stem cell-derived

cardiomyocytes. To offer a safe and sustained therapeutic effect, we aim to generate genetically engineered off-the-shelf allogeneic iPSC-derived cardiomyocytes (iCMs) that are shielded from the patients' immune system (cloaking), carry a drug-inducible kill-switch and possess a safety handle for arrhythmia prevention. Using a validated GMP iPSC line as starting material, we have established a 10-days GMP-compatible 3D differentiation protocol that enables robust and large-scale production of iCMs with very high purity and yield. To ensure reproducible quality and safety of our cells, we perform stringent monitoring of all process stages using a QC panel including, amongst others, flow cytometry, transcriptome analysis, as well as genetic integrity assays. iCMs are tested for their contraction and remuscularization capacity in vitro and in vivo. Differentiated iCM exhibit a marker profile of >97% pure, immature cardiomyocytes. Utilizing our 24-well plate engineered heart tissue platform, we demonstrate contractility and maturation capacity of our iCM in vitro. When injected into cryo-injured hearts of a guinea pig model, the iCM efficiently engraft, mature and form contacts with host myocardium. We confirm that kill-switch engineered iPSC are sensitive to the inducer drug in a dose-dependent manner in vitro.

Keywords: Heart regeneration, iPSC-derived cardiomyocytes, Heart failure

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CHARACTERISATION AND EXPERIMENTAL TREATMENT OF CARDIOMYOCYTES DERIVED FROM HIPSCS FROM A PATIENT WITH BAG3P209L MYOFIBRILLAR MYOPATHY

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BAG3 is a member of the chaperone-assisted selective autophagy (CASA) complex and plays an important role in cellular protein homeostasis. A rare exchange of a single nucleotide in the BAG3 gene, causing the dominant negative missense mutation P209L, leads to myofibrillar myopathy with onset in childhood (OMIM: MFM6). Affected patients suffer from muscle dystrophy, restrictive cardiomyopathy and neuropathy with a life expectancy of around 20 years. For detailed characterisation of the disease and to establish new experimental treatments, we established an induced pluripotent stem cell (iPSC) line by reprogramming fibroblasts from a young patient suffering from BAG3P209L myofibrillar myopathy. These iPSCs were differentiated to cardiomyocytes (CMs) and further analysed by immune-histology, western-blot analysis and live-imaging of beating CMs to get insights into the pathomechanism. Previously studies on mice overexpressing human BAG3P209L showed that a siRNA-mediated knockdown of hBAG3P209L rescued the disease's phenotype. To translate this to the human system, we had to identify a point mutation-specific siRNA that can distinguish between the wild type and mutated BAG3. Therefore, we tested the allele specificity of 19 different siRNAs on the expression levels of hBAG3WT and hBAG3P209L in patient-derived iPSCs. Our goal was to find an siRNA that specifically knocks down the expression level of hBAG3P209L. For detection we used a single nucleotide polymorphism (SNP) -specific quantitative PCR assay which is able to determine the ratio of hBAG3P209L to BAG3WT-mRNA. We are currently testing the most specific siRNAs to analyze whether a specific reduction in BAG3P209L expression level can be achieved and whether we can thereby achieve a change in the phenotype of the pathology.

Keywords: siRNA-mediated allele-specific knockdown, Restrictive cardiomyopathy, MFM6, Patient-specific iPSCs

TOPIC: EPITHELIAL INCLUDES SKIN GUT LUNG

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A TRANSLATION-UP APPROACH USING PLURIPOTENT STEM CELLS TO TREAT CYSTIC FIBROSIS

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Cystic Fibrosis (CF) is the most common lethal autosomal recessive genetic disease in Caucasians. CF is caused by mutations in the cystic fibrosis transmembrane regulator (CFTR) gene, which encodes a chloride ion transporter protein. The CF disease landscape is varied, with over a thousand CFTR mutations grouped into 5 classes, each requiring a different therapeutic approach. Here, we propose a broadly applicable stem cell-based therapeutic approach that, in principle, can benefit CF patients harboring any of the 5 classes of CFTR mutations. The inspiration for this approach came from inspection of the CFTR sequence, which revealed sequences in its 5' UTR suggestive of sub-optimal translation. This raised the possibility that eliminating the sub-optimal elements in the CFTR 5'UTR could increase CFTR translation. The following data supported this hypothesis: First, we found that a single-nucleotide substitution at a key Kozak consensus site following the CFTR start ATG significantly increased reporter expression in transfected 16HBE bronchial epithelial cells. Second, we identified and removed an upstream open-reading frame (uORF) in the CFTR 5' UTR, which also increased reporter expression. Third, a double mutation improving both the Kozak consensus sequence and eliminating the uORF led to a synergistic (3.5 fold) increase in reporter expression. Experiments are in progress to make these 2 mutations in the endogenous CFTR gene in pluripotent stem cell lines harboring CFTR G542X and W1282X nonsense mutations . By differentiating these iPSCs into mature lung cells, we will test their ability to confer therapeutic benefit to the lung, which is the primary site of CF disease pathology. We propose that this "translation-up" stem-cell approach will be universally applicable to all CF patients. Those with only modestly compromised CFTR function may benefit from this approach alone, while those with severely compromised CFTR function will require pairing of the translation-up approach with currently existing therapeutics, such as CFTR modulator drugs and PTC-readthrough drugs.

Keywords: iPSC, cystic fibrosis, guided differentiation

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IDENTIFYING BIOMARKERS OF RETINAL PIGMENT EPITHELIAL CELL STEM CELL-DERIVED RPE CELL HETEROGENEITY AND TRANSPLANTATION EFFICACY

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Transplantation of retinal pigment epithelial (RPE) cells holds great promise for patients with retinal degenerative diseases such as age-related macular degeneration. In-depth characterization of RPE cell product identity and critical quality attributes are needed to enhance efficacy and safety of replacement therapy strategies. Here we characterized an adult RPE stem cell-derived (RPESC-RPE) cell product using bulk and single cell RNA sequencing (sc-RNA-seq), assessing functional cell integration in vitro into a mature RPE monolayer and in vivo efficacy by vision rescue in the Royal College of Surgeons rats. scRNA-seq revealed several distinct subpopulations in the RPESC-RPE product, some with progenitor markers. Our gene expression analysis revealed a lncRNA (TREX) as a predictive marker of in vivo efficacy and integration. TREX knockdown decreased cell integration while overexpression increased integration in vitro and improved vision rescue in the RCS rats. Our scRNA analysis identified specific RPE clusters that express TREX, suggesting that select subpopulations may differentially influence the efficacy of RPE transplantation.

Funding Source: This study was funded by the National Eye Institute, National Institutes of Health (R01EY029281 to J.H.S.)

Keywords: RPE stem cell transplantation, Regenerative medicine, Biomarker discovery

TOPIC: HEMATOPOIETIC, IMMUNE, AND ENDOTHELIAL

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CONTROLLED HEMATOPOIETIC LINEAGE SPECIFICATION USING MORPHOGEN IN HUMAN PLURIPOTENT STEM CELLS

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Human pluripotent stem cells (hPSCs) have infinite potentials for regenerative medicine. Among them, artificial blood technology, which uses stem cells to make various blood components, is currently at the basic level of research. Retinoic acid (RA) signaling is known to regulate definitive hematopoietic development during embryogenesis. However, the role and consequences are not well understood when RA signaling at each step of the blood differentiation. Here we show that RA has an effect on Hemogenic Endothelium (HE). Modulation of RA signaling at the HE formation stage does not affect HE production, but it does make a difference between hematopoietic progenitor cells and mature blood cells by regulating the function of HE. In addition, RA develops the functional properties of hematopoiesis upon temporary treatment on hematopoietic progenitor cells. The potential of RA to differentiate from hematopoietic progenitors into specific blood lineage provides clear signal conditioning for application in specific hematopoiesis process. Our study reveals a previously unexplained RA signaling pathway during early human hematopoiesis, and demonstrates distinct role of fate specification and functional activation through temporal RA regulation.

Funding Source: This research was supported by the KRIBB Research Initiative Program Grants (KGM4562323), the Korea government (MSIT) (NRF-2019R1A2C1086988), and the Korea government (MSIT) (NRF-2022M3A9E4082684)

Keywords: human pluripotent stem cell, hematopoiesis, retinoic acid

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HUMAN PLURIPOTENT STEM CELLS DERIVED ENDOTHELIAL CELLS REPAIR CHOROIDAL ISCHEMIA

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Choroidal atrophy is a common fundus pathological change closely related to the development of age-related macular degeneration (AMD), retinitis pigmentosa, and pathological myopia. Studies suggested that choroidal endothelial cells (CECs) that form the choriocapillaris vessels are the first cells lost in choroidal atrophy. Therefore, restoring the choriocapillaris vessels is crucial to the prognosis of the above diseases. We found that endothelial cells derived from human pluripotent stem cells (hPSC-ECs) through the MESP1+ mesodermal progenitor stage expressed CECs specific markers and can integrate into choriocapillaris. In a rat model of choroid ischemia (CI), transplantation of hPSC-ECs into the suprachoroidal space increased choroid thickness and

vasculature density. Close-up examination showed that engrafted hPSC-ECs formed new blood vessels and integrated with existing rat vessels. Our work demonstrated that hPSC-ECs could be used to repair choroidal ischemia in the animal model, which may lead to a new therapy to alleviate choroidal atrophy implicated in AMD and other ocular diseases such as retinitis pigmentosa, and pathological myopia.

Keywords: Choroidal ischemia, human pluripotent stem cells, endothelial cells

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OPTIMIZATION OF IN VITRO SAFETY ASSAYS FOR GENE THERAPY TO PREDICT RETROVIRAL VECTOR-INDUCED GENOTOXICITY

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Severe adverse events (SAE) in retroviral vector-mediated gene therapy clinical trials urge the field for a more reliable pre-clinical safety assessment. Lymphoid and myeloid leukemia were triggered in some of the treated patients due to the dysregulation of proto-oncogenes neighboring the vector´s insertion sites. We previously developed the In Vitro Immortalization Assay (IVIM) and Surrogate Assay for Genotoxicity Assessment (SAGA) to quantify the genotoxic risk of integrating vectors. Here, murine hematopoietic stem and progenitor cells (mHSPCs) transduced with mutagenic vectors acquire a proliferation advantage under limiting dilution (IVIM) and activate stem cell-like and cancer-related transcriptional programs (SAGA). However, both assays are limited by a myeloid differentiation bias. To broaden the spectrum of insertional mutants detected, we differentiated mHSPCs to mature T cells and analyzed their phenotype, insertion site patterns, and gene expression changes after transduction with retroviral vectors with different genotoxic potentials. We show that a highly mutagenic gammaretroviral vector induced a block in T cell differentiation at an early progenitor stage, which coincided with the uprising of a distinct oncogenic signature detected by the machine-learning algorithm in SAGA. Characteristic insertions nearby the leukemogenic transcription factor Lmo2 were present in the dominant clones, reproducing clinical observations. In this setting, a safer self-inactivating lentiviral vector (SIN-LV) with an internal cellular promoter behaved similarly to untransduced samples. However, further optimization is still needed to bring this assay further in the pre-clinical stage. Currently, we are testing the sensitivity of the new lymphoid pipeline with other SIN-LV designs relevant to the field, e.g., containing strong internal viral-derived promoters (SFFV, MND). Aiming towards a more accessible readout, we are evaluating the expression levels of the lymphoid predictors found by SAGA using a qPCR panel, thus reducing costs and technical complexity. Lastly, ectopic overexpression of the candidates in the lymphoid and myeloid IVIM/SAGA assays will likely lead to a better understanding of the mechanisms underlying insertional mutagenesis.

Keywords: genotoxicity prediction, retroviral vectors, gene therapy

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DEVELOPMENT OF INDUCED PLURIPOTENT STEM CELL-DERIVED T CELLS AS A NOVEL THERAPEUTIC PLATFORM FOR CANCER IMMUNOTHERAPY

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CAR-T therapy has been one of the most highly acclaimed cancer treatments for the past decade. This has been found to be particularly effective against some types of hematological malignancies, and it is anticipated that more simplified, less expensive and more effective cancer immune cell therapies will be realized for clinical application. The most advanced of these is the regeneration of immune cells using induced pluripotent stem cell (iPSC), and significant investments are being made to develop platforms using iPS cell-derived immune cells, such as iPSC-derived NK cells. Clinically validated products, such as autologous CD19 CAR-T have further encouraged these, while many iPS-derived immune cells reported are classified as innate immune system, thus iPS-derived immune cells which mimics adaptive immune system are the next challenge. In this study, we introduce the novel iPSC-derived T cell (iPS-T) platform development which conveys adaptive signatures such as CD4, CD8 and alpha / beta TCR. These iPS-T cells can be generated in organoid-based condition which employs thymic T cell differentiation and don't require murine-derived feeder cells. Robustness, reproducibility and less technical burden are typical improvement for this culture method, suggesting potential application to clinical manufactur-

ing process. To characterize the functional properties of these organoid-derived iPS-T cells, we have harbored CD19-targeted chimeric antigen receptor and found potent anti-tumor effects both in vitro and vivo. Collecting together, these finding provide new insight into the potential of iPS cells for production of a broader range of functional immune system and their potential for clinical applications as the novel off the shelf cancer immunotherapy.

Keywords: iPSC, T cell development, Immunotherapy

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CONTINUOUS PRODUCTION OF FEEDER-FREE HUMAN IPSC-DERIVED DENDRITIC CELL SUBSETS FOR THERAPEUTIC APPLICATIONS

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The number of human clinical trials employing hematopoietic stem cells (HSCs) and their derivatives – including dendritic cells (DCs) – for cell-based immunotherapies is increasing substantially. However, hurdles such as donor availability, donor cell quality, quantity amongst other factors limit the broad applicability of primary donor cells. Instead, human induced pluripotent stem cells (hiPSC) might serve as an alternative approach to generate specific immune cell types in scalable numbers for future innovative cellular immunotherapies. We previously reported the ability to continuously produce hiPSC-macrophages in long-term cultures for several months. Here, we extend this technology and report continuous production of iPSC-derived DCs (iDCs). By nature, DCs are a heterogeneous population of cells that function as sentinels of the immune system and play an essential role in the initiation and regulation of immune responses. Given the importance of these cells in immune regulation and activation, iDCs may pave the way for potential innovative immunotherapies. Our novel method entails pre-culture of iPSC followed by the generation of 3D aggregates (hemanoids) with hematopoietic potential expressing CD309+,TRA-160- (99%±3%) CD34+CD144+ (25%±5%). By adding defined cytokines to control DC fate, we can continuously generate different subsets of iDCs that are produced semi-weekly to weekly in continuous scalable cultures for more than five weeks. Of note, we have developed three different protocols to generate multiple iDC subsets representing DCs from FLT3, GM-CSF, and AhR antagonist cultures. Interestingly, we observed similar immunophenotype profile (CD123+CD141-, CD123+CD141+, CD123-CD141+, CD123-CD141-) in all three iDC cultures and noted differences in the expression ratios of the different populations in the culture conditions. In terms of functionality, the generated iDCs possess antigen-processing capabilities associated with increased MHC II expression. Furthermore, we observed HCMV transfectability in our DCs, which could be used as targets for the generation of HCMV-specific T cells against various naturally occurring HCMV epitopes. In summary, we present innovative methods for the continuous generation of fully functional DC subsets for future therapeutic applications.

Keywords: iPSC derived Dendritic Cell Subsets, Cell Manufacturing, Immunotherapies

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VENETOCLAX-RESISTANT IPSC-DERIVED NATURAL KILLER CELLS FOR IMPROVED LEUKEMIA TREATMENT

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This study investigates the derivation and use of venetoclax-resistant induced pluripotent stem cell (iPSC)-derived natural killer (NK) cells as a cellular therapy treatment for acute myeloid leukemia (AML). AML is one of the most aggressive blood cancers, and less than half of patients are cured. NK cells, known to have potent anti-AML activity, and venetoclax, an anti-cancer drug, are promising treatment options for curing AML in patients. Venetoclax has anti-leukemia activity through its inhibition of the BCL-2 protein—a mitochondrial protein that regulates apoptosis and is often abnormally activated in AML cells. However, treatment of patients with venetoclax has the unintended consequence of killing immune cells important in controlling AML, including NK cells. Here, venetoclax-resistant iPSC-NK cells are generated by use of CRISPR-Cas 9 gene editing to knock-in the BCL2 G101V mutation that has been demonstrated to mediate venetoclax resistance into iPSCs. After this gene editing, iPSCs homozygous for the mutation were selected. The mutant iPSCs were then differentiated into NK cells, using methods standard by our group. The venetoclax-resistant, BCL2 G101V NK cells were found to be approximately 100-fold more resistant to venetoclax than wildtype cells. A 36-hour Incucyte cytotoxicity assay testing the killing activity of NK cells against the MOLM-13 AML cell line found that the mutant NK cells retained their cytotoxicity in the presence of venetoclax, while the wildtype cells were inhibited. Additional degranulation and cytokine production assays also demonstrate the BCL2 G101V NK cells were unaffected by venetoclax. Additionally, BCL2-G101V-iPSC-NK cells demonstrated improved cytotoxicity against both wildtype and venetoclax-resistant MOLM-13 cells, when compared to the wildtype NK cells. We are currently doing studies to treat mice with combination therapy consisting of venetoclax and the BCL2-G101V-iPSC-NK cells, to evaluate the effectiveness of the combined treatment in an in-vivo model. The outcome of this study will contribute to the development of a novel, effective, combination leukemia therapy.

Keywords: NK cell, immunotherapy, iPSC induced pluripotent cell

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COMPARISON OF ISOLATION MODALITIES OF EXTRACELLULAR VESSICLES FROM BIOFLUIDS

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In the search for novel biomarkers of disease, extracellular vesicles (EVs) have emerged as a promising tool for biomarker discovery. As mediators of cell-cell communication, EVs are enriched in many biofluids such as plasma and serum. EVs contain proteins and other biomolecules that regulate both homeostatic and pathological processes. Obtaining blood samples for disease diagnosis is relatively noninvasive, simple, and cost effective. However, methodology for EV isolation is limited by EV heterogenetic and yield. To address these challenges, the goal of the current project is to investigate EV isolation strategies from biofluids for biomarker discovery. For identification of EV-derived prognostic and diagnostic biomarkers, optimal methodology of EV isolation from human biofluids must be established. In this study, serum and platelet poor plasma (PPP) were used. EVs were isolated using size exclusion chromatography (SEC), tangential flow filtration (TFF) and centrifugal filters. Nanoparticle tracking analysis (NTA) was performed to quantify EV yield and purity. PPP and serum isolated from whole blood samples were collected from healthy donors. From 150uL samples, 2.6e10 EVs/mL were isolated using TFF for serum and 2.85e10 for PPP, 1.14e10 EVs/mL using SEC for serum and 7.58e09 for PPP, and 7.35e13 using centrifugal filters for serum and 4.13e13 for PPP. EVs from all modalities had comparable properties with a median size of 149.25nm and average zeta potential of -15.70mV. Preliminary data indicates the highest EV yield from centrifugal filters but low purity of EVs. TFF and SEC had lower EV yield with higher purity. Therefore, TFF and SEC will be explored in future studies with additional EV characterization tools for biomarker discovery applications. This study will have significant implications for early diagnosis and treatment monitoring in a range of diseases.

Keywords: Extracellular Vesicles, Biomarker, Spinal Cord Injury

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TRANSFUSION-READY RED BLOOD CELL GENERATION FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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There is constant need of blood products that are generally rely on blood donations, although the world-wide demand do not meet availability. In vitro red blood cell (RBC) production would substantially supplement this need, and would provide further benefits such as thoroughly screened products, possibility of genetic manipulation and therapeutic loading. IPSC is a promising cell source to derive transfusable RBCs and blood products due to their immortality, donor independency, availability in GMP-grade and as universal source (e.g. HLA-matched, blood group matched). However till to date the field lacks a sufficient iPSC differentiation protocol, that is capable of reaching required erythroid yield suffice transfusion, due to developmental immaturity, inefficient enucleation (5-25%) and traditionally applied small-scale, static culture conditions. RBC-transfusion products generally contain large cell quantity (10^11-12 cells/unit) and to be able to propagate that, feasible iPSC differentiation with high enucleation and scalable, suspension culturing allowing bioreactor applications is required. Here we describe a 4 phase iPSC to RBC differentiation platform and their translation process from static/adherent to dynamic/suspension culture condition, allowing scalability and eventual bioreactor application. Our system gives rise to ~16000 fold increase in cell number, with a constant 50-60% enucleation, thus a mini-transfusion (10^11 RBC, required in phase I trial) according to our calculation could be generated from 6x10^6 iPSC in bioreactors. The iRBC derived here, passed the necessary functional assays, including but not limited to morphological analyses, hemoglobin content, blood group phenotype, deformability, oxygen dissociation. The stability of iRBC is currently tested in mouse transfusion experiments using humanized MISTRG mice. Translation to bioreactors, including novel bioreactor design, culturing parameters, feeding regimen, in house GMP-grade media development is momentarily ongoing. In conclusion, an efficient 4 phase iPSC-RBC differentiation and their translation to dynamic culturing described here for the first time, provides a bridge from small-scale static culturing to large-scale bioreaction RBC production aiding clinical transfusion application.

Keywords: red blood cell, transfusion, cellular therapy

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DEVELOPMENT OF AN OFF-THE-SHELF ALLOGENIC ANTI-CANCER CELL THERAPY USING POTENT IPSC-DERIVED NATURAL KILLER CELLS

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Autologous CAR-T cell therapies have been a game changer in the treatment of specific patient subsets and forms of cancer. However, many challenges remain, including manufacturing complexity for patient-derived cell products, insufficient starting material in immune-exhausted patients, limited therapeutic efficacy against solid tumors, and antigen escape, all of which can limit the durability of response or prevent success in a broad range of cancer types. Human iPSCs can solve some of these hurdles by providing unlimited starting material for manufacturing of allogenic, off-the-shelf cell products, thereby simplifying logistics with lower overall cost per dose. In addition, iPSCs can be genetically modified at the pluripotent stage to give rise to a range of functionally enhanced end products after directed differentiation. Given the innate anti-tumor potential of NK cells against solid tumors due to their immunosurveillance ability, modified NK cells have emerged as a potential alternative to CAR-T cell therapies. CAR-NK cells increase specific tumor killing retaining their natural tumor recognition and killing ability, lack the potential to cause GvHD, and trigger minimal to no cytokine release syndrome (CRS). As part of the EVOcells oncology programs, we have established a scalable feeder- and cell sorting-free process that reproducibly generates highly pure iPSC-derived natural killer (iNK) cells. Our iNK product shows typical NK cell morphology, marker expression profile and function, including ADCC, cytokine secretion, and strong anti-tumor killing activity. Using a GMP iPSC line and our optimized GMP-compatible gene-editing workflow, we have obtained CAR-expressing iPSC clones and differentiated them into CAR-iNK cells. Stringent QC of pluripotent starting material, intermediate and end product confirmed genetic integrity, proper CAR expression, functionality and purity of the CAR-iNK product. Using our scalable EVOcells iNK differentiation process, we generate highly pure and potent iNK cells. Genetic modification of a GMP iPSC line and subsequent differentiation results in CAR-iNKs with strong target-specific cytotoxic activity against primary tumor samples and tumor cell lines in vitro, indicating their great potential as an alternative to autologous CAR cell therapies.

Keywords: iPSCs, NK cells, Cell therapy

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EPITOPE ENGINEERED HEMATOPOIETIC STEM AND PROGENITOR CELLS TO ENABLE MULTI-SPECIFIC CAR-T CELLS FOR ACUTE MYELOID LEUKEMIA

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Despite the use of hematopoietic stem/progenitor (HSPC) cell transplantation (HSCT), acute myeloid leukemia (AML) is associated with a poor outcome for >50% of patients. Whereas novel immunotherapies, such as CAR T cells and bispecific antibodies (Ab), demonstrated efficacy targeting a dispensable lineage antigen (Ag), such as CD19 in B-ALL, this approach cannot be exploited for AML, due to lack of actionable leukemia-restricted Ags and severe on target off tumor toxicity. We reasoned that precise modification of the target epitope in donor HSPC used in HSCT would result in loss of recognition, without affecting protein expression and function. Epitope editing allows targeting genes essential for AML survival, minimizing the risk of immune escape. As proof of concept, we selected the cytokine receptors FLT3, KIT, CD123 and identified amino acid changes in their extracellular domains that preserve surface expression, ligand binding and kinase activation but avoid detection by therapeutic mAbs. These variants were resistant to CAR killing and did not induce CAR activation in co-culture assays. We introduced these mutations by adenine base editing (BE) achieving up to 90%, 85% and 75% efficiency in CD34+ HSPCs on FLT3, KIT and CD123, respectively. To assess if CAR-T cells can eliminate AML while sparing edited hematopoiesis, we sequentially engrafted NBSGW mice with HSPCs, human patient derived AML xenografts (PDX) and FLT3 or CD123 CAR T cells. FLT3 and CD123 edited HSPC sustained long-term multilineage hematopoiesis indicating successful editing of HSCs. Upon treatment with CAR T, we observed eradication of AML PDXs while sparing healthy hematopoiesis (B cells, GMP, LMPP and CD34+38- HSPCs for FLT3 CAR; dendritic cells and myeloid lineages for CD123 CAR) in mice engrafted with edited HSPCs but not in AAVS1BE controls. To assess multiplexing, we

tested all combinations of FLT3, KIT and CD123 BE, which provided superior protection when challenged with dual/triple specific CAR. Dual FLT3+CD123 BE protected hematopoietic lineages in mice treated with FLT3+CD123 CAR, which in turn eradicated a PDX resistant to FLT3 targeting alone. Transplantation of epitope engineered HSPCs endowed with selective resistance to CAR or Ab is a novel approach to enable more effective and safer immunotherapies for high-risk AML patients.

Funding Source: LRF, CCRF

Keywords: Epitope engineering, Acute Myeloid Leukemia, CAR-T cells

TOPIC: LIVER

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ENGINEERING RENEWABLE FORMS OF HUMAN LIVER TISSUE TO TREAT AND MODEL DISEASE

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Liver disease is an escalating global health issue. While liver transplantation is an effective mode of therapy, patient mortality has increased due to the shortage of donor organs. Developing renewable sources of human liver tissue is therefore attractive. Pluripotent stem cell-derived liver tissue represents a potential alternative to cadaver derived hepatocytes and whole organ transplant. At present, two dimensional differentiation procedures deliver tissue lacking certain functions and phenotypic instability. Efforts to overcome these limiting factors have led to the building of three-dimensional (3D) cellular aggregates. Although enabling for the field, their widespread application and adoption is limited due to the reliance on poorly defined biological components. Our studies focus on developing 3D liver tissue under defined conditions. We demonstrate that 3D derived liver tissue is functional, phenotypically stable and can be scaled. Excitingly, when implanted underneath the skin of mice, stem cell derived liver tissue provides key support to mice with metabolic liver disease (Tyrosinemia type 1). Of significance, liver tissue also performs well when delivered to immunocompetent recipients. In 2021 we founded Stimuliver to take this work toward the clinic, building on our revolutionary proof-of-concept data. We believe, that our technology, which does not rely on donor organ formation and modulates the adaptive immune system, has the potential to treat a large spectrum of liver diease indications. In addition to their clinical application, in vitro generated 3D tissues also have important roles to play in developing innovative medicines to treat human disease. The renewable and stable nature of our stem cell derived 3D liver tissue therefore provides an attractive resource for long-term disease modelling. Our current focus is to better understand 2 important disease states, non-alcoholic fatty liver disease and cancer metastasis. Our most recent experimental advances in vitro and in vivo will be presented at the meeting.

Keywords: Pluripotent stem cell, Liver tissue engineering, Preclinical validation

TOPIC: MESENCHYMAL STROMAL CELLS, ADIPOSE, AND CONNECTIVE TISSUE

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ADIPOSE-DERIVED MESENCHYMAL STEM CELLS IMPROVE BRAIN ANEURYSM TREATMENT OUTCOMES

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Brain aneurysms are balloon-like vascular lesions that affect up to 20% of the population. One of the most common treatments is by endovascular coiling, where small metal coils are placed within the aneurysm sac to cause occlusion and redirect blood flow. In up to 30% of patients, coils do not permanently divert blood flow, and many patients need to be retreated. Given their healing capabilities, mesenchymal stem cells (MSCs) are a potential solution. This study aims to analyze the histological and paracrine effects that intra-arterially injected MSCs have on aneurysms. Aneurysms were created in rabbits using the modified elastase model. Blood samples were taken before creation to provide healthy serum levels, before treatment to provide aneurysm containing serum levels, and prior to sacrifice to provide treatment serum levels. Four weeks after creation, treatment was performed. Animals were separated into two groups: Group 1 coils only (n=2) and Group 2 coils+MSCs (n=3). Group 1 animals were packed to completion. Group 2 received 5x10^6 allogeneic MSCs injected into the aneurysm sac after placing 1-2 framing coils and were packed to completion. After 3 months, angiography was performed, then animals were sacrificed and tissues were taken for histology. There were no differences in aneurysm volumes and angiographic outcomes between treatment groups. Coil packing density was not significantly different (p=0.09). Group 2 showed increased neointimal thickness (92um±77.4 vs 52um±37.3, p=0.04). Group 2 also had a significantly increased histologic healing score (13±0.9 vs 8±0.8, p=0.04). Cytokine analysis showed a significantly higher concentration of growth factors in group 2 to group 1 (p=0.026). Compared to healthy and aneurysm serum, pro-inflammatory markers were significantly increased in group 2 while group 1 showed no changes (p=0.013 and p=0.026 vs. p=0.38 and p=0.25) . Conversely, tissue remodelling markers were significantly reduced in group 2, while group 1 showed no changes (p=0.027 and p=0.022 vs. p=0.55 and p=0.48 respectively). These preliminary results demonstrate that MSCs can affect endovascular coiling procedures by improving histological healing and downstream paracrine signalling by upregulating healing through growth factors and inflammatory responses.

Funding Source: Brain CREATE Evolve2Innovate

Keywords: Adipose Mesenchymal Stem Cells, Brain Aneurysms, Tissue Engineering

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SAFETY OF HUMAN EMBRYONIC STEM CELL-DERIVED MESENCHYMAL STEM CELLS FOR TREATING INTERSTITIAL CYSTITIS: A PHASE I STUDY

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There are still no definite treatment modalities for interstitial cystitis (IC). Meanwhile, stem cell therapy is rising as potential alternative for various chronic diseases. This study aimed to investigate the safety of the clinical-grade mesenchymal stem cells (MSCs) derived from human embryonic stem cells (hESCs), code name MR-MC-01 (SNU42-MMSCs), in IC patients. Three female IC patients with (1) symptom duration >6 months, (2) visual pain analog scale (VAS) ≥4, and (3) one or two Hunner lesions < 2 cm in-office cystoscopy within 1 month were included. Under general anesthesia, participants received cystoscopic submucosal injection of SNU42-MMSCs (2.0 × 10^7/5 mL) at the center or margin of Hunner lesions and other parts of the bladder wall except trigone with each injection volume of 1 mL. Follow-up was 1, 3, 6, 9, and 12 months postoperatively. Patients underwent scheduled follow-ups, and symptoms were evaluated with validated questionnaires at each visit. No SNU42-MMSCs-related adverse events including immune reaction and abnormalities on laboratory tests and image examinations were reported up to 12-month follow-up. VAS pain was temporarily improved in all subjects. No de novo Hunner lesions were observed and one lesion of the first subject was not identifiable on 12-month cystoscopy. This study reports the first clinical application of transurethral hESC-derived MSC injection in three patients with IC. hESC-based therapeutics was safe and proved to have potential therapeutic efficacy in IC patients. Stem cell therapy could be a potential therapeutic option for treating IC.

Funding Source: This research was supported by a grant from the Basic Science Research Program through the National Research Foundation of Korea (NRF-2020R1A2C1007789); by an NRF MRC grant funded by the Korean government (NRF-2018R1A5A2020732).

Keywords: interstitial cystitis, embryonic stem cell, mesenchymal stem cell

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PRECLINICAL EVALUATION OF NANOSCAFFOLDS IMPREGNATED WITH HUMAN WHARTON'S JELLY STEM CELLS FOR THE TREATMENT OF SKIN WOUNDS IN A DIABETIC PIG MODEL

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Diabetic foot ulcers (DFU) are a serious consequence of poorly controlled diabetes, and current treatment options are limited. We evaluated the wound healing potential of an aloe-vera-polycaprolactone (AV/PCL) nanoscaffold impregnated with human Wharton's jelly stem cells (hWJSCs + AV/PCL), or hWJSC-conditioned medium (hWJSC-CM + AV/PCL). Following ethical committee review, diabetes was induced in 4 pigs using streptozotocin. 12 full-thickness wounds were created in each pig. 48 wounds were randomised to one of three treatments (n=16 wounds/group) as follows: i) hWJSC + AV/PCL (WJSC Treatment); ii) hWJSC-CM + AV/PCL (WJSC Media Treatment); or iii) empty media (UCM) + AV/PCL (Negative Control). Wound healing was monitored for 28 days. A calibrated digital camera was used to photograph each wound to calculate wound closure rates. All pigs were euthanized on day 28. Full-thickness wound biopsies were sampled for histology and snap-frozen for molecular analyses. All results are mean ± SD. A $p< 0.05$ was considered statistically significant using one-way ANOVA. Full-thickness wounds from diabetic pigs treated with hWJSC + AV/PCL and hWJSC-CM + AV/PCL completely healed by day 28. Wounds treated with UCM+AV/PCL (Negative Control) had slower healing. Wound closure percentages were significantly greater for hWJSCs + AV/PCL (87.5 ± 2.9) and hWJSC-CM+AV/PCL (89.8 ± 2.5) compared to UCM+AV/PCL (75.6 ± 3.4) ($p< 0.05$). Epidermal and dermal thicknesses were significantly greater for hWJSCs + AV/PCL (52.03 ± 1.80 µm and 2388.1 ± 99.5 µm, respectively) and hWJSC-CM+AV/PCL (57.0 ± 4.5 µm and 2387.8 ± 87.8 µm, respectively) treated wounds compared to UCM+AV/PCL (41.0 ± 2.3 µm and 2051.4 ± 70.6 µm, respectively) ($p< 0.05$). Histological studies showed greater re-epithelialization in Treatment Groups compared to Negative Control. mRNA expression of markers associated with wound healing (MMP-9, COL1A1, TGF-β and ANGPTL4) were significantly greater in Treatment Groups compared to Negative Control on day 28. Our results show that hWJSCs nanoscaffolds provide an effective means of treating diabetic wounds in the pig. Interestingly, equivalent wound healing was achieved with the use of nanoscaffolds impregnated with stem cell-conditioned media. The safety and efficacy of this system will be evaluated in a human clinical trial.

Keywords: Human Wharton's jelly stem cells, Pig diabetic wounds, Wound dressing patch

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A NOVEL EXOSOME-DEPLETED XENO-FREE HUMAN PLATELET LYSATE FOR THERAPEUTIC MSC-DERIVED SECRETOME PRODUCTION

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Mesenchymal stem cell (MSC)-derived extracellular vesicles (EVs) play an important role in cell-to-cell communication and have inspired great interest as a new strategy for developing novel therapeutics. Exosomes, one of the subpopulations, comprise various growth factors, cytokines, RNAs, and DNAs. MSC-derived EVs are also now one of the promising therapeutic candidates in both regenerative medicine and drug delivery system based on their unique biological properties. However, existing exosomes derived from supplements of fetal bovine serum or human platelet lysate (hPL) could potentially modulate MSC physiology which subsequently affects the quantity and profile of released EVs. In addition, the mixture of MSC-derived and supplement-derived EVs can also confound downstream isolation and analysis, leading to misinterpretation of the results. Therefore, qualified ancillary materials and a controlled culture environment are crucial for MSCs to achieve consistent EV expression profiles batch-to-batch for cGMP production. Herein, a novel gamma-irradiated hPL (ED hPL Gi) featuring xeno-free with a high level of EV depletion is developed for MSC-derived EV production. Moreover, pathogen reduction treatment (PRT) by gamma irradiation allows the EV production process to comply with regulatory guidance for clinical research and development. In the study, the particle depletion process was performed by using a tangential-flow-filtration system, and then gamma irradiation was the PRT process as the final step to prepare ED hPL Gi. Afterwards, MSC were cultured in 0-5% ED hPL Gi supplemented medium when the confluency reached 50-70% in a T75 flask. The conditioned medium was harvested and refreshed every 2 days. The results showed >98% of the particles were successfully removed in our process. Additionally, MSC could be maintained at >90% viability during the culture period, and billions of EVs were harvested in each cycle in the presence of ED hPL Gi. Therefore, ED hPL Gi provides an effective environment feasible for MSCs to produce a significant amount of EVs and prolong cell activity. Our data support that ED hPL Gi is a promising supplement for MSC-derived EV production in both exosome research and GMP manufacturing for clinical applications.

Keywords: MSC-derived secretome production, Exosome-depleted supplement, Human platelet lysate

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HARNESSING DONOR HETEROGENEITY TO UNRAVEL CELL PERFORMANCE IN AUTOLOGOUS THERAPIES USING PERIOSTEUM-DERIVED CELLS

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Osteoarthritis (OA) is a common degenerative disease of the joints that affects the quality of life of millions of people worldwide. Osteochondral lesions penetrating deeply into the subchondral bone are a major risk factor for OA and a condition that remains a therapeutic challenge to date. The periosteum contains skeletal stem cells capable of healing critical-size bone defects in vivo by endochondral ossification, making these periosteum-derived cells an interesting source for the treatment of osteochondral lesions. However, the biological behavior of these cells has been shown to be age-dependent and variable amongst different harvest sites. Understanding how patient outcomes relate to cell behavior, including cell identity and potency, is paramount for the clinical application of cell-based therapies. In this study, periosteum samples were harvested from the anterior–medial side of the knee of individuals undergoing routine orthopedic surgeries. Human periosteum-derived cells (hPDCs) were isolated from 10 different donors, aged 15 to 45 years. Donor cells showed similar viability, yet differences in proliferation rates and growth curves were observed. While all donors differentiated to the chondrogenic lineage, the osteogenic and adipogenic differentiation profiles presented more diversity. Moreover, donor-derived cells had different expression profiles of the mesenchymal and skeletal stem cell markers CD73, CD105, CD90, CD164 and PDPN. Taken together, these results indicate that hPDCs harvested from the same region differ amongst different donors in terms of cellular properties and behavior. By evaluating donor variability, we will attempt to predict the performance of hPDCs and provide evidence for the reproducibility and the potential outcomes of using these cells in autologous stem cell therapy approaches.

Keywords: osteochondral defects, periosteum, donor variability

TOPIC: NEURAL

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MESENCHYMAL STEM CELLS-DERIVED EXOSOMES AS AN ACTIVE PHARMACEUTICAL INGREDIENT BRINGS A SIGNIFICANT NEURAL RESPONSE IN SPINAL CORD INJURY PATIENTS, A PILOT STUDY

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We have developed a proprietary stem cells-derived exosome active pharmaceutical ingredient (API) containing growth factors and cytokines that is intrinsically stabilised for shelf life over 12 months and could be scaled up by using a fixed bed bioreactor. The API is characterised including for markers such as CD 63, 73, 81, 105 by inhouse optimised nano flow cytometry as well by Western blot. The potency validated by in vitro wound healing assay including optimising the dose and the tubulogenic assay for angiogenesis(VEGF). The safety of the product was based on another double blinded placebo-controlled skin trials completed without any adverse event. This innovative API, Endexome-EX-OTM was the subject of current pilot studies in Spinal Cord Injury (SCI) Patients. Twenty SCI patients were recruited with due ethics approval and with an export permit for API from the Therapeutic Goods Administration (TGA) Australia. Patients were injected API intrathecally every second month for 8 months. Adverse event if any was assessed by blood profiling and efficacy for neural and motor response by sensory dermatomes and muscle reflex scaling. Seventy five percent of the patients responded with a significant progressive increase in neural response, but non-significant motor response compared to patient's base levels. No adverse event was reported. This is the first report of exosomes in SCI pilot trials and data published as PCT AU 2022/050561/WO 2022/256865.

Funding Source: AUSTRALIAN GOVERNMENT *RESEARCH AND DEVELOPMENT* REBATE

Keywords: MSC, EXOSOMES, API, PILOT STUDY, SCI, PILOT STUDY, SCI

Clinical Trial ID number: R#00-103-20

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IDENTIFICATION OF GENES FOR PHOTORECEPTOR CELL ENRICHMENT USING LAMININ BASED PHOTORECEPTOR DIFFERENTIATION

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Background: Aging retinal degenerative disease affects the increasing aging population indiscriminately with increasing prevalence. Blindness caused by retinal degeneration results in irreversible loss of photosensitive photoreceptors. Therefore, replacing the loss of these photoreceptors is considered a potential therapy to restore vision. As shown in our publication, we have developed a laminin based method to generate functional photoreceptor progenitor cells from human pluripotent stem cells. Our day 32 photoreceptor progenitors have shown to partially restore vision in the preclinical animal models. We hypothesize that enriched photoreceptor sub-population containing signature transcript profile could lead to better transplantation outcome as shown in electroretinogram and visual behavioural assays. Methods: We have generated human recombinant retina specific laminin (LN)-323 and 523 and have shown that the human embryonic stem cells grown on these matrices involving two media changes, were able to generate photoreceptor progenitors at day 32. Single cell transcriptomic analysis was used to compare the differentiation efficacy mediated by these matrices. Results: Based on immunohistochemical and single cell transcriptomic analyses, we observed there is different co-expression levels of cone-rod homeobox and recoverin proteins by day 32 for cells cultured on LN323+LN521, LN523+LN521 and LN521 alone, suggesting there is specific matrix mediated efficacy. We have identified 4 candidates out of 194 genes to be playing a role in early photoreceptor specification based on the differential gene analysis from cells differentiated on these different matrix formats. Ongoing experiments are underway to study these candidate genes in our laminin based photoreceptor differentiation system. Conclusions: Our laminin based photoreceptor differentiation method have led to the identification of gene candidates that could be involved in modulating the photoreceptor sub-population. Hence, this system could increase our understanding in human early retina development and thereby generate an improved cell therapeutic product as compared to our earlier report which ultimately brings us closer for future therapies to treat vision loss.

Keywords: cell therapy, retina, photoreceptor

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QUALITATIVE ASSESSMENT OF HUMAN EMBRYONIC- AND PLURIPOTENT STEM CELL DERIVED- NEURAL STEM CELLS UNDER CGMP METHODS

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Neural stem cell therapies represent a promising tool for the development of regenerative medicine and are being tested in clinical trials for several neurological disorders. However, the clinical applicability of stem cell therapies is dependent on the implementation of good manufacturing practices (GMPs) to ensure the quality, safety, and consistency that stem cell products need to meet FDA regulatory requirements. As such, there is a need for a shift to xeno-free methodologies so experimental conditions are cGMP compliant. The purpose of this study is to test a GMP compatible production method to generate multipotent neural stem cell (NSCs) from different cell sources. Comparability of NSCs is dependent on critical quality attributes (CQAs) such as sterility, stability, purity, and multipotent neural differentiation. Accordingly, we will test human embryonic cells (hESCs) Shef6 and induced pluripotent stem cell (iPSC) ADRC76 lines for their suitability to produce CD133-positive neural stem cells. Due to potential epigentic differences found in hiPSCs, we predict that hESCs will produce higher quality NSCs compared to hiPSCs. To test this, both cell lines will be cultured under identical reagents and methods for expansion and neuralization. For NSC characterization, trophic factor and cytokine secretion and neural differentiation will be compared between the processing runs. Clinical and manufacturing controls (CMC) standards will be applied throughout the downstream processing such as sterility, stability and purity validations. This manufacturing protocol will be compared with a prior derivation of Shef6 hNSCs. In the future, we would test several additional hESC and hiPSC lines to confirm if cell source is a significant factor in generating cGMP neural stem cell products. The adoption of cGMP-compliant methods early in the research environment will improve the replication of results across cell lines and may increase translatability of preclinical studies as the protocols are transferred to GMP facilities.

Funding Source: A Tran 1 and a Bridges Grant from California Institute of Regenerative Medicine (CIRM)

Keywords: Pluripotent Stem Cells, Current Good Manufacturing Process (cGMP), Neural Stem Cells (NSCs)

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NEURAL CELLS FOR NEURODEGENERATIVE DISEASES IN CLINICAL TRIALS

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Neural stem cells (NSCs) are multipotent cells found within the central nervous system (CNS), capable of differentiating into the various neural lineages. Neurodegenerative (ND) diseases, many of which have no effective cure, affect hundreds of millions worldwide, and increasingly represent a major global health challenge today. We aimed to collate the latest clinical trials using NSCs and cells of neural lineage for the treatment of ND diseases. For each disease, a search of the disease name, neural stem cells, neural cells and cell therapy were performed on clinicaltrials.gov. There are18 trials listed for Parkinson's with four being neural and 32 cells transplantation clinical trials recorded for Amyotrophic Lateral Sclerosis of which five are with neural related cells. A total of 48 studies involving cell therapy for Multiple Sclerosis was retrieved, with five cell sources being of neural lineage (10.4%). Four trials proposed direct CNS delivery of CNS derived NSCs (n=3) or Umbilical Cord Blood derived oligodendrocyte-like cells (n=1) for a group of rare diseases including Pelizaeus-Merzbacher, Batten and a few enzymes deficiency disorders and another four trials retrieved for Huntington, one of which neurons from fetal tissues were used as a donor. For macular degeneration, there are 22 trials listed of which 16 involved neural related cells. Finally, six of the 19 cell therapy trials for retinitis pigmentosa are with cells of neural lineage. In our review, we noted 33 trials involving neural stem/ progenitor cells, 8 involving differentiated neural cells and 109 trials utilising non-neural cells for ND diseases. Cells of neural lineage have the advantage of contributing to direct differentiation in replacing neural cell types. However, there are many more trials involving non-neural cell types, particularly MSCs indicating the importance of secretion of trophic factors and/or neuroprotective growth factors as well as the preference for immune compatibility to avoid rejection. Better characterization of well-defined human NSCs as well as understanding the immunology of cell therapy will need to continue to widen the range of diseases treatable with NSCs and their downstream differentiated cells as well as to improve the efficacy of the treatments.

Keywords: Neural stem cells, neurodegenerative disease, clinical trial

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IMPROVING THE FUNCTIONAL INTEGRATION OF HUMAN PLURIPOTENT STEM CELL DERIVED NEURAL GRAFTS THROUGH ENVIRONMENTAL ENRICHMENT

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Evidence that human pluripotent stem cell (hPSC)-derived dopaminergic (DA) neurons can structurally and functionally integrate into the host brain when transplanted into Parkinson's disease (PD) models has led to a series of ongoing clinical trial. However, preclinical studies have highlighted poor graft survival and plasticity. We recently demonstrated that the viral delivery of Glial-Derived Neurotrophic Factor (GDNF), can significantly improve the survival, plasticity, and function of hPSC-derived grafts. Of relevance, numerous studies have reported that environmental enrichment can increase neurotrophic levels, including GDNF, in the rodent

brain. We therefore examined whether an enriched environment (including enhanced sensory, cognitive, and/or motor stimulation) could improve hPSC-derived midbrain graft outcomes. We show that the housing of animals receiving hPSC-derived neural grafts in an enriched environment resulted in a significant increase in the number of surviving DA neurons, their innervation of target nuclei and density of graft-derived synapses – with motor stimuli having the greatest impact. These findings were supported by a significant increase in phosphorylated extracellular signal-regulated protein kinase (pERK) expressing cells in the graft, indicative of mitogen-activated protein kinase (MAPK-ERK) signalling, a downstream target of GDNF. We subsequently demonstrated that voluntary wheel running could significantly enhance recovery of motor deficits (amphetamine-induced rotational, cylinder and adjusted stepping tasks) in animals receiving ectopic/striatal, but not homotopic/midbrain placed grafts. Together, these results demonstrate the remarkable capacity for achieving superior efficacy within hPSC-derived DA transplantation using clinically relevant exercise and enrichment approaches.

Keywords: Parkinson's Disease, Neural Transplantation, Environmental Enrichment

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SAFETY, EFFICACY, AND DOSE-RANGING ASSESSMENT OF HESC-DERIVED DOPAMINERGIC PROGENITORS FOR PHASE1 CLINICAL TRIAL IN PARKINSON'S DISEASE

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Transplantation of human pluripotent stem cell-derived midbrain dopaminergic (mDA) progenitors has gained enormous attention as a promising therapeutic strategy for Parkinson's disease (PD). A critical step toward clinical application of cell therapeutics is to demonstrate that the cells manufactured under cGMP conditions satisfy numerous non-clinical prerequisites regarding safety and efficacy, guided by the regulatory agency of every country. Here, we adapted our mDA neuronal differentiation protocol to GMP-compliant conditions under rigorous quality controls, generating high purity of clinical mDA progenitors derived from hESCs on a large-scale as cell therapeutics for PD. We then conducted preclinical safety assessment of clinical mDA progenitors to evaluate toxicity, biodistribution and tumorigenicity under the GLP-compliant facilities. A year-long and large-scale transplantation study performed by an independent CRO using immunodeficient rats revealed that the mDA neuron graft did not cause significant toxicity, ectopic integration outside the injection site, or any tumorigenicity. Next, to investigate the therapeutic potential, clinical mDA progenitors were transplanted in the striatum of toxin-induced hemi-parkinsonian rats with various cell doses. The results showed that the cells ranging from 10,000 to 350,000 engrafted and matured into functional mDA neurons in host brains, allowing behavioral improvement in the parkinsonian rat models. The present pre-clinical profiles provided us with a verification of the therapeutic safety and efficacy of the clinical mDA progenitors as well as an insight into designing cell dosage for human trials. Based on the results, we submitted an investigational new drug (IND) application to the Korean Ministry of Food and Drug Safety (KMFDS) for a clinical trial and received the final approval.

Keywords: Parkinson's disease, Cell therapy, dopaminergic neurons

TOPIC: NO TISSUE SPECIFICITY

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LARGE KNOCK-IN IN PRIMARY T CELLS WITH OPTIMIZED CAS9 HDR METHODS AND DESIGN

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Knock-in of large transgenes in primary T lymphocytes has previously relied on methods that insert via random integration, not allowing for precise editing and making the genome susceptible to insertional mutagenesis (off-target effects, chromosomal rearrangements, translocations, etc.). As such, there is a need for precision gene editing for introduction of stably expressed transgenes such as chimeric antigen receptor (CAR) variants. CRISPR-Cas9 is an expedient tool for precision editing and can mediate targeted HDR-based insertion. Here we present optimized reagents, donor design, and delivery methods for CRISPR HDR in primary T cells with chemically synthesized short HDR donors for small inserts and PCR-based long HDR donors for knock-in of up to 2kb. These methods and reagents together in primary human T cells achieve high-efficiency precise genome editing with low off-target integration.

Keywords: CAR, CRISPR, knock-in

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NOVEL ONCOGENIC EXOSOMAL MIRNA IDENTIFIED IN TRIPLE NEGATIVE BREAST CANCER AND STEM CELLS

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Triple-negative breast cancers (TNBCs) are defined by lack of hormone receptors and human epidermal growth factor receptor type 2 (HER) leading to a dearth of targeted therapies, poor prognosis, high rates of recurrences and mortality. TNBC is also characterized by an abundance of treatment-resistant breast cancer stem cells (CSCs). Exosomes and exosome-secreted miRNAs have emerged as critical mediators of intercellular communication in the tumor microenvironment. Tumor-derived exosomes have been linked to the initiation and progression of various cancers.

In the present study we sought to analyze oncogenic exosomal miRNAs secreted by TNBC cell lines and TNBC stem cells and their possible role in tumor diagnosis/ prognosis. Meta analysis was first carried out to identify all differentially expressed miRNAs in breast cancer and TNBC. From the data pool we selected a set of five highly expressed miRNAs namely miR 6803, miR 1180, miR 4728, miR 1915 and miR 940 and their expression in exosomes isolated from TNBC cell lines were analyzed by RTqPCR. We subsequently performed target prediction of the selected miRNAs and carried out GO and KEGG pathway enrichment analyses to explore the potential effects of these miRNAs on TNBC progression. To reconstruct the microRNAs-genes regulatory network in TNBC, we employed the expression data from The Cancer Genome Atlas (TCGA) related to these five novel miRNAs. Survival analysis revealed high expression of these miRNAs is significantly associated with decreased overall survival. Exosomal transcriptomes are reflective of the cells from which they were produced, therefore they should serve as a platform for the identification of tumor-specific markers. These specific secretory miRNAs could serve as a liquid biopsy tool for diagnosis and may provide reference value for the early detection and monitoring of TNBC progression. In summary, our study identifies novel oncogenic exosomal miRNAs expressed both in TNBC and TNBC CSCs and highlights the potency of exosomal miRNAs as therapeutic targets for TNBC

Keywords: TNBC, CSC, miRNA, exosomes

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DOWNREGULATION OF ADAMTS3 SUPPRESSES STEMNESS AND TUMORIGENICITY IN GLIOMA STEM CELL

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Glioblastoma multiforme (GBM) is the most aggressive type of human brain tumor, with a poor prognosis and a median overall survival of fewer than 15 months. Glioma stem cells (GSCs) have recently been identified as a key player in tumor initiation and therapeutic resistance in GBM. ADAMTS family of metalloproteinases is known to cleave a wide range of extracellular matrix substrates and has been linked to tissue remodeling events in tumor development. Here, we investigate that ADAMTS3 regulates GSC proliferation and self-renewal activities, and tumorigenesis in orthotopic xenograft models. ADAMTS3 mRNA expression levels in normal human astrocyte (NHA), glioma, and GSCs cell lines were compared. After knockdown of ADAMTS3, alamarBlue assay, in vitro limiting dilution, and orthotopic xenograft assays were performed. To investigate the tumor-associated roles of ADAMTS3, several statistical assays were conducted using publicly available datasets. ADAMTS3 level was remarkably higher in GSCs than in NHA, glioma cell lines, and their matched differentiated tumor cells. Interestingly, knockdown of ADAMTS3 disrupted GSC's proliferation, self-renewal activity, and tumor formation in vivo. Furthermore, ADAMTS3 could be used as an independent predictor of malignancy progression in GBM. We identified ADAMTS3 as a potential therapeutic target for GBM.

Keywords: Glioma Stem Cell, GBM, ADAMTS3

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ROBUST LENTIVIRUS AND AAV MANUFACTURING PLATFORMS FOR CELL AND GENE THERAPIES

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Chimeric antigen T cell receptors (CAR-T) are receptor proteins that have been engineered to give T cells the new ability to target a specific antigen on cancer cells. To enable success of such therapies in the clinic, viral vectors such as lentivirus and AAV have to be designed, and manufactured according to GMP standards. Cellvec a leading CDMO in Singapore has developed a duet of robust lentivirus and AAV viral vector manufacturing platforms for clients to meet their viral vector requirements suitable for all phases of clinical development (pre-clinical to GMP manufacturing). For the lentiviral vector platform, we have developed patented helper plasmids for the production of CD19, BCMA and CD20 CAR transgenes, produced in a fully characterised bacterial cell bank free from animal components that meet FDA and EMEA regulatory standards. Designing of the vector construct to GMP manufacturing in a closed system, downstream purification and analysis of quality attributes can be accomplished between 6-9 months. Functional (FACS) and genomic (qPCR) titers of the purified product are typically 10E+11 TU/ml in 15L compared to competitive technologies that only achieve 1.5E+11 TU/ml in 50L suspension cultures. The purified lentiviral vectors are highly potent, and is able to transduce 30% of CD3+ T cells at a low multiplicity of infection equivalent to 1, with a low vector integrant copy number. This manufacturing platform can be readily scaled currently to 40L with a potential to scale to 100L in the future. For suspension based viral vector technologies, Cellvec has partnered with SGVector. Using rAAV2-RPE65 as a gene of interest, SGVector has developed an efficient 6 day total HEK293 suspension bioreactor process (3 days of cell expansion + 3 days for viral transfection). This upstream platform suspension process at the 2L scale has consistently demonstrated harvest viral titers (qPCR) yields of 2E+12 vg/ml, 20-fold higher compared to a leading CDMO's published AAV2 process yield of 1E+11 vg/ml. The robust AAV downstream process demonstrates effective clearance of impurities that meets FDA and EMEA regulatory expectations for safety, identity, strength, purity and quality attributes (SISPQ).

Keywords: Lentiviral Vector, Adeno-associated virus, T-cell, Bioreactor, GMP, Virus production, Contract manufacturing

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RECOMBINANT LAMININ 521 IMPROVES SINGLE CELL SURVIVAL AND MAINTAINED PLURIPOTENCY OF PLURIPOTENT CELLS DURING GENE EDITING

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The CRISPR/Cas9 technology revolutionized the field of gene editing. So far, obtaining single-cell clones of edited human pluripotent cells (hPSC) has been a major problem. Laminins are an extracellular matrix (ECM) protein family of around 16 different isoforms and a major component of the basement membrane, thus vital for tissue homeostasis. The laminin-521 isoform is the major ECM protein expressed in the inner cell mass of the pre-implanted embryo and offering a biologically relevant support for PSCs in vitro, actively stabilizing cell identity and proliferation. Recombinant Biolaminin® 521 can support different gene editing applications. Here, we are summarizing several studies using the matrix for CRISPR/Cas9 technology. In feeder-free cultures, Biolaminin 521 increased the single cell cloning efficiency compared to e.g. tissue-extract based substrates and improved survival of single cell seeding at low density. Additionally, the purity of Biolaminin proved advantageous for imaging purposes. Due to the tissue-specific expression of laminins, Biolaminin 521 and -111 supported the effective differentiation of CRISPR/Cas9-edited human induced PSC (hiPSC) into cortical neurons via targeting the neuroprotective E3-ubiquitin ligase CHIP, which is important in healthy brain aging. Moreover, Biolaminin 521 and -111 were efficient substrates for CRISPRi-mediated transcriptional silencing in hiPSCs and the differentiation of hiPSC into forebrain neural progenitor cells for loss-of-function studies in brain development research. The CRISPRi cells differentiated in cerebral organoids with steady transcriptional silencing after 4 months. In conclusion Biolaminins are highly suitable matrixes for CRISPR/Cas9 gene editing of hPSC in a variety of applications including PSC maintenance and differentiation. Biolaminins improve single cell survival and pluripotency and help achieving high transduction and silencing efficiency.

Keywords: Gene-editing, cell therapy, extracellular matrix

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NEXT-GENERATION DMSO-FREE & SERUM-FREE CRYOPRESERVATION FOR CELL AND GENE THERAPY MANUFACTURING AND PROCESSING

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The regenerative medicine field is rapidly advancing, with many pivotal trials underway. As the field moves into large-scale commercial manufacturing, cryopreservation becomes an even more critical component for ensuring maximum efficacy and long shelf-life during end-to-end production. However, the potency and yield of fragile cell types, such as induced pluripotent stem cells and genetically modified cell-based immunotherapies, are significantly reduced post-cryopreservation due to ice damage and genomic and in vivo toxicity associated with current cryoprotectants (e.g. DMSO). These issues further limit the realization of off-the-shelf advanced regenerative medicine products, such as those being developed in allogeneic cell therapy and tissue engineering. Existing standards in cryopreservation use 5-10% dimethyl sulfoxide (DMSO) and often serum. DMSO exhibits acute toxicity in patients and causes adverse cell mutation at low levels including irreversible cell chromosome damage and alterations in the epigenetic landscape. X-Therma applies convergent biomimetic nanoscience to solve this unmet need in biopreservation, pioneering a novel chemistry that is inspired by natural antifreeze protein, developed with modern drug discovery methods. Our fully synthetic molecules are non-toxic, chemically stable, and exhibit surprising dual ice control function, superior to antifreeze proteins and 500x more effective than non-colligative small molecule cryoprotectants. The resulting product is XT-Thrive. Third-party validations have demonstrated superior post-thaw cryopreservation outcomes for both cell viability and functionality with a variety of engineered cell lines and therapeutic cell-based products. XT-Thrive is extremely process-friendly and can be directly plugged into current workflows without requiring any specialized instrumentation, replacing the leading DMSO-based cryopreservation solutions. Empowered by negligible toxicity, XT-Thrive removes bottlenecks for large batch production and enables a highway of premium quality cell products for the many patients in need. Moreover, the lack of DMSO is expected to result in a better patient experience during clinical trials and the use of final cell therapy products.

Keywords: Regenerative Medicine, Cryopreservation, Cell and gene therapy

TOPIC: NEURAL

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NEURONAL PROTEOSTATIC STRESS AND GLIAL RESPONSES IN ALS REVEALED BY SINGLE NUCLEUS RNA-SEQUENCING OF PRIMARY SAMPLES AND HUMAN IPSC MODELLING

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder characterised by a progressive loss of motor function. The eponymous spinal sclerosis observed at autopsy is the result of the degeneration of extratelencephalic neurons/Betz cells (ETNs/Cortico-Spinal Motor Neuron). To understand the unique molecular properties that specifically sensitise these cells to ALS, we performed RNA sequencing of 79,169 single nuclei from cortices of patients and controls. In patients, we found that genetic risk factors connected to ALS, as well as genes involved in protein homeostasis and stress responses, were significantly upregulated in a collection of subcerebral projection neurons, including layerV-ET neurons. We used hiPSC-derived neurons and RNA-seq to dissect changes driven by proteostatic stress and then compared it to the signature identified in patients' neurons. This comparison allowed us to underline the importance of the ubiquitin-proteasome system and autophagic responses in stressed neurons connected to disruption of synaptic biology relevant to ALS. Examination of microglial nuclei from patients revealed gene expression changes that were at least in part a response to disease-associated alterations in neurons. Disease modelling using hiPSC-derived microglial-like cells confirmed that dying neurons trigger changes in phagocytosis and inflammatory responses in microglial cells similar to the ones seen in ALS patients. Our findings suggest that selective vulnerability of ETNs to ALS is connected to both intrinsic molecular properties sensiting them to genetic and mechanistic factors of degeneration and our human iPSC in vitro systems confirmed these changes in neurons are connected to alterations to proteostasis that trigger microglial activation.

Keywords: ALS, Motor neurons, Microglia

TRACK: CLINICAL APPLICATIONS (CA)

Session 5: Even

2:00 PM – 2:45 PM

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EPIGENETIC MODIFICATION ENHANCES HAIR CELL REGENERATION AND RESTORES FUNCTION IN MICE WITH VESTIBULAR DISORDERS

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The sense of balance relies on motion sensing vestibular hair cells within the inner ear. Hair cells accumulate damage with age, resulting in balance disorders, the number one health complaint of individuals over age 70. The lack of significant hair cell regeneration in the adult vestibular system makes balance disorders essentially irreversible, and patients have few treatment options. In this work, we sought to develop a pharmacological treatment to enhance hair cell regeneration in the balance organ of adult mice. We used mice expressing the diphtheria toxin receptor in hair cells (Pou4f3-DTR) for targeted ablation of hair cells. After diphtheria toxin administration, mice were injected via the semi-circular canal with a glycogen synthase kinase inhibitor and a histone deacetylase inhibitor, a combination previously shown to stimulate hair cell differentiation in the auditory organ. We observed a significant increase in hair cell number relative to spontaneously regenerated hair cells. Drug treatment resulted in regeneration of 58% of the normal number of hair cells. Regenerated hair cells from drug treatment re-established afferent connections with the vestibular neurons as assessed by single unit recording, resulting in a significant functional improvement measured by the vestibulo-ocular reflex. The drug combination described here may pave the way toward a novel therapeutic approach suited for hair cell replacement to restore vestibular function in patients with vestibular disorder.

Funding Source: This work was supported by NIH grant DC0014089.

Keywords: Hair cell regeneration, Epigenetic modification, Balance disorders

TOPIC: CARDIAC

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ENGINEERED EPICARDIAL CELLS FROM HUMAN EMBRYONIC STEM CELLS PROMOTE ENGRAFTMENT AND MATURATION OF HUMAN CARDIAC PROGENITOR CELLS IN THE RODENT KIDNEY CAPSULE MODEL

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Despite major advances in the treatment of ischemic heart failure, the root cause of impairment is still unaddressed due to the inability of the injured mammalian heart to regenerate the loss of contractile myocardial tissue. Human embryonic stem cell (hESC)-derived cardiac progenitors (CPCs) engraft, differentiate into cardiomyocytes (CMs), and prevent disease progression after myocardial infarction (MI) in the murine and porcine heart (reference). As such CPCs are a promising source of cardiomyocytes for cardiac regeneration of the adult mammalian heart. However, the extensive myocardial remodeling post-injury makes it a particularly harsh environment that negatively affects graft size. In this study, we are exploring strategies to further enhance robust cell engraftment and maturation. To unlock the full potential of CPCs, we have tested the ability of hESC-derived epicardial cells (EPIs) to promote CPC-derived CM graft size and maturation in the rodent kidney subcapsular transplant model. During development, EPIs and their deriving cells play a crucial role in the expansion, and support of the embryonic myocardium. To further promote the grafting of CPCs, we engineered EPIs to secrete cardiac supporting growth factor(s) which promote CPC proliferation. The growth factor(s) were selected based on their ability to affect the proliferation of CPCs in vitro measured by EdU and Ki67 assays. Luciferase-expressing CPCs were co-transplanted with engineered and naïve EPIs in vivo. Bioluminescence imaging and histological analysis revealed that the presence of EPIs significantly promotes the expansion of the grafted CM population over time. Most strikingly, co-transplantation of engineered EPIs further improves graft size severalfold. Lastly, in all conditions, EPIs promote a robust increase in the expression of CM maturation markers. In summary, the ability to promote engraftment and maturation presents EPIs, especially engineered EPIs, as an important companion cell enhancing the therapeutic effect of CPCs in cardiac repair.

Keywords: cardiovascular, cell therapy, ischemic heart failure

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PROTEIN-FREE, LOW-COST MEDIUM FOR EFFICIENT HPSC-CARDIOMYOGENIC DIFFERENTIATION IN MULTIPLE CULTURE PLATFORMS INCLUDING UPSCALING TO STIRRED-TANK BIOREACTORS

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Harnessing the full potential of human pluripotent stem cell (hPSC)-derived cardiomyocytes for cell therapy, tissue engineering and drug discovery, will require the cost-effective, large-scale production of cell products. Chemically defined, low-cost media of constant quality represent an important factor in such processes. The broadly utilised “Chemically defined medium, 3 components” (CDM3) for cardiac differentiation presents a first step towards this. However, one main component of CDM3 is the costly and batch-quality dependent recombinant human albumin (RHA), utilised in high concentrations. In our preceding studies, we showed that the variability of the cardiomyogenic hPSC differentiation process is substantially influenced by cell-secreted paracrine factors. Thus, investigating the differentiation stage-dependent secretome is of substantial interest to further optimize and control the process, and for revealing novel biomarkers for process monitoring. However, high concentrations of protein, in particular, RHA, in the culture medium interfere with a proper and sensitive secretome analysis by mass spectrometry, which is especially critical for low-abundant, yet differentiation-directing, factors such as secreted cytokines. To overcome these drawbacks we have developed entirely protein-free, chemically defined media formulations that efficiently support cardiomyocyte yield and purity equivalent or even better than CDM3 medium. Applicability of these novel media formulations is demonstrated across numerous differentiation culture platforms, including conventional 6-well plates (3 mL volume) and Erlenmeyer flasks (20 mL volume), followed by process upscaling to 150 mL stirred tank bioreactors (STBRs) and ultimately 2000 mL STBR scale, supporting cell production for clinical translation. The achieved CM yield of about 1.5×106 cells/mL on average is superior to previous data in the field. All components of the newly developed media are commonly utilized in the medical industry and are known to be highly biocompatible, in line with the clinical applicability of hPSC-derived cardiomyocytes; moreover, media cost reduction of at least 30% compared to CDM3 medium is achieved, further promoting commercial aspects of cell therapies for heart regeneration.

Keywords: Cardiomyocyte-bioprocessing, Protein-free Medium, Secreteomics

TOPIC: EPITHELIAL INCLUDES SKIN GUT LUNG

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AUTOLOGOUS IPSC THERAPY AND THE DEVELOPMENT OF AN RPE-PLGA TREATMENT FOR AGE-RELATED ACUTE MACULAR DEGENERATION

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Age-related macular degeneration (AMD) is one of the most common causes of blindness in individuals over the age of 55. The “dry” stage of this retinal degenerative disease is characterized by degeneration of the retinal pigment epithelium (RPE), retinal photoreceptors, and vasculature of the retina. Our lab uses induced pluripotent stem cells (iPSCs) to generate autologous iPSC-RPE to treat patients with the “dry” form of AMD. Patients’ blood was collected to isolate peripheral blood mononuclear cells (PBMCs). PBMCs were reprogrammed into iPSCs and subsequently differentiated into RPE using our tri-phasic protocol. The iPSC-RPE were matured on a biodegradable polylactic co-glycolic acid (PLGA) scaffold for five weeks. The maturity and purity were verified by FLOW-based assay for RPE-specific markers (BEST1, TYRP1, CRALBP), and pluripotency markers (TRA-1-81 and OCT-4). The safety and efficacy of the iPSC-RPE patch was tested in small (rat) and large (pig) animal models, respectively. The 0.5mm patch was transplanted in immune-compromised rats and no signs of tumor formation was observed, confirming the safety of the product. For testing the efficacy, the iPSC-RPE patch was transplanted in the pig after laser injuring their RPE layer. Optical coherence tomography (OCT) and histological analysis confirmed the integration of the patch within the pig RPE layer. In conjunction with OCT, multi-focal electroretinogram (ERG) was used to show that the retina over the transplanted iPSC-RPE patch had a higher electrical response as compared to the lasered area without the patch. Recently, the FDA has cleared our product for a phase I/IIa IND application that will allow us to transplant the iPSC-RPE patch in patients to treat “dry” AMD. This ongoing clinical study will allow us to test the safety and feasibility of the autologous iPSC-RPE patch in twelve “dry” AMD patients.

Keywords: Autologous iPSC therapy, Age-related macular degeneration, iPSC-RPE

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IDENTIFICATION OF MARKERS FOR OCULAR SURFACE CHARACTERIZATION IN LSCD PATIENTS TREATED BY ORAL MUCOSA

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Total bilateral Limbal Stem Cells Deficiency is a pathologic condition of the ocular surface due to loss or impairment of corneal stem cell function, altering the corneal epithelium's homeostasis and bringing discomfort and blindness. Cultivated Oral Mucosa Epithelial Transplantation (COMET) is the only autologous successful treatment for this pathology in clinical applications. However, peripheric corneal vascularization often occurs. Proper characterization of the transplanted cornea is needed for accurate follow-up and understanding of the underlying regenerative and neovascularization processes. So far, the univocal identification of transplanted oral mucosa has been challenging due to the absence of tissue-specific markers. Previously proposed markers were shown to be co-expressed by the different ocular surface epithelia in a homeostatic or perturbated environment. In this scenario, we investigated new unique markers that distinguish the transplanted oral tissue from the other epithelia of the ocular surface. We compared the transcriptome of stem cells from the human oral mucosa, limbus and conjunctiva and found factors uniquely upregulated in oral epithelium. Moreover, we identified angiogenesis-related factors responsible for vascularization in oral mucosa-treated patients. Different molecular biology techniques validated the upregulated transcripts at RNA and protein levels. Finally, the proposed markers were used to investigate corneal samples with 10 years of follow-up of aniridia patient treated by COMET. These new findings have several implications: a) they will support the follow-up analysis of patients transplanted with non-ocular epithelia; b) they will provide the knowledge on the process of neovascularization to prevent it on patient corneas; c) they will help to shed lights on the mechanism of repair and regeneration of the cornea.

Funding Source: This study was funded by the Awards "Lombardia è Ricerca 2018" and "Louis Jeantet Award 2020" and by Holostem Terapie Avanzate s.r.l. and by Regione Emilia-Romagna (area 1b, medicina rigenerativa) and POR-FESR 2007-13-Tecnopolo.

Keywords: Oral Mucosa, Cornea, Neovascularization

TOPIC: GERMLINE AND EARLY EMBRYO

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AN OPTIMIZED APPROACH TO GENERATE OVARIAN SUPPORT CELLS FROM INDUCED PLURIPOTENT STEM CELLS: A PLATFORM TO DEVELOP THERAPEUTICS FOR DISEASES OF THE FEMALE REPRODUCTIVE SYSTEM

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An in vitro model of the human ovary would advance our understanding of female sexual development and reproduction, enabling development of novel fertility treatments such as robust oocyte in vitro maturation technology. A crucial functional component of such a model are ovarian support cells (OSCs), including granulosa, theca and stromal lineages. Together, OSCs are responsible for shaping the ovarian signaling and oocyte growth environment throughout development and adult life. Recently, our group reported an efficient 5 day protocol to generate FOXL2+ OSCs from human induced pluripotent stem cells (hiPSCs) through transcription factor (TF) overexpression. Resultant differentiated cells have transcriptomes resembling human in vivo OSCs and recapitulate key ovarian phenotypes in vitro, including follicle formation and steroidogenesis. As the induced OSCs are a heterogeneous cell population, differentiation outcomes, while high, exhibit significant variability (75.4 ± 23.3% FOXL2+ cells) and scRNA-seq analysis indicates that cell subpopulation composition varies across production batches. Aiming to increase reproducibility and ensure translatability to clinical settings, we applied design of experiments (DOE) to further optimize multiple factors known to be associated with intermediate mesoderm induction and OSC differentiation along with our standard TF overexpression. We assessed cell viability and FOXL2, AMHR2 and CD82 expression as indicators of successful OSC differentiation. We demonstrated that optimized conditions determined by DOE result in less variability in OSC induction than our previous differentiation protocol. The improvements in the differentiation process combined with quality by design approaches allow for reduced manufacturing costs and improve compliance with cGMP regulations. Further investigation will establish whether specific optimized conditions and associated OSC characteristics would give

a more robust clinical outcome in assisted reproductive technologies applications. Generation of a robust and consistent protocol to produce OSCs for oocyte in vitro maturation applications have the potential to widen access to IVF and egg freezing, as well as reduce the duration and hormonal burden to which women are subject during these processes.

Keywords: Ovarian support cells, Granulosa cells, Cell Manufacturing Directed Differentiation, Design of experiments, Quality by Design

TOPIC: HEMATOPOIETIC, IMMUNE, AND ENDOTHELIAL

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DEVELOPMENT OF AN IN VITRO POTENCY ASSAY OF IMMUNE EFFECTOR CELL-MEDIATED CYTOTOXICITY AND KINETICS

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Immunotherapy harnesses the power of the innate and adaptive immune system to seek out and attack cancer. Engineering chimeric antigen receptors (CAR) into T cells or Natural Killer (NK) cells to target tumor-associated or neo-antigens can lend high specificity. Stem cell-derived allogeneic therapies offer advantages over autologous products, particularly for manufacturing, off-the-shelf availability, and patient access. One such therapy is oNKord® NK cells developed by Glycostem Therapeutics, which are derived from umbilical cord blood hematopoietic stem cells (HSCs). Immunotherapy development relies on the use of in vitro potency assays—which are key for understanding complex interactions between immune (effector) cells and cancer (target) cells—to evaluate the function, specificity, and sensitivity of a product. Here, we describe an in vitro potency assay that quantifies the killing kinetics of various immune effector cells. We show examples using HER2-CAR T cells against SKOV3 target cells, as well as antibody-dependent cellular cytotoxicity (ADCC)-specific killing using the monoclonal antibody trastuzumab and oNKord NK cells against SK-BR-3 target cells. Target cells were first seeded into CytoView-Z plates. Electrical impedance measurements from the electrodes in each well detected cell attachment and proliferation. Immune effector cells, as well as antibody for ADCC experiments, were then added and co-cultured with the target cells for 24-72 hours. Cytolysis of the target cells was calculated by comparing treated wells to no treatment control wells, and the kinetics of cell death were determined by the time required for effector cells to kill half of the target cells (kill time 50, KT50). For CAR-T experiments, antigen-specific cytolysis of target cells was evaluated by comparing SKOV3 cytolysis from HER2-specific CAR T cells (93.5 +/- 1.5%) to donor-matched mock CAR T cells (52.1 +/- 9.4%) and non-transduced T cells 24 hours post-effector cell addition. The kinetic readout from the assay indicates a KT50 of 7.1 +/- 0.6 hours for HER2 CAR T cells and 22.6 +/- 4.8 hours for mock CAR T cells. For NK experiments, ADCC was observed with trastuzumab and oNKord NK cells from three different donors. Future work will further evaluate cytolysis kinetics using additional immune therapies and cancer models.

Keywords: Immunotherapy, Potency assay, Impedance

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DEVELOPMENT OF TRANSLATABLE PROCESSES FOR VIRAL & NON-VIRAL T-CELL MODIFICATION

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Immunotherapy has been revolutionized by advancements in Chimeric Antigen Receptors (CAR) T-cell manufacturing processes using viral, nonviral, or a combination of the two methods. However, further process improvements must be implemented for CAR T-cell therapies to become an “off-the-shelf” therapy in the clinical setting. Challenges associated with viral modification include the need for high-quality virus with adequate purity and potency, while nonviral methods rely on efficient delivery methods and components. Additionally, the design of the designated clinical study defines the scalability at which the CAR T-cell manufacturing process is to be performed. This calls for a scalable and translatable approach to attain regulatory-compliant production under good manufacturing practice (GMP) conditions. In this study, we investigated flexible cell processing methods and gene delivery tools to generate CAR T-cells to best translate to GMP conditions. CAR T-cell workflows were performed using either single-step automated, multistep semi-automated, or modular protocols and assessed for improvements in cell phenotype, cell expansion, and gene editing efficiency. Using viral or non-viral modification platforms in combination with different donor materials, our efficiency in generating CAR T-cells ranged between 8.5% to 40.7%. Subsequent expansion of these CAR T-cells resulted in a 50 to 80-fold expansion rate with >90% viability. To further access the efficiency of our CAR T-cell process and identify additional factors impacting variability besides donor or lentivirus lot; we established flow cytometry and image-based in-process testing at various stages of processing. T-cell enrichment (~50% CD3(+) pre-process vs >95% CD3(+) post-process); the presence of contaminating cell types such as monocytes (18.2% pre-process vs <1% post-process); and activation status (CD25 >70%, CD69 >50%) of cells prior to transduction were established as key checkpoints to ensure successful runs. A systematic approach to understanding sources of variability will help specify control

measures and criteria which are critical for translation to scalable manufacturing.

Keywords: Immunotherapy, Good Manufacturing Practices (GMP), Bioprocessing

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HIGH-EFFICIENCY TARGETED TRANSGENE INTEGRATION VIA PRIMED MICRO-HOMOLOGUES

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Due to the difficulties in precisely manipulating DNA repair pathways, targeted integration of large transgenes triggered by double-strand breaks (DSBs) is inherently inefficient and non-specific. Here, we exploited prime editors (PE) in donor template processing and devised a robust knock-in (KI) strategy: Primed micro-homologues-assisted integration (PAINT). PAINT utilizes the reverse-transcribed single-stranded micro-homologues (MHs) to boost targeted KIs in various cell types. PAINT exhibits highest editing efficiency and minimized off-target integration effects, especially in dealing with scarless in-frame KIs. By the PAINT method, we achieved KI frequencies of up to 85% with a 2.5-kb CAG-EGFP reporter transgene at several therapeutically relevant genomic loci, indicating its application potential in clinical trials. We also devised a DSB-independent version of PAINT, nPAINT, which is triggered with a double-nick at the genomic target site, and achieved up to 40% editing efficiencies with much higher KI/indels ratios. Thus, we establish that the PAINT/nPAINT system is a powerful gene editing tool for large transgene integrations and will pave a new avenue for cell and gene therapies and genome writing technologies.

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Keywords: gene editing, targeted knock-in (KI), PAINT

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IMPROVING CD34 + YIELDS LEADS TO A ROBUST AND REPRODUCIBLE FEEDER-FREE HUMAN INK DIFFERENTIATION PROCESS

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Natural Killer (NK) cells show promise as a cancer immunotherapy to target both hematological and solid tumors. However, current sources of NK cells such as umbilical cord (UCB) or Peripheral blood (PB) cannot easily expand to clinically relevant numbers and are heterogeneous in cell composition. Theoretically, human iPSCs-derived-NKs (iNKs) may provide a uniform and potentially unlimited source of NKs for allogeneic cell therapy but current protocols are not robust or easily reproducible due to highly variable differentiation efficiency. Using small molecules and recombinant animal free proteins, we developed a feeder free differentiation process to reproducibly generate iNKs at scale by optimizing for CD34+ hematopoietic stem cells (iHSC) expression prior to downstream NK differentiation. These optimizations led to vastly improved iNK yields. Within 30 days, cells reached a purity of more than 95% CD56+, CD3- with a single iPSC yielding up to 500 iNKs. Subsequent activation and expansion of iNKs showed potent (>90%) killing of target cancer cells (including K562 and SKOV3) via an Incucyte based killing assay. Additionally, we transfected iPSCs with a CD19-CAR construct using TC-Buster to generate CAR-iNKs and obtained cells with increased B-cell lymphoma targeted cytotoxicity. These results show that optimizing for CD34+ HSCs is a critical step for robustly differentiating iNK from iPSCs. This protocol should greatly increase reproducibility and uniformity of clinically relevant iNK production for allogeneic cancer therapy.

Keywords: iPSC derived Natural Killer cell (iNK), iPSC derived Hematopoetic stem cell (iHSC), Differentiation optimization

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ESTABLISHMENT OF AN EXTERNAL QUALITY ASSURANCE PROGRAMME FOR VIABLE CD34 CELL ENUMERATION: TRANSLATION TO CLINICAL PRACTICE

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Blood and marrow transplant centers use CD34+ cell enumeration as the primary determinant of hematopoietic progenitor cell (HPC) content of the infused cells. A viable (rather than total) CD34+ assay should be performed on products that have been manipulated and/or cryopreserved. Although there are external quality assurance programs (EQAP) for total CD34+ enumeration using fixed cells, there is an urgent need to develop one to determine inter-laboratory accuracy and reproducibility of viable CD34+ enumeration. We have recently published a sustainable EQAP method which utilizes discarded cryopreserved HPC that have been approved and consented for research use. The current work is to translate research to clinical practice. An initial survey identified 28 laboratories across Australia that are interested in participating. Cryopreserved HPC were thawed, washed and re-frozen in 100 de-identified aliquots for use as the EQAP cryopreserved reference material. Aliquots were maintained in the vapor phase of liquid nitrogen apart from approximately 24 hours on dry ice during transit to participating centers. Ten of these samples were tested up to 3 months after transit by two different laboratories. The total CD34 counts between aliquots were reproducible (sample 1 total CD34 CV = 10.1%; sample 2 CV = 12.6%), but greater variation in viable CD34 counts was observed, particularly in sample 2 which had lower viability (sample 1 CV for viable CD34 = 21.2%; sample 2 CV = 30.5%). The viable CD34 counts in sample 2 did not show a progressive decrease over time suggesting that the low viability is due to sample quality rather than storage time post transit. The high CV is likely due to subjectivity in placement of the viability gate. The distribution of EQAP samples and collection of data for the viable CD34 EQAP will be coordinated through Royal College of Pathologists of Australasia Quality Assurance Programs. We expect to complete these feasibility and logistic studies within next few months and to launch the first EQAP for viable CD34+ cell enumeration in cryopreserved HSC products in continental Australia by mid-year.

Funding Source: Royal College of Pathologists of Australasia (RCPA) Foundation Grant

Keywords: Viable CD34, Quality assurance program, Hematopoietic transplantation

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GENERATION OF FUNCTIONAL T CELLS FROM IPSCS VIA RECAPITULATION OF THYMIC NOTCH SIGNALLING USING DLL4/VCAM1 MICROBEADS

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Adoptive cell therapy (ACT) with CAR-T cells derived from autologous or allogeneic donors has demonstrated success with hematological malignancies. However, the high cost and lengthy manufacturing times of autologous T cells and a risk of graft versus host disease (GvHD) with allogeneic cells have been initial roadblocks for therapeutic applications. Human induced pluripotent cells (iPSCs) can differentiate into T cells and are amenable to multiplexed gene edits and clonal expansion. Current methods for generating iPSC-derived T cells rely on plate bound or feeder-based presentation of Notch ligands which limit the scalability of the process. Hence, developing a defined, efficient, and bioreactor-compatible process to generate hematopoietic precursor cells (HPCs) with lymphoid potency is key to making iPSC derived T cells as a universal off-the-shelf product for ACT. Here we report the development of a proprietary, scalable Engineered Thymic Niche (ETN) comprising of DLL4-VCAM-conjugated paramagnetic microbeads that allow precise control of Notch signaling during T cell differentiation. In a previous study using the ETN we demon-

strated the generation of Pro T cells and further differentiation to CD8αβ+ cells in the presence of sustained high concentration of Notch. The present study focused on recapitulating the canonical T cell differentiation pathway in the thymus using the ETN platform. First, we generated multipotent CD34+CD43+CD45+ HPCs across multiple iPSC lines with a high yield and purity that resemble definitive HPCs in the human embryo. Next, we optimized the ratio of ETN dose and cell density to recapitulate high Notch signaling required to drive HPCs to the Progenitor T-cells stage of T-cell development. Then, we fine-tuned the decline of Notch signaling intensity using the ETN to generate double positive CD4+CD8+, which then mature to single positive CD4-CD8+ αβ T cells from unmodified and engineered iPSCs. T cells generated from iPSC expressing CAR demonstrated multiple rounds of in vitro tumor cell killing. The stage specific transition from HPCs to T cells was supported by phenotypic characterization and analysis of Notch target genes. The results demonstrate that the modular ETN platform is a powerful system that can generate clinical batches of iPSC derived T cells for ACT applications.

Keywords: iPSC-derived T cells, Notch signalling, Engineered Thymic Niche

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REACTIVATION OF FETAL HEMOGLOBIN BY GENERATING HPFH3 GENOTYPE IN SICKLE CELL DISEASE AND BETA-THALASSEMIA PATIENTS-DERIVED HSPCS TO TREAT THE DISEASE

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Beta hemoglobinopathies are a set of inherited blood disorders affecting beta-globin production, a constituent of hemoglobin. These diseases include sickle cell disease (SCD) and beta-thalassemia (BT), which result from genetic mutations in the HBB gene that play a role in producing adult hemoglobin (HbA) in red blood cells (RBCs). SCD leads to the creation of sickle hemoglobin (HbS), which gives the RBCs a sickle shape, while over 200 mutations are responsible for BT, resulting in the reduced or no production of HBB. Designing donor DNAs for each type of mutation in beta-thalassemia (BT), which involves over 200 mutations, and sickle cell disease (SCD), is a difficult and impractical approach for gene-correction-based therapies. Furthermore, such therapies are restricted by the limited efficiency of homology-directed repair, while utilizing the naturally occurring HPFH3 genotype to reactivate fetal hemoglobin (HbF) has the potential to alleviate symptoms of the disease. HPFH3 genotype is a deletion of 50 kb region of the beta-globin gene cluster encompassing HBD, HBB, and some repressors of HBG1/2. The deletion is associated with the reactivation of HBG1/2 gene causing HbF induction. HPFH3 genotype were recapitulated in patients' HSPCs using efficient NHEJ-based outcomes of CRISPR/Cas9, and the edited cells were differentiated into erythroid cells to assess the cellular and molecular aspects of the disease. We found that HPFH3 genotype reactivated HbF in edited HSPCs-derived erythroid cells, resulting in reduced symptoms such as improved erythropoiesis and decreased oxidative stress in both SCD and BT cells. In our study, we observed that edited sickle cell disease (SCD)-derived cells showed a noteworthy decrease of 81% in the number of sickled cells, while edited beta-thalassemia (BT)-derived cells showed the restoration of globin chain balance. The HPFH3 genotype bears several advantages as a genome editing strategy for beta-hemoglobinopathies, including its therapeutic potential as a naturally occurring genotype, and its suitability for treating both SCD and BT. Additionally, the strategy relies on the efficient NHEJ-based outcome of CRISPR/Cas9.

Funding Source: Department of Biotechnology, India CSIR-IGIB, Delhi, India

Keywords: beta-hemoglobinopathies, fetal hemoglobin, genome editing in hematopoietic stem cells

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GENERATION OF HUMAN IPSC-DERIVED NATURAL KILLER CELLS FOR ALLOGENEIC CANCER IMMUNOTHERAPY

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Natural Killer (NK) cell-based immunotherapies have gained significant traction to treat several cancer types including B-cell lymphoma as they have achieved appreciable and durable responses without eliciting the adverse toxicity events that have been associated with CAR-T therapies. NK-based therapies do not require complete HLA-matching and therefore can be manufactured from allogeneic cell sources. Generating functional NK cells from a potentially unlimited source like iPSCs alleviates many manufacturing and affordability constraints. iPSC-derived NK cells have so far proven as effective as those from primary cell sources. Facile genetic modification and the capability to create clonal iPSC lines provides huge potential for 'off-the-shelf' therapies with increased functionality. We have used a combinatorial screening platform specifically developed for use with stem cells, CombiCult®, to test thousands of cell culture protocols in parallel to discover efficient, cost-effective, feeder-free protocols for differentiation of hiPSCs into functional NK cells. Our screening strategy was focused on development of serum-free and feeder-free GMP-compliant protocols for production of functional NK cells from human iPS cells using defined media compositions that effectively substitute exogenous signals necessary for NK cell-poiesis. In a CombiCult® screening experiment the starting iPS cells are encapsulated in alginate microbeads then subjected to a predefined combinatorial cell culture regime through serial split-pool cycles. Altogether, different beads were exposed to 4,032 protocols, and screened for the presence of proliferative leukocyte progenitors at Day 12 of differentiation (using CD45 staining and EdU-Click incorporation) and mature NK cells at Day 32 (expression of CD45+/CD56+). Using the proprietary bioinformatics software (Ariadne) we deconvoluted hits and deduced the optimal differentiation protocols through statistical analysis of high performing media components. Ten serum-free feeder-free protocols were further validated in both alginate-encapsulated and suspension cultures. The phenotype of differentiated NK cells was assessed by expression of maturation and functionality

markers, as well as their ability to kill tumor cells in cytotoxicity assays.

Funding Source: Innovate UK: EUREKA GlobalStars Singapore CRD – Round 2 Innovate UK: Biomedical Catalyst 2021 Round 2: Feasibility & Primer Awards

Keywords: Natural Killer cell-based immunotherapies, human iPSC-derived Natural Killer (NK) cells, combinatorial screening platform

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USING THE CTS XENON ELECTROPORARION SYSTEM WITH A TAL-BASED GENE EDITING PLATFORM ON A PATH TO CLINICAL T CELL THERAPY APPLICATIONS

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TALXcell™ platform is a novel, programmable TAL-based nuclease for genome editing. TALXcell™ platform consists of a DNA binding domain that is specifically engineered to target your site of interest and that is fused with a highly effective, proprietary nuclease to cleave the DNA. Unlike CRISPR-Cas9, TALXcell™ platform has no sequence restrictions so it can target any desired sequence within a genome without PAM site. Recently, the advanced cell and gene therapeutics sector has been undergoing significant transformations, driven by the clinical success of CAR T cell therapy. To minimize risk for therapeutic developers, it is essential to ensure the manufacturing process is suitable for clinical production. Thermo Fisher Scientific has been providing solutions for cell therapy manufacturing by incorporating innovative technologies into the Gibco™ Cell Therapy Systems™ (CTS) product line. One such product is the Gibco™ CTS™ Xenon™ Electroporation System, which supports closed, scalable GMP-compliant cell therapy manufacturing. The CTS™ Xenon™ electroporation system delivers non-viral gene editing through reliable delivery of DNA, RNA, proteins and other molecules into cells, with exceptional cell viability and recovery. The workflow is as follow: On day 0, PBMCs from 3 different donors were thawed and activated using Gibco™ CTS™ Dynabeads™ CD3/28 and expanded in Gibco™ CTS™ OpTmizer™ T Cell Expansion SFM, supplemented with Gibco™ CTS™ Immune Cell SR and other components as instructed per product insert. On day 3, activated T cells were debeaded and electroporated using the Xenon electroporation system with TALXcell™ technology. As a result, functional CAR T cells were generated with high viability (>89%), high knockout efficiency (>90%) and similar gene editing efficiency (25-32%) 7 days post-electroporation for 3 different donors. Additionally, those CD19+ CAR T cells demonstrated the ability to efficiently kill GFP-labeled target cells. Through our analysis of electroporation efficiency and functionality of the resulting CAR T cells, we showcase that the integration of the CTS™ Xenon™ electroporation system and TALXcell™ platform can be used for non-viral cell therapy applications.

Funding Source: Thermo Fisher Scientific

Keywords: Xenon Electroporation, T cell therapy, TALXcell

TOPIC: KIDNEY

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ADVANCED ORGAN THERAPEUTICS AS A NOVEL TREATMENT FOR RENAL FAILURE

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Over two million patients worldwide depend on renal replacement therapy due to end stage renal disease. Transplantation is the only cure, yet available only to a small fraction of patients due to the shortage of donor organs and the need for chronic immunosuppression. As an alternative to both hemodialysis and transplantation, we aim to leverage recent progress in hypo-immune stem cell technology, and to replace renal function with advanced organ therapeutics. To date, our team has achieved four milestones: (1) Scalable manufacturing of a robust biomimetic filtration membrane that supports water and solute transfer and can be maintained and remodeled by cells to prevent fouling and ensure long term function in direct blood contact.(2) A tunable hierarchical blood distribution system that accommodates sufficient blood flow to the filtration membrane at a resistance that is compatible with the boundaries of the human cardiovascular system. (3) Integration of membrane and channel system in a biomimetic fully cell lined biologic system of human scale that is resilient and capable of self-repair like a human organ. (4) A functional architecture that enables filtration and subsequent self-balancing separation of water and solute transport to enable sufficient toxin removal without the need for adjustment and dialysate. By combining these building blocks, we have created a scalable biologic blood purification device that achieves toxin and fluid removal in vitro. Two devices were subsequently anastomosed to the ab-

dominal aorta and inferior vena cava in large animal pilot studies and produced a filtrate under blood perfusion by the recipient.

Keywords: bioengineered kidney, organ engineering, biologic blood purification

TOPIC: LIVER

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STUDYING THE EFFECT OF SMALL MOLECULE DRUG ON ALAGILLE SYNDROME PATIENTS CELLS DERIVED SPHEROIDS AND ORGANOIDS

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Alagille syndrome is a rare genetic disorder caused by a mutation in the notch ligand gene JAG1, which leads to lower activation of the Notch2/Sox9 signaling axis. The global reported incident is 1 in about 30,000-40,000 births, yearly. Furthermore, the survival rate without a liver transplanted is less than 30% by 18.5 years old. The Jag1/Notch2/Sox9 signaling is widely involved in developmental events, including to germ layer specification, axis formation, and organogenesis. The failure of JAG1/Notch2 activation causes intrahepatic biliary paucity (no or less liver duct formation), liver cirrhosis, slow development/mental growth retardation, cardiac ventral septum defects, weight loss, jaundice, and many other birth defects. Several studies have shown that the Notch2/Sox9 signaling is a key player in liver duct development. In ALGS patients, the notch signaling is failed to activate the Notch2 downstream gene regulatory network (GRN), resulting in no or less expression of Sox9 in a Notch2-depedent manner. The project focuses on studying the molecular mechanism of Notch2 signaling activation in hepatocyte cells (HepG2) and heterozygous hepatocytes (HepG2-Jag1+/-). The goal is to identify a Notch2 agonist and its clinical validation to regenerate the bile network in ALGS patients to target the bile paucity in liver. A small molecule drug (NoRA1) is being used to study its effect on bile duct regeneration in-vitro and in-vivo model systems. The results indicate that NoRA1 activated Notch2 signaling and increased Sox9 expression in WT-HepG2 and Jag1+/- cells (invitro). Moreover, NoRA1 has shown an increase in the expression of Sox9 in zebrafish's liver with mutant JAG1 (in vivo) (Zhao et al, 2022). Additionally, NoRA1 also augmented the Sox9, and Hes1 expressions in mice liver. In spheroid, NoRA1 rescued MDR1 (Bile Canaliculi marker) level in a Jag1+/- spheroid which was decreased due to Jag1+/- knockout spheroid compared to untreated group (fig.4). Thereafter, the MDR1 expression remarkably reduced in double heterozygous (Jag1+/- and Notch2+/-) mice liver. At the last, results suggested that NoRA1 has Notch2 agonist therapeutic potential, leading to duct regeneration in ALGS patients. Currently, we are focusing to validate its clinical aspect in organoids model and trigger duct regeneration in ALGS patients.

Keywords: Bile Ducts Notch Signaling Pathway Alagille Syndrome Protein Expression SOX9, Notch 2, MDR1 Notch receptor Notch ligand Jagged 1+/- Intrahepatic biliary paucity spheroids, Alagille Syndrome Protein Expression SOX9, Notch 2, MDR1 Notch receptor Notch ligand Jagged 1+/- Intrahepatic biliary paucity spheroids, Rare autosomal genetic disorder

TOPIC: MESENCHYMAL STROMAL CELLS, ADIPOSE, AND CONNECTIVE TISSUE

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ENHANCED CHONDROGENIC DIFFERENTIATION OF IPS CELL-DERIVED MESENCHYMAL STEM/ STROMAL CELLS VIA NEURAL CREST CELL INDUCTION FOR HYALINE CARTILAGE REPAIR

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To date, there is no effective long-lasting treatment for cartilage tissue repair. Primary chondrocytes and mesenchymal stem/stromal cells are the most commonly used cell sources in regenerative medicine. However, both cell types have limitations, such as dedifferentiation, donor morbidity, and limited expansion. Here, we report a stepwise differentiation method to generate matrix-rich cartilage spheroids from induced pluripotent stem cell-derived mesenchymal stem/stromal cells (iMSCs) via the induction of neural crest cells under xeno-free conditions. The genes and signaling pathways regulating the chondrogenic susceptibility of iMSCs generated under different conditions were studied. Enhanced chondrogenic differentiation was achieved using a combination of growth factors and small-molecule inducers. We demonstrated that the use of a thienoindazole derivative, TD-198946, synergistically improves chondrogenesis in iMSCs. The proposed strategy produced controlled-size spheroids and increased cartilage extracellular matrix production with no signs of dedifferentiation, fibrotic cartilage formation, or hypertrophy in vivo. These findings provide a novel cell source for stem cell-based cartilage repair. Furthermore, since chondrogenic spheroids have the potential to fuse within a few days, they can be used as building blocks for biofabrication of larger cartilage tissues using technologies such as the Kenzan Bioprinting method.

Funding Source: This research was supported by the Incubation Program of Kyoto University, HEALIOS K.K., Ajinomoto Co., Inc., Takeda Pharmaceutical Company Limited, AMED, JSPS, and iPS Cell Research Fund.

Keywords: induced pluripotent stem cells, chondrogenesis, cartilage

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BONE MARROW DERIVED MESENCHYMAL STEM CELLS COMBINED WITH PLATELET RICH PLASMA VERSUS PLATELET RICH PLASMA IN KNEE OSTEOARTHRITIS: A RANDOMISED COMPARATIVE STUDY

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Conservative management fails to repair the articular cartilage in knee osteoarthritis (KOA). Biological agents such as Platelet-rich plasma (PRP) has been found to be promising treatment option, however short-term effects and limited efficacy with increasing age are the limitation. Hypothesis was that autologous bone marrow derived mesenchymal stem cell (BM-MSCs) enhanced PRP would improve the clinical outcome and healing of articular cartilage. Thirty-two patients with bilateral early KOA of Ahlbacks grade I &II, were randomly allocated to either PRP with MSCs treatment (group I; 16) or PRP only (Group II; 16). Follow up clinical evaluation was done on 6th week, 6th month and 12 months by visual analogue scores for pain (VAS), Western Ontario and McMaster Universities Index of Osteoarthritis (WOMAC) and Knee injury and Osteoarthritis Outcome Score (KOOS). Mean articular cartilage thickness was evaluated before and after 12 months. VAS at 1st follow-up after PRP combined with MSCs (41.88±11.67) was significantly lower than PRP alone (52.50±9.30) ($p < 0.006$) and percentage change of VAS score from baseline to 12 months follow-up after MSCs and PRP was significantly more than PRP alone (46.36±14.19 vs 31.60±10.24; p=0.002). Decrease in stiffness from baseline to 1st and 2nd follow-up was significantly more in group I compared to group II. However, at final follow-up difference was not significant. Percentage change in KOOS ADL score from Baseline to 12month follow up was significant. Percentage change in KOOS sports and recreational score in group I (32.11±44.72) was significantly ($p< .037$) more than group II (-11.04±65.22). Global WOMAC score was significantly better at 6 months follow-up. However, at 12 months the difference was not significant. MSCs enhanced PRP increased cartilage thickness from 3.62±0.56 mm at base line to 4.06±0.38mm at 12 months follow-up. PRP alone increased cartilage thickness from 3.61±0.32 to 3.77±0.33. Increase in mean articular cartilage thickness in group I was significantly higher than in group II ($p < 0.030$). This study suggests that pain relief, functional outcome and healing of articular cartilage were better after intraarticular injection of BM-MSC with PRP than PRP alone.

Funding Source: No funding involved

Keywords: Osteoarthritis, Cartilage, bone marrow derived mesenchymal stem cell

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EXPANSION OF HUMAN MESENCHYMAL STROMAL/STEM CELLS ON DECELLULARIZED ADIPOSE TISSUE SCAFFOLDS PRESERVES PRIMITIVE MSC PHENOTYPE AND ENHANCES PRO-ANGIOGENIC SECRETORY FUNCTION

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Due to limited treatment options for individuals with critical limb ischemia (CLI), cellular-based therapies have been investigated to induce collateral blood vessel regeneration. Bone marrow-derived mesenchymal stromal/stem cells (BM- MSC) have shown pre-clinical success in animal models of CLI as they possess pro-angiogenic and immunomodulatory secretory functions. However, clinical translation has been hindered by inadequate expansion and poor survival in the ischemic limb after injection. This project aimed to characterize the phenotype and pro-angiogenic secretory function of human BM-MSC cultured on decellularized adipose tissue (DAT) bioscaffolds as an expansion and delivery platform. Human fat samples were decellularized through an established detergent-free treatment protocol and then processed to generate DAT coatings. BM-MSC seeded onto DAT coatings were assessed in vitro for cell survival, proliferation, surface marker phenotype, mass spec-based proteomic analyses, and pro-angiogenic secretory function. In vivo function was characterized via the Directed in vivo Angiogenesis Assay (DIVAATM). Compared to cells grown on tissue-culture plastic (TCP), DAT coatings increased BM-MSC proliferation and regenerative marker expression (Aldehyde dehydrogenase and CD271). Conditioned media (CM) generated by BM-MSC cultured on DAT coatings significantly increased human endothelial cell survival under starvation conditions in vitro, and proteomic analysis of the CM showed enrichment with factors associated with wound healing and angiogenesis in the BM-MSC expanded on DAT relative to TCP. Similarly, BM-MSC cultured on DAT coatings increased endothelial cell infiltration in DIVAATM inserts implanted in NOD/SCID mice. BM-MSC expansion on DAT coatings represents a promising approach to retain primitive cell phenotype and enhance pro-angiogenic secretory function.

Funding Source: CIHR and NSERC

Keywords: Vascular Regeneration, Mesenchymal Stem Cells, Bioscaffolds

TOPIC: NEURAL

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MICROTISSUE CELL THERAPY FOR PARKINSON'S DISEASE: SCALE-UP IN BIOREACTORS AND LATEST IN VIVO RESULTS

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A breadth of preclinical studies (Doi et al, 2020; Piao et al, 2021; Hiller et al, 2022) is now backing the rationale of cell replacement therapies as a disease-modifying treatment to restore motor function in Parkinsonian patients. As programs move to the clinic - with several phase I trials initiated over the past 5 years - cell therapy players are still facing several issues with progenitors derived from pluripotent stem cells, including batch-to-batch variability, low post-transplantation survival, uncontrolled in situ neurodifferentiation and proliferation risks. To ensure accurate dosing and enhance functional reproducibility, one solution may consist in changing the graft format, moving away from single cell suspensions to neural microtissues that are resistant to the stress of transplantation. However, this 3D approach typically requires the use of well-plates, and thus remains unscalable. TreeFrog Therapeutics created a scalable approach to generate 3D neural microtissues from human induced pluripotent stem cells in standard bioreactors using a proprietary C-Stem technology. In this presentation, we will describe the manufacturing process up to cryopreservation, the latest in vivo results with cryopreserved products demonstrating safety and dose-dependent efficacy, as well as our roadmap to the clinic, including large animal studies to validate transplantation procedure, toxicity studies and Phase I.

Keywords: Parkinson's disease, 3D neural microtissues, Cell therapy

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QUALITY CONTROL AND PRECLINICAL STUDIES OF HUMAN IPSC-DERIVED RETINAL SHEETS FOR TISSUE-TRANSPLANTATION THERAPY

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Retinitis pigmentosa, a group of hereditary diseases characterized by a loss of photoreceptors, is the major cause of untreatable blindness in developed countries. Pluripotent stem cell (PSC)-derived three-dimensional retinal organoid (3D-retina) is anticipated as a promising graft source for retinal tissue-sheet transplantation therapy. We have previously developed a self-organizing culture technique based on SFEBq to generate 3D-retina. Here we present a quality control strategy and preclinical study results for tissue-sheet transplantation. We found that self-organizing human PSCs differentiated into both retinal and off-target tissues. Gene expression analyses identified the major off-target tissues as eye-related, cortex-like, and spinal cord-like tissues. We established qPCR-based quality test using a fragment of the retinal tissue-sheet (retinal sheet). Main part of each retinal sheet, to be used for transplantation, were preserved with non-freezing preservation method during the quality test. We performed a tumorigenicity study of these retinal sheets and observed no transplant-related adverse events. We also confirmed that retinal sheets subretinally transplanted into retinal degeneration model rats differentiated into mature photoreceptors and exhibited light responses in electrophysiology assays. These results demonstrate our rationale toward self-organizing retinal sheet transplantation therapy. Based on these technologies, we generated clinical grade human allogeneic iPSC-derived retinal sheets and supplied them to Kobe City Eye Hospital on October 2020 for the first-in-human clinical research for retinitis pigmentosa.

Funding Source: This work was funded by AMED and by Sumitomo Pharma Co., Ltd.

Keywords: self-organizing organoid culture, retinal tissue sheet transplantation, regenerative medicine

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TRANSPLANTATION OF HUMAN EMBRYONIC STEM CELL-DERIVED RETINAL SHEET IN A PRIMATE MODEL OF MACULAR HOLE

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A macular hole (MH) is a retinal break involving the fovea and causes impaired vision including metamorphopsia and central scotoma. Although routine vitreoretinal surgical protocols can achieve more than 90% MH closure rate after primary surgery, refractory cases such as high myopic MHs, large MHs, and recurrent MHs still exist. Recently, in such difficult cases, autologous retinal transplantation became an optional therapy, which allows for good anatomic success after surgery, but visual improvement is rare and peripheral visual field defects are inevitable after harvesting the transplant sheet. This study aims to evaluate if hESC-derived retinal organoid sheet transplantation can be an effective treatment for MH in the primate model. We used the retinal organoid sheet derived from Islet1-/- CRX::Venus hESC cell line (KhES-1) and transplanted it in the eye of the Japanese Macaque with idiopathic MH. Anatomical closure and foveal structure were assessed by optical coherence tomography (OCT). Subjective and objective visual functional examinations were also conducted. As for the subjective examination, we tried eye fixation tasks on this model before and after transplantation. Focal macular electroretinograms (FMERGs) were adapted as an objective test before and after transplantation. After seven months, the monkey was euthanized and the transplanted eye was studied by immunohistochemistry (IHC). Immediately after transplantation, MH was successfully closed and no obvious graft rejection was observed early after surgery; however, OCT findings showed slight macular edema around the graft at four months after transplantation and subtenon steroid injection was conducted. The fixation tasks were conducted at six months after transplantation, and the correct answer ratio significantly increased [from 1.5% (7/461) to 31.0% (54/174), $p < 0.001$]. The b-wave amplitude of FMERGs obviously increased at the same time. IHC showed that the grafted tissue was present in the host MH space and developed mature

photoreceptors inside. Also, GFAP-positive activated glial cells, possibly as a response to slight rejection, were observed segmentally in the graft. In summary, hESC-derived retinal organoid sheet transplantation would be effective for treating refractory MH and may be applicable in macular disease.

Funding Source: This research was supported by AMED grant JP20bm0204002.

Keywords: Macular Hole, Human ESC-derived Retinal Organoid, Xenotransplantation

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THERAPEUTIC POTENTIAL OF HUMAN IPSC-DERIVED RETINAL SHEETS IN RESTORING VISUAL FUNCTION IN A SWINE MODEL OF MACULAR DEGENERATION

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Retinal degenerative diseases (RDs) affect millions of people worldwide, and legal blindness is commonly associated with the loss of cone photoreceptors (PRs) in the central macular region. Currently, no treatment is available to stop or reverse these diseases. Induced pluripotent stem cells (iPSCs) are a promising tool for regenerative medicine, as they can differentiate into any cell type in the human body, including retinal neurons. We have developed a method to produce large amounts of cone photoreceptor precursors from human ESC and iPSC using the Cerberus-like family member COCO/DAND5, allowing us to generate a polarized human retinal sheet with a high proportion of cone precursors. We transplanted round retinal sheets derived from human iPSCs into a chemically-induced swine model of macular degeneration, which showed complete destruction of the outer nuclear layer (ONL) without affecting the inner nuclear layer (INL) in an 8mm diameter area around the macula. The grafted retinal sheets integrated into the host retinal tissue and survived for several months. OCT and histological studies indicated the formation of a new polarized “hiPSC-derived ONL” and the generation of synaptic connections between grafted cells and the host INL. Multifocal ERG analysis suggested some restoration of bright-light responsiveness at the grafted site in one of the transplanted animals. No residual pluripotent stem cells were detected in the graft. This work demonstrates the therapeutic potential of iPSC-derived retinal sheets in restoring visual function by replacing degenerated photoreceptors and provides new insights into the treatment of RDs such as age-related macular degeneration, retinitis pigmentosa, and photoreceptor degeneration caused by traumatic injury or inherited genetic disorders.

Funding Source: Stem cell network, Healios, StemAxon

Keywords: Preclinical study, regenerative medicine, Macular Degeneration (MD), photoreceptors, Retinal sheets, transplantation

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DIFFERENTIAL MONOLAYER FORMATION OF HIPSC-DERIVED RPE SUBPOPULATIONS AFTER SUBRETINAL TRANSPLANTATION INTO RPE-DEPLETED MICE

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Retinal pigment epithelium (RPE) degeneration leads to photoreceptor dysfunction and loss causing visual impairment and blindness. Cell replacement using human RPE generated from pluripotent stem cells in vitro represents a potential therapeutic strategy, however cell cultures can be heterogenous. We therefore identified cell surface markers to detect, isolate and transplant RPE subpopulations and examined how the groups differ with respect to cell survival, monolayer formation and maturation after suspension transplantation into RPE-depleted mice. A combination of two markers was used for MACSQuant® Tyto® based cell sorting. Suspensions containing 50,000 cells from unsorted, target-enriched or target-reduced fractions were transplanted subretinally into RPE-depleted C57BL/6JRj mice, which had intravenously received 30 mg/kg NaIO3 one week before. 80 µg triamcinolone acetonide were injected intravitreally for local immune suppression. Three weeks later, eyes were analyzed by IHC staining, electron microscopy and bulk RNA sequencing. All cell fractions expressed RPE-specific markers, showed survival for three weeks and were able to generate a RPE-specific phenotype in vivo with a basal labyrinth, basal nuclei, melanosomes and apical microvilli. While all groups formed correctly polarized monolayers the extent of retinal coverage and monolayer formation varied greatly between groups. Target-enriched cells covered the largest retinal area (35%) and exhibited the highest fraction of monolayer (72% of graft area). Unsorted and target-reduced cells showed similar retinal coverage (30% and 24%), yet a significantly lower proportion of polarized monolayers (32% and 38%). Here, we show that increased homogeneity of hiPSC-derived RPE cell compositions after surface marker based flow sorting greatly affects the capacity for monolayer formation after transplantation into RPE-depleted mouse retinas. Remarkably, target-enriched human RPE formed monolayers covering up to 72% of the graft and 35% of the entire retinal surface, an important prerequisite for proper RPE function. Our results thus suggest

that cell transplantation approaches for the treatment of retinal degenerative diseases require careful selection of appropriate donor cell populations.

Funding Source: ReSight BMBF

Keywords: RPE, sorting, Tyto

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IMPROVED DOPAMINERGIC NEURON DIFFERENTIATION FROM LINEAGE-RESTRICTED UNDIFFERENTIATED STEM CELLS

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The differentiation of human pluripotent stem cells (hPSCs) into mesencephalic dopaminergic (mesDA) neurons requires a precise combination of extrinsic factors that recapitulate the in vivo environment and timing. Current methods are capable of generating mesDA neurons after long-term culture in vitro; however, when mesDA progenitors are transplanted in vivo the resulting mesDA neurons are only minor components of the graft. In this study, we genetically modified pluripotent stem cells to generate a novel type of stem cell that we named lineage-restricted undifferentiated stem cells (LR-USCs). LR-USCs are prevented from differentiating into a broad range of nondopaminergic cell types due to the knockout of genes critical for the specification of alternate lineages. When LR-USCs were differentiated using a state-of-the-art protocol, significantly more of the LR-USCs generated bona fide caudal midbrain floor plate progenitors, compared to human embryonic stem cells (hESCs) differentiated under the same conditions. Furthermore, LR-USCs generated significantly more dopaminergic (DA) neurons in vitro compared to hESCs from the same genetic background. We show that the LR-USC-derived mesDA neurons were electrophysiologically active in vitro and restored motor function eight weeks after transplantation into 6-hydroxydopamine (6-OHDA)-lesioned rats. This novel strategy improves the efficiency, reliability and thus scalability of mesDA neuron generation for clinical use.

Keywords: Dopaminerigc neurons, human pluripotent stem cells, Lineage restriction

TOPIC: PANCREAS

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STEM CELL DERIVED ISLET CELLS SHOW ROBUST SURVIVAL AND FUNCTION WHEN TRANSPLANTED IN THE MUSCLE WITHOUT NEED FOR ADDITIONAL BIOSCAFFOLDING

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The recent clinical result shown in a single patient using stem cell-derived islet cells (SC-islets) with immune suppression has highlighted the potential such therapies may hold for a broadly accessible cure to diabetes. However, much like the cadaveric islet transplant field, these clinical trials with non-encapsulated SC-islets utilize portal vein infusion to deliver the therapy - an invasive procedure with the potential to introduce complications, including instant blood mediated immune response (IBMIR). Moreover, diffuse engraftment of cells within the liver makes monitoring the entire graft in situ difficult, thus limiting the safety and functionality data that can be gathered about the cells following implantation. Intramuscular (IM) administration of SC-islets could avoid IBMIR, simplify monitoring, and increase patient accessibility but has not been thoroughly studied. To evaluate the potential of the IM site of implantation using hypoimmune-edited (HIP) SC-islets, 1 million to 10 million cells were transplanted into the hamstring of immunodeficient mice made diabetic via streptozotocin treatment (n=49 total). An additional pilot study tested implants in the gastrocnemius and abdominal wall skeletal muscle locations (n=1 each). Graft success was assessed by sustained euglycemia (< 200 mg/dL), as well as by stimulated human c-peptide production and glucose tolerance test compared to cadaveric islets implanted in kidney capsule. IM-injected SC-islets corrected disease phenotype at multiple cell doses, cell batches, and skeletal muscle locations. Euglycemia was observed across the dose ranges and implant locations tested, and 100% of mice receiving 5 million cells or more were cured when dosed in the hamstring. Time to euglycemia was dose dependent, with doses of 10 million cells normalizing blood glucose as early as 4-weeks post implant. Clinical translation of these methodologies may result in cell therapies that are easier to monitor in situ and that may be administered in an outpatient setting. Moreover, the HIP edits, which have been shown in other experiments to protect cells in immunocompetent animal models without the need for immunosuppression, did not impede the functionality of the islets. HIP SC-islets thus represent a potentially accessible, functional cure for type 1 diabetes.

Keywords: Diabetology, iPSC, Intramuscular

TOPIC: NO TISSUE SPECIFICITY

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SELECTIVE VULNERABILITY OF HUMAN-INDUCED PLURIPOTENT STEM CELLS TO DIHYDROOROTATE DEHYDROGENASE INHIBITION DURING MESENCHYMAL STEM/STROMAL CELL PURIFICATION

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The use of induced mesenchymal stem/stromal cells (iMSCs) derived from human induced pluripotent stem cells (hiPSCs) in regenerative medicine involves the risk of teratoma formation due to hiPSCs contamination in iMSCs. Therefore, eradicating the remaining undifferentiated hiPSCs is crucial for the effectiveness of the strategy. The present study demonstrates the Brequinar (BRQ)-induced inhibition of dihydroorotate dehydrogenase (DHODH), a key enzyme in de novo pyrimidine biosynthesis, selectively induces apoptosis, cell cycle arrest, and differentiation; furthermore, it promotes transcriptional changes and prevents the growth of 3-dimensional hiPSC aggregates. Contrastingly, BRQ-treated iMSCs showed no changes in survival, differentiation potential, or gene expression. The results suggest that BRQ is a potential agent for the effective purification of iMSCs from a mixed population of iMSCs and hiPSCs, which is a crucial step in successful iMSC-based therapy.

Funding Source: (AMED) under Grant Number JP15bm0104001 and JP22bm1123006, grants-in-aid JSPS (#22H03275, #22H03204), the iPS Cell Research Fund, and Takeda Pharmaceutical Company, and by JST SPRING (grant number JPMJSP2110)

Keywords: Mesenchymal stem/stromal cells (MSCs), Induced pluripotent stem cells (hiPSC), brequinar (BRQ)

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DEVELOPMENT OF CELL THERAPY SET PROTOTYPE FOR MY IPS

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We are preparing to launch the my iPS® project in Japan in 2025, a custom-made cell therapy method in which iPS cells are generated from the patient's own blood, differentiated into cells suitable for the patient's treatment, and transplanted into the patient.

A closed culture system is under development to produce therapeutic cells via iPS cells from the patient's own cells. The "Cell Therapy Applications for my iPS®" are a closed culture set that is equipped with 10 culture ports for each culture process, and the cells are moved to the next culture port according to the step of the culture process. When using blood collected in a hospital outpatient setting as a source for iPS cell establishment, it is desirable to be able to inject the desired cells into a closed culture system without opening the blood collection tube. Therefore, we chose BD Vacutainer mononuclear cell preparation tubes for our blood collection tubes. After blood collection, PBMCs (peripheral blood mononuclear cells) were separated as supernatant by centrifugation, and then PBMCs in the blood collection tube were connected to the culture port using the BD Vacutainer Luer-Lok™ Access Device as a connection connector to complete injection. Next, in the process of establishing iPS cells and inducing differentiation, the cells were attached to atelocollagen beads so that the cells and beads could move between the culture processes without cell detachment. As a result, the therapeutic cells are loaded into a syringe together with atelocollagen beads at the final stage for commercialization. As a pilot study using the my iPS® cell therapy set prototype, we first established iPS cells on atelocollagen beads using PBMCs and evaluated their characteristics (mRNA expression analysis, induction of 3-hybrid differentiation, flow cytometry analysis, karyotyping analysis, microarray analysis, and PluriTest, and cell proliferation test were performed. A series of processes was reproduced in a closed culture system to examine cell characteristics. Besides the above studies, we reproduce the series of processes using whole blood in a cell therapy set and verify the cell characteristics. In conclusion, we have developed a prototype closed culture system for use in the my iPS® project.

Funding Source: Agency for Medical Research and Development (AMED) under Grant Number JP22bm0104001.

Keywords: iPS cell, Closed culture system, Custom-made cell therapy

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VITRONECTIN IS A XENO-FREE AND DEFINED SURFACE FOR THE DERIVATION OF HUMAN EMBRYONIC STEM CELLS

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Human embryonic stem cells (hESCs) have unique abilities that enable their use in cell therapy, disease modelling, and drug development. Their derivation is usually performed using a feeder layer, which is undefined and potentially contagious, and because of these difficulties, there is a tendency to replace feeders with xeno-free defined substrates in recent years. We used truncated vitronectin for the derivation of hESCs for the first time, derived three hESC lines, and confirmed their undifferentiated state, hESC morphology, and standard karyotypes together with their potential to differentiate into three germ layers. These results support the conclusion that truncated vitronectin is a defined xeno-free substrate that is suitable for the derivation of hESCs.

Keywords: human embryonic stem cells, vitronectin, derivation

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CLINICALLY COMPLIANT MATRIX FOR HUMAN ES AND IPSC CULTURE FOR PRECLINICAL AND CLINICAL THERAPY

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Laminins influence phenotypic stability, differentiation, and functionality of all cells. Laminin 521 is a key basement membrane protein naturally expressed by human pluripotent stem cells (hPSCs) and is a critical factor of the PSC niche, promoting attachment, high survival, and robust long-term self-renewal of hPSCs. The lack of defined, xeno-free and robust matrixes for efficient expansion and differentiation of hPSC for cell therapy has been a drawback for the development of promising cell therapies. The cell therapy Biolaminin® 521 grades MX/CT521 are human, full-length, recombinant Laminin-521 substrates and the only products on the market which provide an optimal environment for xeno-free culture of hESCs and hiPSCs and most other anchorage-dependent cell types. MX/CT521 enables efficient single cell passaging of genetically stable hPSCs without any apoptosis inhibitors, e.g. Rocki. hPSCs reach 10 populations doubling already after 10days on MX/CT521, compared to 20 days on other matrices. The simplicity and reliability of the culture procedure, the rapid cell amplification and the genetic stability of the cells make MX/CT521 suitable for each stage throughout the cell therapy development process, from research development to commercialized therapy. Scientific publications have proven MX/CT to be an excellent matrix for culture of iPSC and differentiation into astrocytes, dermal fibroblast and islet-like cluster cells for various cell therapy treatment development. We showed MX/CT521 supports long term hESC and hiPSCs cultivation while maintaining normal cell karyotype. In conclusion, we demonstrated that MX/CT521 is an optimal matrix for human ESC and iPSC culture in cell therapy processes, due to its biological relevance that allows derivation, clonal cultivation, stable long-term pluripotent cell growth, scalability, and differentiation. We showed that Biolaminin® 521 grades are an optimal matrix for human PSC and ES culture due to its biological relevance that allows derivation, clonal cultivation, stable long-term pluripotent cell growth and scalability. We showed that hiPSC and hESC exhibit a normal morphology on Biolaminin® 521 can reach confluency by day3. Cells can keep pluripotent for more than 10 passages which are confirmed by immonocytology , flowcytometry and PCR with pluripotency markers :OCT4,NANOG, SSEA4 and TRA1-81 with maintaining normal karyotype and capability of EB formation. The robust method allows minimum culture maintenance and standardized protocols, which can easily be adapted

to automation platforms, making CT521 a suitable reagent choice for human cell therapy trials.

Keywords: stem cell niche, cell therapy, extracellular matrix

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FRIZZLED-7 BIOMARKER DETECTION IN CANCER LIQUID BIOPSIES

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Cancer stage at diagnosis is one of the most potent indicators of patient prognosis; however, screening methods are costly, invasive, and infrequently used in individuals younger than age 45. Though breast cancer in younger individuals is rare, it carries a poor prognosis relative to breast cancer in older individuals. Due to its poorer prognosis, impact on life expectancy, advanced stage at presentation, and higher prevalence of unfavorable tumor features compared to breast cancer at older ages, the disease carries a disproportionate burden on younger patients. We have identified the protein Frizzled-7 (FZD7) as a tumor-specific antigen in various cancers, including breast cancer. We propose to examine whether FZD7 can serve as a biomarker for the early detection of these different types of cancers. Toward this objective, we will test various liquid biopsies, including culture media from FZD7- expressing cancer cell lines, mammary tumor-bearing mouse sera, and cancer patient serum samples. We will process these samples to isolate exosomes, a type of vesicle abundantly produced and released from cancer cells. Exosomes are present in serum and are capable of carrying a variety of proteins, such as FZD7. In preliminary studies, we analyzed cell conditioned media (CCM) from an ovarian cancer cell line, MA-148, by immunoblotting for FZD7 and for the common exosome markers CD9, CD63, and CD81. We are investigating several protocols to optimize exosome isolation, including ultracentrifugation, size exclusion chromatography (SEC), commercial exosome isolation kits, and Nanoparticle Tracking Analysis. To date, the most reliable FZD7 detection was accomplished by performing SEC exosomal isolation and FZD7 immunoprecipitation on 48-hour CCM. The preliminary findings support the hypothesis that FZD7 is detectable in cancer cell CCM. FZD7 biomarker detection could provide a platform for a highly sensitive, inexpensive, and non-invasive screening method for certain solid tumor cancers at a broader range of ages, thus leading to improved survivability and treatment outcomes.

Keywords: cancer, biomarker, exosome

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UNDERSTANDING THE FACTORS INVOLVED IN U.S. PATIENT ATTITUDES ON UNPROVEN STEM CELL INTERVENTIONS TO IMPROVE PERSUASIVE EDUCATION

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Given the increasing availability of unproven stem cell interventions (SCIs) and the potential associated harms, understanding patient attitudes for acquiring SCIs is necessary and urgent to inform interventions to correct misinformation and discourage participation in unproven SCIs. We aimed to understand the attitudes, beliefs, and knowledge of patients interested in unproven SCIs. We conducted semi-structured interviews with 31 U.S. patients and families recruited from Mayo Clinic's Regenerative Medicine Consult Service and ResearchMatch databases. Participants were categorized as high or low SCI seekers based on intent to pursue unproven SCIs. We identified 6 factors (Hope, Desperation, Knowledge, Trust, Pre-existing Attitudes, and Personality Traits) that influence patient willingness to undertake an unproven SCI. Feelings of desperation and desire for hope were reported as main drivers for pursuing unproven SCIs among high seekers. Many patients expressed that desperation was influenced by experiencing chronic and unbearable suffering, feeling abandoned by the medical establishment, and not knowing where else to turn for treatment. Both low and high seekers reported low scientific knowledge of SCI and frequently relied on mental shortcuts or simple rules for decision-making. Many expressed they cannot understand scientific messaging about SCI efficacy or safety but relied on information from their trusted sources, including family, friends, and for-profit clinicians. Among most high seekers, higher tolerance of risk and uncertainty, the need to control health, willingness to diverge from medical norms, and mistrust of mainstream health and medical institutions contributed to higher intent to pursue unproven SCIs. Among most low seekers, skepticism of business practices among SCI clinics, respect of medical expertise, and trust in the medical establishment and regulatory agencies was involved in reducing intent to pursue unproven SCIs. Our findings suggest that persuasive education on unproven SCIs should empathize with patient experiences, use trusted message sources, and guide critical thinking. Warnings from gov-

ernment agencies are unlikely to be successful at deterring patients towards unproven SCIs and may cause reactance.

Funding Source: National Institute on Aging, National Institutes of Health (R21AG068620 and R03AG078555)

Keywords: unproven stem cell interventions, U.S. patient perceptions, Persuasive education and policy

VIRTUAL POSTERS

TRACK: CELLULAR IDENTITY (CI)

TOPIC: CARDIAC

2001

WNT-HISTONE DEUBIQUITINASE LINK AND MODULATION OF MESODERMAL CELL FATES

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Embryogenesis involves a fine-tuned orchestration of developmental events modulated by selective activation and repression of many genetic and epigenetic players that exert their influence in a precise and coordinated fashion. During heart development, the role of Wnt signalling remains contentious with reports spanning both positive and negative influences of Wnt during the same. Earlier findings from our group have suggested a crucial and temporal influence of canonical Wnt signalling during mesoderm specification and its further differentiation into the cardiomyogenic derivative, wherein it induces mesoderm and attenuates cardiomyogenesis during murine embryonic stem cell (ESC) differentiation. Hence, further mechanistic underpinning on Wnt influence during mesoderm specification from ESCs has revealed USP3, a deubiquitinating (DUB) enzyme, as a downstream target of canonical Wnt. While USP3 seems dispensable during ESCs' maintenance, as revealed by the retention of normal colony morphology and pluripotent gene signatures in both USP3-deficient and efficient ESC clones, it plays a crucial modulatory role by displaying differential fate-switching between neuroectoderm and mesoderm differentiation. Under USP3 deficient condition, the overall mesoderm induction was promoted depicting its negative modulatory influence and the same was also true for the generation of Mesp1+ cardiac progenitors and subsequent cardiomyocytes. In contrast, USP3 positively regulated hemangioblasts differentiation that further promoted hematopoietic differentiation at the expense of endothelial ones. The differential fate modulation by USP3 was proven to be mediated by deubiquitination and contextual deposition of H2AUb/H2BUb in the promoters of mesoderm genes, reflecting its histone deubiquitinase attribute. Collectively, our study has revealed the crucial Wnt-DUB link in manifesting differential cell-fate modulation. Hence, unveiling its potential chromatin regulatory functions would greatly enhance our knowledge of epigenetic machinery underlying Wnt-mediated differential fate choice during ESCs differentiation into various mesodermal derivatives.

Keywords: Embryonic stem cell, Wnt, DUB, Mesoderm

2002

FOXG1 REGULATES EPICARDIAL CELL PROLIFERATION IN RODENTS

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The epicardium contributes precursor cells for coronary vascular development and also secretes paracrine factors that support cardiac growth. Although normally quiescent in the adult heart, epicardial cells are activated by ischemic cardiac injury; this promotes their proliferation, EMT, migration, and differentiation to aid in tissue repair. Thus, epicardial cells are a potential source of reparative cells to regenerate injured cardiac tissue. We investigated the transcriptome of epicardial cells during physiological (beneficial) cardiac remodeling to identify gene targets that regulate epicardial cells. We compared native epicardial cells isolated directly from hearts of running-exercised mice to age-matched, non-running littermates. To obtain epicardial cells, we performed Magnetic-Activated Cell Sorting (MACS) with antibodies against CD104 (integrin β4). By cDNA microarray assays, we identified genes with increased transcription in epicardial cells after running exercise; these included Snord116, a small non-coding RNA that coordinates expression of genes with epigenetic, circadian, and metabolic functions, and FoxG1, a transcription factor that controls neural progenitor cell proliferation during brain development. By immunohistochemistry, FoxG1 localized to the nuclei of proliferating (PCNA+) epicardial-derived cells. In culture, shRNA-mediated FoxG1 knockdown significantly decreased epicardial cell proliferation and Snord116 expression. Our results demonstrate that FoxG1 regulates epicardial cell proliferation and likely acts upstream of Snord116 expression. In vivo modulation of FoxG1 levels in epicardial cells has potential to improve cardiac remodeling and outcomes after ischemic injury.

Funding Source: This work was supported, in part, by National Institutes of Health (NIH) grant HL132264 (to J.L.S.). Additional support was provided by the Cardiovascular Research Institute of Vermont (CVRI-VT).

Keywords: Epicardial cell, Proliferation, FoxG1

2003

A CARDIAC FIBROSIS DETERMINATIVE FACTOR, MEOX1, BLOCKS DIRECT REPROGRAMMING OF CARDIAC MYOFIBROBLASTS TO CARDIOMYOCYTE-LIKE CELLS

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Direct reprogramming of cardiac fibroblasts (CFs) into induced cardiomyocyte-like cells (iCMs) by expressing transcription factors involved in cardiomyocyte fate determination is a promising strategy under development for future in situ regenerative medicine therapies for myocardial infarction (MI) patients. However, injury-induced cardiac fibroblasts (MFs) show lower reprogramming efficiency to these factors in MI model mice in vivo compared with that in vitro. This study explores possible molecular barriers influencing MF to iCM reprogramming in the MI microenvironment. We conducted a targeted shRNA library screen of transcription factors (TFs) enriched in MFs with single cell RNA sequencing to identify the endogenous barriers blocking MF or in vivo cardiac reprogramming. We identified Meox1, a TF downstream of TGF-β1 reportedly involved in driving cardiac fibrosis, as a barrier to iCM reprogramming. In MFs induced with MGT (Polycistronic Mef2c, Gata4 and Tbx5) or MGT plus Myocd (MGTM), Meox1 knockdown results in an approximate 4-fold increase in reprogramming efficiency compared to that in the vector control group in vitro. By contrast, Meox1 overexpression results in a 10-fold decrease in cardiac reprogramming efficiency in vitro. RNA-seq and CHIP-seq analysis showed that Meox1 mainly binds to DNA sequence motifs in the promoter region of fibrosis-related genes, supporting its function in stabilizing a fibrosis-associated transcriptomic program. Furthermore, mutations disrupting the MEOX1 DNA binding domain or fusing it to a transcriptional repressor domain attenuate its inhibition of cardiac reprogramming. Dual recombinase lineage tracing showed that iCM reprogramming efficiency is higher and cardiac function can be restored in MI model mice with Meox1 knockdown. Finally, we found that Meox1 knockdown leads to improved reprogramming efficiency in human CFs in vitro. In summary, our findings illustrate the molecular basis through which factors regulating cardiac fibrosis, especially Meox1, antagonize cardiac reprogramming in situ, providing a robust combination of reprogramming factors that can be further developed for direct in situ cardiac regeneration therapies.

Funding Source: Supported by the National Key Research and Development Program of China (2018YFA0800504), the National Natural Science Foundation of China (31922020), and fundings provided by Plastech Pharmaceutical Technology Co., LTD

Keywords: cardiac reprogramming, heart regeneration, cardiac fibrosis

TOPIC: EPITHELIAL INCLUDES SKIN GUT LUNG

2004

REDOX CONTROL OF THE PROGRESSION AND INVASION OF HUMAN GASTRIC CANCER ORGANOIDS

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Gastric cancer (GC) organoids are frequently used to examine the tumorigenic ability of human GC and screen the novel antioxidant drugs to prevent the progression of GC. In general, GC organoids were used to examine cell proliferation and death as well as cancer development. Here, the GC organoids can be used in the identification of novel antioxidant drugs to prevent the progression of GC. Invasion-migration, xenotransplantation, and reactive oxygen species (ROS) production assays were used to examine the effects of antioxidant drugs including perillaldehyde (PEA), cinnamaldehyde (CA), and sulforaphane (SNF), on GC. We found that both PEA and CA repressed the proliferation of human GC organoids, whereas SFN enhanced it. Caspase 3/7 activities were also repressed on treatment with PEA and CA. In addition, the tumor formation and invasive activities were also repressed on treatment with PEA and CA, whereas they were enhanced on treatment with SFN. The ROS production and the expression of p53, nuclear factor (erythroid-derived 2)-like 2, and Jun dimerization protein 2 were downregulated on treatment with PEA and CA, but not SFN. The new type of antioxidation reagents such as PEA and CA inhibited the development of the human gastric cancer through the decrease of ROS, indicating they have the potential to be new antitumorigenic therapeutics for GC. Taken together, we provide the first experimental evidence using GC organoids to show the functional heterogeneity of the antioxidant drugs on GC. PEA and CA have the potential to be new antitumorigenic therapeutics for GC.

Funding Source: Ministry of Science and Technology (MOST 111-2314-B-037-009), the National Health Research Institutes (NHRI-EX109-10720SI), Kaohsiung Medical University Hospital (KMUH110-0R86; KMUH-DK (A)-111001)..

Keywords: Antioxidation, Organoid, ROS

2005

DESCRIPTION OF A NOVEL INTERMEDIATE MELANOCYTE PROGENITOR SUBPOPULATION

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Current prevention of cutaneous melanoma is passive. In part, this is because the early biological and molecular events that transform normal melanocytes to melanomas are poorly understood. Based on the cell-of-origin model of tumor initiation, a given melanomagenic stimulus may be more or less transforming depending on the type of melanocyte exposed to it. To develop sophisticated strategies for melanoma prevention, understanding is essential of how melanocytes develop, are maintained, and respond to mitogenic stimuli. Towards this, we performed scRNAseq on CKIT+ human skin melanocytes purified by flow cytometry. One cell cluster displayed increased expression of NTRK2, which is linked to neural progenitor function and oncogenesis. The gene expression profile of NTRK2-expressing cells had progenitor cell characteristics including increased ribosome biogenesis. Splicing analysis revealed that the isoform of NTRK2 expressed in human melanocytes is the short-truncated form, TrkB.T1. NTRK2+ melanocytes were detected by immunohistochemistry in epidermis and hair follicles in human and mouse skin. NTRK2+ melanocytes were less pigmented and more clonogenic than NTRK2- melanocytes, and dominated primary melanocyte cultures. NTRK2+ melanocytes had increased expression of nucleolin (NCL), a regulator of cell proliferation, DNA damage responses, and ribosome biogenesis, which was suppressed with siNTRK2s and in NTRK2-knock out mouse melanocytes. Following UVB irradiation, NCL, pATR, γH2AX and Bcl-2 were upregulated in melanocytes in vitro while this UVB-induced upregulation was suppressed by treatment with siNTRK2s. NTRK2+ CKIT+ melanocytes were increased in mouse skin in vivo and in ex vivo whole human skin culture, implicating regulation by NTRK2 of melanocytic ribosome biogenesis, DNA damage and proliferative responses to UVB. Consistent with this, survival of siNTRK2-treated melanocytes after UVB was inhibited in vitro. These data implicate NTRK2+ CKIT+ melanocytes as intermediate progenitors that regulate melanocyte stress and mitogenic responses to UVB-irradiation.

Funding Source: This project is partially funded by Department of Defense U.S. Army Medical Research and Development Command Congressionally Directed Medical Research Programs Fiscal Year 2021 Melanoma Research Program Idea Award.

Keywords: Melanocyte, NTRK2, Single cell RNA-seq

TOPIC: GERMLINE AND EARLY EMBRYO

2006

ABERRANT BROAD H3K4ME3 DOMAIN IMPAIRS REPROGRAMMING EFFICIENCY OF SOMATIC CELL NUCLEAR TRANSFER EMBRYOS

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Somatic cell nuclear transfer (SCNT) enables terminally differentiated somatic cells reprogrammed into totipotent embryos. However, developmental defects such as early-stage-arrest and epigenetic abnormalities usually occur in SCNT embryos and the underlying mechanisms are largely unknown. Here, we generate genome-wide map of H3K4me3 and H3K27me3, two critical epigenetic markers which regulate gene expression in SCNT preimplantation embryos. We found distinct profiles of H3K4me3 and H3K27me3 between SCNT embryos and naturally fertilized embryos. Notably, over-establishment of H3K4me3 domains, especially those wider than 5 kb which we termed broad H3K4me3 domains, exist in SCNT two-cell-stage embryos. Injection of histone demethylase Kdm5b and incubation with histone methyltransferase inhibitor WDR5-0103 both successfully narrowed the width of H3K4me3 domains in SCNT embryos to the same level of naturally fertilized embryos. Furthermore, incubation of WDR5-0103 rescued gene expression level and improved developmental capacity of SCNT embryos. Our findings reveal genome-wide H3K4me3 and H3K27me3 profiles in SCNT preimplantation embryos and suggest approaches for improving reprogramming strategies.

Keywords: Somatic Cell Nuclear Transfer, Histone Modification, Reprogramming

2007

DEVELOPMENT OF TERATOMAS IN MICE PRODUCED FROM ESCS MAINTAINED IN CHEMICALS-BASED CULTURES

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A mouse can be produced entirely from pluripotent embryonic stem cells (ESCs) by tetraploid embryo complementation (TEC) assay, demonstrating that ESCs held the capacity to differentiate into all cell types in a body, thus are truly pluripotent. Various culture conditions by small molecules have been explored to extend pluripotency of the stem cells to maximize their potential clinical therapy in regenerative medicine, but their impacts on cell fate in vivo remain elusive. We systematically and directly compared the effects of various culture conditions on the pluripotency and cell fate in vivo of the ESCs originated from the same mouse genetic background by TEC assay. Conventional ESC cultures in serum/LIF-based condition produced the complete ESC-mice and also the survival to the adulthood at the highest rates than those of all other chemical-based cultures. Moreover, long-term examination of the adult ESC-mice demonstrated that conventional ESC cultures did not lead to visible abnormality for up to 1.5-2 years, whereas, unexpectedly, the chemicals-based cultures developed retroperitoneal atypical teratomas or leiomyomas. The chemicals-based cultures exhibited the transcriptome typically differed from that of conventional ESC cultures. Our results warrant further refinement of the culture conditions in promoting the pluripotency as well as the safety of ESCs in future applications.

Funding Source: This research was supported in part by the National Key *Research and Development* Program of China (2018YFA0107000), the National Natural Science Foundation of China (32030033) and Tianjin Science and Technology Plan Key Project (20JCZDJC00550).

Keywords: Pluripotent stem cells, teratoma, tetraploid embryo complementation

2008

NEUROTROPHIN-4 CONTRIBUTES TROPHECTODERMAL LINEAGE SPECIFICATION DURING PORCINE PREIMPLANTATION EMBRYONIC DEVELOPMENT

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Neurotrophins are associated with successful implantation and maintenance of pregnancy. Here, we investigated the effect of neurotrophin-4 (NT-4) on porcine preimplantation embryonic development in vitro. We confirmed for the first time that NT-4 and its receptor proteins (TrkB and p75NTR) were localized to both the inner cell mass (ICM) and trophectoderm (TE) in porcine parthenogenetic activation (PA)-derived blastocysts (BLs). Porcine zygotes were treated with different concentrations of NT-4 (0, 1, 10, and 100 ng/mL) during in vitro culture (IVC). The optimal concentration of NT-4 to enhance the porcine preimplantation embryonic developmental capacity during IVC was identified as 10 ng/mL. NT-4 supplementation during porcine IVC significantly ($p < 0.05$) increased proportion of the TE cells by inducing the transcription of TE lineage markers (CDX2, PPAG3, and GATA3 transcripts). In addition, NT-4 can reduce apoptosis by regulating the transcription of apoptosis-related genes (BAX and BCL2L1 transcripts). Interestingly, supplementation of NT-4 during IVC significantly ($p < 0.05$) increased YAP1 transcript levels and significantly ($p < 0.01$) decreased LATS2 transcript levels in the porcine PA-derived BLs. It was also confirmed that intensity of YAP1 protein expression significantly ($p < 0.001$) increased in the NT-4-treated BLs compared with control. Thus, NT-4 contributes to promoting differentiation into the TE lineage rather than the ICM lineage in the porcine preimplantation embryonic development.

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Keywords: Neurotrophin-4, Trophectoderm, Blastocysts

2009

MULTI-OMICS ANALYSIS OF STEM CELLS ESTABLISHED FROM AGED INDIVIDUALS

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Assisted reproductive technology (ART) has become increasingly important among fertility treatments in recent years due to the declining birthrate. However, ART is not immune to the effects of aging, and improving embryo quality is a major issue. Proliferative potential, expression of pluripotent markers, and DNA methyltransferases such as Dnmt3a of embryonic stem cells (ESCs) established from aged mice (8-month-old) are decreased compared to those derived from young mice, suggesting that the aging effect on oocytes is inherited by stem cells as well. Therefore, we established ESCs from blastocysts to capture the mechanism of embryonic senescence, and iPSCs from isogenic individuals to examine the effects of differences in the initialization mechanism on stem cells. In order to examine the effects of aging on iPSCs in detail, we established iPS cell lines up to 24 months and performed multi-omics analysis to identify aging regulatory factors. Transcriptomic analysis followed by gene ontology analysis revealed changes in the expression of genes involved in cell defense. Accordingly, we conducted a radiation-induced genome stability test. The results showed that the number of γH2AX foci in iPSCs significantly increased with aging, suggesting that genomic instability was increased. Furthermore, the metabolomic analysis revealed that glutathione was more abundant in aged iPSCs, while oxidized glutathione was inverted, suggesting that the scavenger pathway works in aged iPSCs to regulate oxidative stress. These results suggest the existence of a compensatory gene cluster in aged iPSCs, which suppresses oxidative stress through the scavenger pathway in aged iPSCs. We would identify candidate factors for regulating senescence in iPSCs and investigate gene transfer methods using viral vectors to demonstrate ex vivo gene therapy aimed at restoring the developmental potential of aged embryos.

Keywords: Assisted reproductive technology, Aging, Metabolome

2010

IDENTIFICATION, CHARACTERIZATION, AND LINEAGE TRACING OF MESENDODERM PROGENITOR CELLS IN MAMMALIAN EMBRYO

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Multipotent embryonic cells are capable of contributing to a multitude of cell types in the body. As the development progresses, these progenitor cells become restricted in lineage potential for a specific range of tissue derivatives. At gastrulation, the epiblast cells are allocated to the progenitor cells for the ectoderm, the mesoderm, and the endoderm, which is driven by lineage-specific transcriptional activity and regionalized signalling input. However, when and whereabouts of the allocation of the progenitors of specific germ layers and the trajectory of their differentiation towards multiple lineages are not clearly understood. Previous studies conducted in nematode and zebrafish embryos identified a subset of germ layer progenitors that display dual propensity for mesoderm and endoderm. The existence and lineage propensity of such bipotential mesendoderm progenitors in mammalian embryos remains unclear. Our study focuses on the identification and characterization of the putative bipotential progenitor cells of mesoderm and endoderm in mouse embryos using single-cell transcriptome analysis and imaging of the lineage descendants of these progenitors in embryos and in vitro mouse and human embryo models. Identification and characterisation of the bipotential progenitors will enable efficient generation of biologically relevant cell types for tissue engineering applications.

Keywords: Mesendoderm, Early embryo, Confocal Microscopy

2011

UNREPROGRAMMED H3K9ME3 PREVENTS MINOR ZGA AND LINEAGE COMMITMENT IN SCNT EMBRYOS

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Somatic cell nuclear transfer (SCNT) can be used to reprogram differentiated somatic cells to a totipotent state but has poor efficiency in supporting full-term development. H3K9me3 is considered to be an epigenetic barrier to zygotic genomic activation (ZGA) in 2-cell SCNT embryos. However, the mechanism underlying the failure of H3K9me3 reprogramming during SCNT embryo development remains elusive. Here, we performed genome-wide profiling of H3K9me3 and found it to be persistently redundant throughout SCNT embryo development, with most marks inherited from the donor cells. These redundant H3K9me3 marks directly blocked the transcription of minor ZGA genes and delayed the activation of long terminal repeats (LTRs) during ZGA. Moreover, SCNT blastocysts showed severely indistinct lineage-specific H3K9me3 deposition. Max and Mcrs1 were identified as potential lineage-specific H3K9me3-related transcription factors and were found to be essential for early embryogenesis. Compensatory expression of Max and Mcrs1 significantly benefited the early development and improved the implantation capability of SCNT embryos. Notably, Mcrs1 improved the differentiation of SCNT embryos, with reorganized lineage-specific H3K9me3 allocation, and further improved the efficiency of full-term development.

Keywords: cell fate decision, SCNT, histone modifications

TOPIC: HEMATOPOIETIC, IMMUNE, AND ENDOTHELIAL

2012

IDENTIFYING REGULATORS OF HEMOGENIC REPROGRAMMING WITH CRISPR/Cas9 SCREENING

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Hematopoietic stem cells (HSCs) are capable of self-renewal and continuous production of mature blood cells. For these reasons, HSC transplantation is the only curative treatment for a variety of hematologic malignancies. However, HSC numbers are often insufficient to meet transplantation demands, and expansion of HSCs in vitro remains challenging. The generation of patient-tailored HSCs by direct cellular reprogramming has the potential to overcome these limitations. We have previously shown that the ectopic expression of the transcription factors (TFs) GATA2, GFI1B, and FOS in fibroblasts generates hematopoietic stem and progenitor cells (HSPCs) through a dynamic endothelial to hematopoietic transition. Nevertheless, a comprehensive understanding of the molecular regulators underlying this dynamic process in humans is needed to improve the efficiency and fidelity of the process. Here, we optimized a CRISPR/Cas9 knock-out (KO) screening toolbox to map genes encoding for positive and negative regulators of hemogenic reprogramming. We fined-tuned the conditions for efficient KO with a constitutive Cas9 and a sgRNA library targeting 116 genes implicated in HSC self-renewal and expansion. In parallel, we established the delivery of the hemogenic TFs to human dermal fibroblasts using a polycistronic lentiviral vector allowing antibiotic-mediated selection of transduced cells. We tested 6 polycistronic constructions and showed that the order of GATA2, GFI1B, and FOS which is translated to elevated levels of GATA2 and GFI1B, results in the highest reprogramming efficiency measured by the activation of the early hemogenic markers CD9 and CD49f. After 15 days of inducing reprogramming of edited fibroblasts, we purified reprogrammed (CD9+CD49f+) and non-reprogrammed (CD9-CD49f-) populations and performed deep sequencing. We identified candidate genes that may function as barriers or facilitators of hemogenic reprogramming including signaling interactors and TFs, for future exploration of molecular mechanisms. Overall, our findings provide the foundation for CRISPR/Cas9 screening to define drivers

of human hematopoietic reprogramming and ultimately use this information for the efficient generation of patient-specific HSCs.

Keywords: HSCs, Cellular Reprogramming, CRISPR/Cas9

2013

RUNX3 DEPENDENT GENE EXPRESSION PROFILES IN PLURIPOTENT STEM CELL DERIVED MYELOID CELLS

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The transcription factor RUNX3 is an important regulator of several hematopoietic cell lineages. For example, RUNX3 can facilitate the terminal differentiation and the maturation of cytotoxic T cells and some dendritic cell (DC) subsets. We have previously described that forced expression of Runx3 positively regulated the mouse embryonic stem cell (ESC) derived myeloid DC (ES-DC) development and this factor promoted the ES-DC maturation and immunogenicity. In this study we analyzed RUNX3 mediated gene expression changes in ESCs and ES-DC progenitors with bulk RNA sequencing. Large number of differently expressed mRNA transcripts were identified in the presence of the transgenic Runx3. For example, members of the granzyme b gene family were coordinately upregulated by RUNX3 during the early phase of the ESC differentiation. We also employed gene expression profiling of RUNX3-instructed and control ESC derived mesodermal and myeloid cells by single-cell RNA sequencing. We observed a very diverse set of cell types and states during the ES-DC differentiation. Importantly, we identified RUNX3 dependent cell populations with unique gene expression profiles. In summary, our genome-scale transcript analysis provides a catalogue for exploration of the RUNX3 regulatory network in murine ESC derived myeloid cells. Moreover, this work provides unique insights for studying the molecular mechanisms of the RUNX3-dependent cell fate modulation.

Keywords: RNA sequencing, RUNX3, myeloid cells

TOPIC: KIDNEY

2014

SEXUAL DIMORPHISM IN THE MAMMALIAN KIDNEY IS REGULATED BY ANDROGEN RECEPTOR IN PROXIMAL TUBULE SEGMENTS

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Previous studies have demonstrated sexual dimorphism in the mammalian kidney's physiology, susceptibility to disease and response to injury. In the mouse, proximal tubule (PT) cell types show distinct gene expression between the sexes. Here, we investigated the molecular mechanisms regulating dimorphic gene expression in the murine kidney. Gene- and isoform-level analysis highlighted differential functions related to peroxisomal lipid metabolism and nuclear receptor pathways, suggesting a sex-dependent role for metabolic regulation in renal function. Whole-kidney bulk RNA-seq from neonates to aged adult C57BL/6 mice demonstrated differential gene expression was established with the onset of sexual maturity from 4-8 weeks under gonadal control and stably maintained. Nephron-specific ablation of androgen receptor (AR) and estrogen receptor activity, and hormonal injection studies, showed testosterone-driven Ar activity in proximal tubule cells is the primary regulator of sexually distinct gene expression in the mouse kidney, distinguishing predominant sex-specific control mechanisms between the kidney and liver. Single-nuclear multiomic analysis of chromatin accessibility (snATAC-seq) and gene expression (snRNA-seq) demonstrated decreased chromatin accessibility for male-biased genes and increased accessibility for female-biased genes following Ar removal in male PT segments. Integrating published AR-ChIP-seq

and these multiomic data identified putative androgen response elements near sex-biased genes. Examining human kidney datasets identified evidence of conserved dimorphic gene expression in the male and female human kidney. Collectively, these studies highlight organ-specific regulatory programs differentiating gene activity between organs in the mouse and provide a foundation for mechanistic analysis of sex difference in renal physiology and injury and disease modeling.

Keywords: Renal Sexual Dimorphism, Androgen Receptor, Gene Regulation

TOPIC: LIVER

2015

THE EFFECT OF HYPOXIA INDUCIBLE FACTOR-1A ON THE METABOLIC FUNCTIONS OF HEPATOCELLULAR CARCINOMA

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Hypoxia is a pathological state in solid tumors, characterized by low oxygen levels due to the imbalance between oxygen supply and oxygen demand. Hypoxia inducible factor-1A (HIF-1A) mediates the effect of hypoxia by binding to the hypoxia responsive element (HRE) at the promoter site of the targeted genes. HIF-1A modulates different cancer-related properties including genomic instability, metastasis, invasion, proliferation, angiogenesis, and metabolic reprogramming. In this study, we utilized computational analytical tools to determine the altered genes and enriched pathways due to hypoxia in hepatocellular carcinoma (HCC). Data acquired from TCGA were analyzed for differential gene expression, protein-protein interaction (PPI) network, and pathway enrichment analysis. Our results showed the enrichment of 7 pathways under the metabolic, disease, and signaling pathways categories. Since metabolic reprogramming is one of the cancer hallmarks in HCC caused by hypoxia, the 9 target genes of HIF-1A in those metabolic pathways including SLC2A1, SLC2A2, ENO2, ENO3, PKM, PFKP, HK2, PFKPB3, and GCK were subjected to further validation using liver cancer organoids as a 3 dimensional (3D) in-vitro model. Gene expression analysis of the HIF-1A targeted genes demonstrated the metabolic reprogramming of liver cancer organoids under hypoxic conditions from oxidative phosphorylation into glycolysis. Data were confirmed by the consumption of glucose and the production of lactate and pyruvate assays. Our data highlight specific HIF-1A targeted genes that can be considered therapeutic targets for HCC treatment.

Funding Source: Funded by: 1- Scientists for Next Generation (SNG) cycle 7 from the Academy of Scientific Research and Technology (ASRT) 2- JESOR ASRT 5275 3- STDF FLUG Call 1- Project ID 46721

Keywords: Hypoxia inducible factor-1A (HIF-1A), Hepatocellular carcinoma (HCC), Glycolysis

TOPIC: MESENCHYMAL STROMAL CELLS, ADIPOSE, AND CONNECTIVE TISSUE

2016

A NOVEL XENO-FREE PROLIFERATION CONTROL CULTURE SYSTEM FOR HUMAN ADIPOSE DERIVED STEM CELLS

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Human mesenchymal stem cells (hMSCs) have shown great promise as a cell source not only for cell therapy but also for exosome therapy. However, when cells are cultured or transported alive, it is vital to keep the cells on a routinely passage in order to avoid overcrowding which can cause poor cell growth, senescence even death. We have developed the Xyltech cell proliferation control system for human pluripotent stem cells. In this study, we developed the novel Xeno-Free proliferation control medium (Xyltech, MSC-01) on the basis of “Xyltech technology”, and validated its applicability on human adipose-derived stem cells (hADSCs). Xeno -free growth medium (Xyltech Growth, MSC) was used as a control. Cellular proliferation of hADSCs cultured in proliferation control medium (MSC-01) was suppressed compared with that in growth medium (MSC). hADSCs resumed proliferation immediately after replacement with MSC. Moreover, these proliferative suppressed cells cultured in MSC-01 presented with remarkable lower positive for the senescence marker, SA-β-GAL than crowded cells cultured in MSC. The expression analysis of hMSC-specific markers by flow cytometry showed that hADSCs in the presence of MSC-01 maintained a positive rate of more than 95% for expressed markers (CD73, CD90, CD105) and less than 2% for unexpressed markers (CD34, CD45). Furthermore, hADSCs cultured in MSC-01 were capable of differentiating into adipocytes, osteoblasts, and chondrocytes. These findings indicated that hADSCs cultured in MSC-01 retained the same characteristics of hMSCs seen with MSC medium. In addition, the proliferation control culture using this “Xyltech system” can support a long term maintenance of human stem cells without frequent media changes and cell passages during experiments and live cell transport, thereby reduce the workload of researchers.

Keywords: Mesenchymal Stem cells, Cell Proliferation Suppression, Xeno-Free

2017

ANALYSIS OF NEURAL CREST-LIKE PROPERTIES OF CD271+ WHARTON JELLY-DERIVED MESENCHYMAL STEM/STROMAL CELLS

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Mesenchymal Stem/Stromal Cells (MSCs) is a highly heterogenous group of cells used in the regenerative medicine. Existence of small population expressing CD271 (low-affinity nerve growth factor receptor) suggest partial distinct origin of MSCs, as this marker is typical for Neural Crest Derived Stem Cells (NCSCs). According to the literature, such cells characterize with neural differentiation in vitro and in vivo, but there have been no evidences described for CD271+ subpopulation isolated from MSCs yet. Here, we would like to describe properties of CD271+ MSCs to confirm whether such population exhibit higher neural properties than heterogenous MSCs. In our research, we used MSCs derived from Wharton jelly (WJ-MSCs) to isolate CD271+ cells with Fluorescence Activated Cell Sorting (FACS). Received results from positive population (WJ-MSC-CD271+) were compared to negative (WJ-MSC-CD271-) and initial (unsorted WJ-MSC) populations. We analyzed the CD271 content before and after FACS sorting, clonogenicity of WJ-MSC-CD271+ subpopulation and expression of neural markers performed with qPCR and immunocytochemistry staining. We observed, that WJ-MSCs contained a sparse subpopulation of CD271+ cells, less than 5%. FACS sorting allowed for enrichment of CD271+ subpopulation for 1 passage. WJ-MSC-CD271+ cells exhibited higher expression of neural genes (B3Tubulin, Nestin and GFAP) than negative and initial populations. We confirmed that WJ-MSCs contain cells exhibiting CD271+, but population size makes it difficult to isolate and it disappears with the duration of culture. WJ-MSC-CD271+ cells exhibit increased expression of markers associated with neural tissue.

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Keywords: Mesenchymal Stem/Stromal Cells, Neural Crest derived stem cells, CD271

2018

PRODUCTION AND IN-DEPTH QUALITY ASSESSMENT OF MULTIFACTORIAL REPROGRAMMED SKELETAL MUSCLE CELLS FROM HUMAN STEM CELLS BY ADVANCED INTEGRATIVE TECHNOLOGIES.

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Skeletal muscle tissue engineering aims at generating biological substitutes that restore, maintain or improve normal muscle function; however, the quality of cells produced by current protocols remains insufficient. Here, we developed a multifactor- based protocol that combines adenovector (AdV)-mediated MYOD expression, small molecule inhibitor and growth factor treatment, and electrical pulse stimulation (EPS) to efficiently reprogram different types of human-derived multipotent stem cells into physiologically functional skeletal muscle cells (SMCs). The protocol was complemented through a novel in silico workflow that allows for in-depth estimation and potentially optimization of the quality of generated muscle tissue, based on the transcriptomes of transdifferentiated cells. We additionally patch-clamped phenotypic SMCs to associate their bioelectri- cal characteristics with their transcriptome reprogramming. Overall, we set up a comprehensive and dynamic approach at the nexus of viral vector-based technology, bioinformatics, and electrophysiology that facilitates production of high-quality skeletal muscle cells and can guide iterative cycles to improve myo-differentiation protocols.

Funding Source: This work was supported by the European Union Structural Fund [ESF/14-BM-A55-0026/18, ESF-14BM-A55-

0025–18-A02], DFG [PU 188/19–1), and the Research award of the Deutsche Duch- enne-Stiftung to A.S and H.B.

Keywords: Multipotent stem cells, Myogenic differentiation, Myo-informatics

2019

HUMAN IPSCS DIFFERENTIATION TO TENOGENIC LINEAGE INFORMED BY SINGLE CELL TRANSCRIPTOMICS

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Tendon injuries contribute to 45% of musculoskeletal consultations in the US alone and can be attributed to tendons' poor innate healing capacity. Conservative approaches and/or surgical intervention is the current standard of care, but rehabilitation is prolonged and re-injury rates are high. To date there is no consensus on a biological or pharmacological approach for the healing process that is clinically practical. Bone marrow mesenchymal stromal cells and adipose stem cells have been explored as potential cell therapies, but they have limited self-renewal capacity and phenotypic heterogeneity. iPSCs have potential because they do not have these limitations. However, there are very few protocols for tenogenic differentiation from human iPSCs with high efficiency rates, largely in part to the limited understanding of tendon development origins and how they differentiate from precursors. We sought to investigate a method of differentiation from human iPSCs to tenocytes that is more efficient and specific. Human iPSCs were differentiated to Syndetome (SYN) in a stepwise manner through the presomitic mesoderm and somite. Successful differentiation was confirmed through classical markers at each developmental stage with gene expression analysis, immunofluorescence, and single cell RNA-sequencing (scRNA-seq). However, trajectory analysis revealed off-target differentiation towards a neural phenotype. Because Wnt family members were identified as crucial in the generation of neural by-products, a WNT signaling inhibitor Wnt-C59 was added to the later stages of the differentiation. Further transcriptomics post-treatment showed that blockade of the Wnt pathway not only dramatically increased the SYN population but also eliminated the side-products, resulting in a more specific cell population differentiating towards the tenogenic lineage. This study demonstrates that iPSCs can be differentiated to tenocytes more specifically in a stepwise manner by blocking the Wnt signaling pathway in the later stages of the differentiation. Elucidating the Wnt pathway mechanism using a development-inspired protocol can lead to more powerful and specific differentiation protocols for cell therapy applications and has potential as an off-the-shelf cell source for tendon injuries.

Keywords: iPSCs, Differentiation, Single cell RNA-sequencing

2020

A SUBTYPE OF HUMAN NUCLEUS PULPOSUS CELLS DRIVES PAIN SYMPTOMS IN DEGENERATING INTERVERTEBRAL DISCS

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Low back pain (LBP) is a leading cause of disability affecting almost 80% of the adult population, and 40% of LBP is attributed to intervertebral disc (IVD) degeneration. However, the mechanisms

that determine which degenerating discs drive the pain are yet to be fully revealed. Loss of nucleus pulposus cells (NPCs) correlates with disc degeneration. We hypothesize that NPC affected by specific environmental stressors trigger the onset of low back pain. Degenerated IVD tissue freshly harvested from surgical discards or human cadavers with approved protocols were compared with single-cell RNA-seq (scRNA-seq), including 3 back pain-inducing IVDs (bpIVD) and 4 asymptomatic IVDs (aIVD) from different individuals. Further subset NPCs from bpIVD (bpNPC) or aIVD (aNPC) for further analyses. Replated aNPCs in 2D tissue culture and transformed them from non-stressed status (nsNPC) into a stressed status (sNPC). Pain-inducing effect of sNPC was determined in vitro in a co-culture with iPSC-derived nociceptors (iNOC) using microfluidic devices and in vivo using healthy rat models. For statistics, 3-way ANOVA and a critical significance level of 5% was used. Some clusters, particularly NPC1MMP3+, were found associated with bpIVDs because 62% cells of this subtype were derived from bpIVDs (Fig. 1A). A pseudo-time trajectory analysis shows the trend of differentiation (Fig. 1B). Within NPC1 subtype, the cells solely derived from bpIVD were annotated as bpNPC1 and further examined for pathway network (Fig. 1C). These data suggest that NPC1 cluster might be the pain-inducing cell subtype. We recreated the NPC1 subtype with combinatory stimulation of NPCs in vitro with cell stressors (Fig. 1D) resulting in an upregulation of degenerative markers (Fig. 1E). sNPC showed bpNPC1-like transcriptomes as evidenced by Fig. 1F-N. In the animal study, the intradiscal injection (Fig. 1O) led to an expression of nociceptive markers only in sNPC-injected group (Fig, 1P) and increased nociceptive behavior compared to nsNPCs or saline-injected groups (Fig. 1Q). This study discovered that a subtype of NPC generated under stress is responsible for triggering low back pain. This NPC-mediated pain mechanism will inform future NPC-focused clinical therapies that are more approachable than regenerating the entire IVDs.

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Keywords: Low back pain, Intervertebral disc, Single cell RNA-seq

2021

SATELLITE CELL SPECIFICATION INTO BROWN ADIPOSE TISSUE IS REGULATED BY P53

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Brown adipose progenitors are derived from the muscle stem cell population in adult skeletal muscle, where the switch from myogenic to brown adipogenic fates is regulated by microRNA-133 (miR-133). However, little is known about the control of miR-133 expression during adipogenic fate-switching. Using a biosensor for miR-133 activity, we conducted a screen for regulators of miR-133 expression. We found the p53 inhibitor Pifithrin-α to be a potent repressive agent for miR-133 expression in primary myoblasts and muscle stem cells. Notably, p53 inhibition stimulates precocious brown adipose formation within regenerating skeletal muscle, and mice exposed to cold temperatures exhibit reduced p53 expression in muscle. Moreover, we found pri-miR-133 processing was inhibited when p53 is down-regulated. Together, our experiments provide new insight into muscle stem cell fate determination and suggest modulating pri-miRNA-133 processing may be beneficial to manage obesity through selective induction of brown fat.

Keywords: satellite cell, muscle stem cell, adipogenesis

TOPIC: NEURAL

2022

SNRNA-SEQ DISSECTION OF NEUROG2-INDUCED GLIA-TO-NEURON REPROGRAMMING INDICATES PROGRESSIVE ACQUISITION OF HOMEOSTATIC GENE EXPRESSION RESPONSES TO NEURONAL ACTIVITY MODULATION

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Direct cell reprogramming, whereby somatic cells are induced to acquire a desired (e.g. neuronal) identity, holds promise as a strategy to address cell replacement therapeutic needs and as a tool to dissect processes influencing cell identity acquisition and maintenance. Diverse cell types can be reprogrammed to induced neurons (iNs) by forced Neurogenin2 (Neurog2) expression, but it is unclear if iNs acquire functional properties typical of

mature endogenous neurons (eNs) which ensure integrated contribution to the activity of neural networks, such as homeostatic synaptic plasticity (HSP). To address this, we pharmacologically inhibited for 48hrs the activity of co-cultured mature cortical eNs and postnatal glia-derived Neurog2-iNs, followed by single-nucleus transcriptional profiling. eNs displayed strong transcriptional responses to inhibition, suggestive of homeostatic synaptic upscaling and predicted to be driven by well-established activity-dependent transcriptional regulators. iNs comprised a molecularly heterogeneous population of neurons mapping to cortical glutamatergic subtypes at various stages of maturation. As a population, iNs displayed a limited transcriptional response to inhibition suggestive of morphological reorganization, which we confirmed experimentally. Interestingly, the most mature iNs more closely resembled eNs in their response to inhibition, upregulating synapse-associated signatures, thus suggesting progressive acquisition of the ability to undergo HSP by iNs. Immature and mature iNs displayed high basal levels of eN-upregulated maturation-related genes, intriguingly resembling early stages of in vivo gene expression dynamics observed in mice and humans during neuronal maturation, when maturation- and synapse-related genes are transiently upregulated. This raises the possibility that an evolutionarily conserved early event in the maturation of cortical neurons, recapitulated during glia-to-neuron direct reprogramming, may share regulatory mechanisms with homeostatic synaptic plasticity. Overall, our observations indicate that Neurog2 drives reprogramming of cortical glia to iNs which progressively acquire core functional attributes of endogenous neurons, in a process reminiscent of endogenous neuronal maturation.

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Keywords: Glia-to-neuron Reprogramming, Neurogenin2, Homeostatic synaptic plasticity

2023

IN VITRO PIGMENTATION OF HUMAN IPSC-DERIVED RETINAL PIGMENT EPITHELIUM CELLS DOES NOT INDICATE THEIR QUALITY FOR CELL TRANSPLANTATION

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Retinal pigment epithelium (RPE) cells show heterogeneous level of pigmentation when cultured in vitro. To know whether their color in appearance indicates any functional qualities of the RPE, especially in terms of clinical use for cell transplantation, we analyzed human-induced pluripotent stem cell-derived RPE cells (iPSC-RPE) that passed our quality management criteria for clinicals use, for the correlation between their color intensities and gene expression profiles at single cell level. For this purpose, we utilized our recent invention, Automated Live imaging and cell Picking System (ALPS), which enabled photographing each cell before RNA-sequencing analysis to profile the gene expression of each cell. While our iPSC-RPE were categorized in 4 clusters by gene expression, the color intensities of iPSC-RPE did not project any specific gene expression profiles. The results suggest the degree of pigmentation of iPSC-RPE in vitro does not specifically correlate with the functional characteristics of the cells.

Keywords: retinal pigment epithelium (RPE) cell, single cell RNA sequencing, cell transplantation

2024

REPROGRAMMING TRAJECTORIES DURING THE DIRECT CONVERSION OF HUMAN FIBROBLASTS INTO INDUCED NEURAL STEM CELLS ANALYZED BY SINGLE-NUCLEI MULTIOME SEQUENCING

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Mammalian tissue regeneration, particularly in the central nervous system, is limited. Comparative analysis with regeneration in fish and axolotl indicates evolutionary conserved transcriptional programs, that are not fully elucidated. The cellular reprogramming of human somatic cells into induced pluripotent stem cells (iPSCs) provides new opportunities to analyze regeneration trajectories. During iPSC-type reprogramming, cells get epigenetically rejuvenated, whereas direct conversion of adult dermal fibroblasts (ADFs) into induced neurons (iNs) circumvents the rejuvenation and maintains the majority of epigenetic ageing marks. The direct conversion into induced neural stem cells (iNSCs) provides another novel system for studying human neural regeneration. However, the transcriptional programs underlying the iNSC-type rejuvenation are poorly investigated. In this study, we aim to assess the reprogramming trajectories during the conversion of ADFs into iNSCs. For this purpose, we employed a lentiviral cell barcoding method, CellTagging, to label cells with barcodes at multiple stages during the direct conversion. Subsequently, samples were collected along the iNSC conversion, in order to analyze the transcriptome and epigenome on a single-cell level by multi-omics. Single-cell RNA sequencing data revealed the generation of a highly homogeneous cell population, enriched for bona fide NSC markers, like PAX6, indicating a successful conversion. Analysis of markers for the anterior/posterior and ventral/dorsal axis of the neural tube revealed a hindbrain, dorsal cellular identity of the converted iNSCs. The transcriptomic temporal data indicated intermediate conversion stages, characterized by relevant identity marker gradients of expression, like COL1A2 for the initial fibroblast population and CXCR4 for later iNSC clusters. Furthermore, we also identified trajectory pathways that lead to non-productive conversion outcomes, i.e., neural progenitor-like cells with inactive cell cycle. Currently, we are deciphering the key genes and pathways affecting the conversion efficiency and putative conversion bifurcation points. Ultimately, we expect our study to provide novel insights into the transcriptional programs regulating human neural ageing and regeneration.

Keywords: Human neural ageing and regeneration, Neural stem cells, Conversion pathways

TOPIC: NO TISSUE SPECIFICITY

2025

DERIVATION OF INDUCED PLURIPOTENT STEM CELLS IN BORNEAN ORANGUTANS

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Bornean orangutans (Pongo pygmaeus) is an endanged non-human primate species. Here we report the derivation of induced pluripotent stem cells (iPSC) from peripheral blood mononuclear cells (PBMCs) in Bornean orangutans. Non-integrative Sendai virus expressing human OCT4, SOX2, NANOG, and c-MYC were transduced into the Bornean PBMCs. Putative orangutan iPSCs (o-iPSCs) colonies appeared three weeks after induction, manifesting a dome-shape morphology in E8 and hESM culture conditions. Moreover, these colonies showed typical characteristics of pluripotency, including positive staining of alkaline phosphatase activity, normal karyotypes, and expression of pluripotent markers OCT4, SOX2, NANOG, and SSEA3/4. The teratoma assay demonstrated these o-iPSCs were capable of differentiation into all three germlayer lineages in vivo. We also confirmed that these o-iPSCs are of normal karyotypes. In sum, the o-iPSCs were successfully derived from the non-human primate species Bornean orangutans. Given the genomic similarity between this species and Homo sapiens, the o-iPSCs are valuable tools to study regenerative biology.

Funding Source: This work was supported by the Ministry of Science and Technology Taiwan (MOST 109-2313-B-002-003-MY2) and Chang Gung Memorial Hospital Taiwan (CMRPG3L0171).

Keywords: Bornean orangutans, non-integrative Sendai virus transduction, induced pluripotent stem cells

2026

EPITHELIAL-MESENCHYMAL TRANSITION AFFECTS AMNIOTIC DERIVED STEM CELL IMMUNE RESPONSE

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Epithelial-mesenchymal transition (EMT) deeply affects many biological properties of stem cells, including those of placental amniotic epithelial cells (AEC). AEC experience EMT during pregnancy, at labor or during in vitro expansion. EMT affects AEC properties by impacting on mechanism controlling placental immune response, however gene regulation involved remains still unknown. Here, to fill the gap related to the transcriptome dynamics regulating immune response of the AEC we adopted a compelling method allowing to link data derived from RNA seq, system biology and ex vivo cultural approaches. We demonstrated that epithelial (eAEC) and mesenchymal (mAEC) counterpart of AEC, collected before and after EMT in vitro induction, showed a divergent expression of genes implied in the immune response. In particular, eAEC acted as antigen-presenting cells and were able to induce a higher inhibition of PHA-stimulated lymphocyte proliferation. Conversely, even though mAEC showed an enhanced production of inflammatory mediators (e.g. upregulating IL6, IL12B, IL10, PDGFB) they were less effective in damping lymphocyte proliferation. Consistently, we found that genetic ablation of the identified key drivers of the immune response of eAEC (CIITA, the HLA class II presenting antigen related gene) and mAEC (Nrf2, the key regulator of cellular response to oxidants) caused an impaired immune response of mAEC and eAEC, respectively. This new scenario, open to the hypothesis that eAEC and mAEC could co-exist in vivo and synergistically collaborate to lead the different immune responses towards the resolution of the inflammation, an essential event characterizing the remodeling mechanism of the amniotic membrane during pregnancy and labor, which might be managed to improve the current approaches in stem cell therapy and regenerative medicine.

Keywords: EMT of amniotic stem cells, Immunomodulation, RNA-seq

2027

ESTABLISHMENT OF PORCINE EMBRYONIC STEM CELLS CAPABLE OF SERUM-FREE AND FEEDER-FREE CULTURE FROM IN VITRO BLASTOCYSTS

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Stable porcine embryonic stem cells (pESCs) obtained from blastocysts in vitro can be a promising tool enabling beneficial applications in animal breeding as well as modeling human development as pigs are similar to humans in terms of embryonic development and physiology. However, most pESCs require complex media compositions and feeder layers. We derived pESCs using simplified culture media (F12-FIW) consisting of small molecules FGF2, IWR-1, and WH-4-023 in knockout serum replacement medium. Blastocysts obtained in vitro on day 6 were seeded on the feeder cell layer with F12-FIW medium. We established several pESCs lines (pESCs-FIW) capable of single cell passaging with short cell doubling time. These cells exhibited alkaline phosphatase (AP) activity and expressed pluripotency markers, OCT4, SOX2, and NANOG, as well as cell surface markers, SSEA1, SSEA4, and TRA-1-60. Established pESCs showed negative expression for the naive marker KLF4 and primed marker T, whereas strong expression for the formative marker OTX2. When the pESCs were maintained until late passages (above p50), they showed similar proliferation rates and single-cell clonal efficiencies with the same morphology as those of early passages (below p30). When using mTeSR™, a feeder-free culture medium for human pluripotent stem cells, pESCs could be maintained on Matrigel, fibronectin, rhLaminin-521, and Vitronectin. Expression of pluripotency marker genes in pESCs maintained on each of the four matrices showed similar levels to those in pESCs under the feeder condition. These results indicate that the canonical WNT inhibitor (IWR-1) and the SRC inhibitor (WH-4-023) under serum-free conditions are sufficient to establish pESCs capable of single-cell passaging and feeder-free expansion. Easy to maintain-pESCs can be used to develop complex genetic modifications useful for agriculture and biomedicine, and manufacture cell-derived meat and other products.

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Agriculture, Forestry and Fisheries (318016-5, 320005-4)", Republic of Korea.

Keywords: Pigs, Embryonic stem cells, Serum-free media

2028

CELLS LINES AUTHENTICATION FOR THEIR DEPOSIT IN BIOBANKS

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Cell lines authentication is part of the Good Cell Culture Practice principles, so it's necessary to establish cells identity and tracking provenance prior to the initiation of experimental research. Cell lines authentication is promoted by the International Cell Line Authentication Committee (ICLAC). Currently, funding agencies and scientific papers are increasingly requiring evidences of cell lines authentication. Although several techniques have arisen in order to assess cell lines identity, STRs (Short Tandem Repeats) profiling is the preferred method for cell lines authentication. The Andalusian Public Health System Biobank (BBSSPA) is part of the National Stem Cell Bank (BNLC), and participates in the deposit and traceability control of human pluripotent stem cell lines. STRs genotyping is based on the detection of DNA microsatellites containing variable lengths of STRs through the genome. The analysis of those repeated regions is performed by PCR and bioinformatics software. Hence, when the STRs profile of a cell line matches ≥80% with its original source we may confirm its authenticity. Currently, the minimum number of STR loci to accept cell lines authentication is thirteen, thus some authors test sixteen STRs to improve the specificity of the analysis. During the last 6 years, traceability analysis of 44 pluripotent stem cell lines was carried out, by analyzing 10 STRs fragments (GenePrint-10). All cell lines showed a correct authentication, except one who manifest the mixture of two different pluripotent stem cells lines. Currently, this method has been replaced by a more precise one that includes 16 STRs (CLA IdentiFiler™ Plus PCR Amplification Kit) following the recommendations of the ANSI/ATCC ASN-0002. The BBSSPA performs a fundamental task of traceability of cell lines deposited in the BNLC for their distribution. Cell lines traceability must be checked during different passages such as at the beginning of the generation, regularly during the culture or before distribution, among others. Finally, as a node of the BNLC, it's essential to promote standardized and coordinated methods for international authentication of stem cells lines.

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Keywords: Authentication cells lines, stem cells lines, Short Tandem Repeats

TOPIC: NO TISSUE SPECIFICITY

2125

RE-ENGINEERING THE METHYLATION READER FUNCTION OF KLF4 ENABLES PLURIPOTENCY INDUCTION WITHOUT THE SOX2/OCT4 DUO

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Krüppel-like factor 4 (KLF4) is a pioneer transcription factor directing reprogramming towards pluripotent and multipotent stem cells. This activity is tied to its capacity to bind silenced chromatin such as methylated CpG and nucleosome core particles. To dissect the basis for KLF4's reprogramming activity and to enhance its function, we designed saturation mutagenesis libraries by randomising positions that are critical to bind epigenetically modified DNA. Using pooled screens in mouse pluripotency reprogramming, we identified several evolved KLF4 (eKLF4) variants that change the reprogramming activity of wild-type KLF4. Two KLF4 double mutants support iPSC generation without the otherwise essential SOX2/OCT4 duo. The iPSCs derived from eKLF4 and c-MYC (eKM iPSCs) exhibit all the molecular, cellular and functional characteristics of embryonic stem cells (ESCs). Mechanistically, eKLF4 shows a higher sensitivity to the methylation of its binding motif compared to the wild-type protein in vitro. Further analysis will elucidate the molecular basis for the pioneer factor function of KLF4, decode its sequence-structure-function relationship, and inform the functional enhancements of KLFs to optimize strategies for the generation of functional cell types for regenerative medicine.

Keywords: iPSC, KLF4, Epigenetics

TRACK: CLINICAL APPLICATIONS (CA)

TOPIC: HEMATOPOIETIC, IMMUNE, AND ENDOTHELIAL

2029

IMPROVING THE LONG-TERM ENGRAFTMENT OF GENETICALLY MODIFIED HEMATOPOIETIC STEM AND PROGENITOR CELLS IN THE BONE MARROW BY TRANSIENT UPREGULATION OF ENGRAFTMENT ENHANCERS

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Autologous transplantation of genetically modified hematopoietic stem and progenitor cells (HSPCs) is becoming a promising

treatment for patients suffering from blood disorders. The HSPCs gene editing (GE) procedure is mostly performed ex-vivo through a process in which HSPCs are harvested from the patient, genetically corrected in vitro and re-infused back to the patient by intravenous transplantation. Effective treatment requires the successful homing and engraftment of transplanted HSPCs into the bone marrow (BM) niche, where they undergo self-renewal and establish a population of genetically modified cells that pass a correct copy of the gene to the blood cell progeny. Despite the excellent levels of gene correction currently attainable in vitro, the translational potential of this approach has been hampered by a poor engraftment of engineered HSPCs in vivo after transplant. Most likely, this is caused by some cell intrinsic defect such as reduced homing capacity or the loss of HSPCs repopulating potential due to the ex vivo manipulation. This represents an impellent hurdle to overcome for the therapeutic success of GE technologies. To improve the long-term engraftment of GE-HSPCs in the BM, we devised an innovative epigenome platform for the transient up-regulation of chemokines and integrins involved in stem cell homing and engraftment. We used a highly specific CRISPR activation system composed by a catalytically dead Cas9 protein fused to the tripartite transcriptional activator VPR (dCas9-VPR) and sgRNAs specifically designed to recruit dCas9-VPR to the promoter region of the target genes and activate their transcription. The time-course analysis revealed a peak of gene activation after 24h and the restoration of baseline expression levels after 96h, demonstrating that this platform is compatible with the kinetics of HSPCs homing and engraftment after transplant. We also tested the efficiency of this system by in vitro migration assay of HSPCs gene-edited with a CRISPR/Cas9 platform for the correction of WAS mutation developed by our group. By integrating our epigenome platform with GE platforms for gene correction, it will be possible to ensure high levels of engraftment of corrected HSPCs providing a bench-to-bedside translation of GE technologies to treat blood disorders.

Keywords: HSPCs, Engraftment, Epigenome editing

TOPIC: MESENCHYMAL STROMAL CELLS, ADIPOSE, AND CONNECTIVE TISSUE

2030

GENOME EDITING OF MESENCHYMAL STEM CELLS: IMPLICATIONS FOR TISSUE REGENERATION IN CHRONIC INFLAMMATORY MUSCULOSKELETAL DISORDERS.

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Musculoskeletal disorders are major healthcare concerns that lead to disabilities in the aged population. Progression of multiple musculoskeletal disorders is associated with the accumulation of inflammatory cytokines causing destruction of extracellular matrix and cellular apoptosis in the affected tissues. Current pharmacological recommendations for chronic inflammatory musculoskeletal disorders are palliative and fail to reconstruct the damaged tissue. Thus, existing therapies have limited clinical potential with variable outcomes. Stem cell-based tissue reconstruction is a promising approach wherein Mesenchymal stem cells (MSCs) have shown most promising results in human and animal clinical trials for various disorders of bone, cartilage and muscle. However; these clinical trials state that exogenously delivered MSCs get eliminated fast due to deleterious effects of inflammatory environment. Inflammatory cytokines down-regulate the transcription factors involved in differentiation and up-regulate the apoptotic pathways in MSCs. Thus, successful application of MSCs for chronic inflammatory and autoimmune disorders relies upon the efficient localization and retention of cells within appropriate tissue. Present study hypothesizes an approach to improve engraftment efficiency and survival of exogenously infused MSCs to increase their therapeutic effects, based on a genetic engineering approach. This study documents generation of TNF-α and IFN-γ receptor knockdown in human MSCs (hMSCs) using shRNA-mediated technology. MSCs isolated from bone marrow, adipose tissue and gingiva showed the expression of TNFR1 and IFNGR1 as evidenced by flow cytometry and western blotting. Further, it was studied that exposure of hMSCs to TNF-α and IFN-γ synergistically induced apoptosis and inhibited osteogenesis. Therefore, a set of shRNAs were used to knockdown TNFR1 and IFNGR1. We generated engineered hMSCs which showed about 50% knockdown of TNF-α and IFN-γ receptors than that of control. Currently, we are investigating the growth, survival and differentiation potential of these engineered MSCs in presence of an inflammatory environment. In the subsequent studies, we propose to use CRISPR-based epigenome editing for generation of inflammation-resistant hMSCs.

Funding Source: The author is a recipient of research grant from Department of Science and Technology, Government of India under “Women Scientist-A” scheme.

Keywords: mesenchymal stem cells, genome editing, inflammatory musculoskeletal disorders

2031

ADIPOSE TISSUE DERIVED MESENCHYMAL STROMAL STEM CELL (MSC) TRANSPLANTATION SHOULD BE CONSIDERED WITH CAUTION IN PROTHROMBOTIC CONDITIONS

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The conventional thought is that liver produces Factor VIII/ anti-hemophilic factor-an important blood-clotting protein. Here we show that adipose tissue synthesizes FVIII and its expression is increased significantly in cirrhosis/Chronic Liver Disease (CLD). Since the mass of adipose tissue is large, the net contribution of adipose tissue towards circulating factor VIII is significant. This is clinically important in obesity, diabetes, fatty liver disease, which are associated with high fat mass. This may also partly explain increased incidence of blood clots, myocardial infarction, and stroke in obesity. We have shown the factor VIII levels in adipose tissue is increased in CLD by multiple methods-immunohistochemistry (IHC), western blot (WB), reverse transcriptase real time PCR and flow cytometry of freshly isolated cells from adipose tis-

sue. Further we showed that these cells retain FVIII positivity in culture by immunofluorescence (IF) and WB. This fact is important when we consider MSC transplantation in prothrombotic conditions such as stroke or CLD. We tested the difference between MSC from CLD patients versus healthy subjects and found that MSC from CLD patients proliferated faster in culture but they also attained senescence faster (β-galactosidase assay). We found that MSC from patients differentiated less efficiently into osteocyte (silver stain), adipocyte (Oil Red O stain) and chondrocyte (Alcian Blue stain) lineages. This means MSC from patients aren't equal to healthy subjects and this fact should be a consideration for autologous MSC transplantations. Human subjects: Healthy subjects, n=15; cirrhosis patients, n=15, Inclusion criteria: decompensated liver cirrhosis who were referred for liver transplantation. Exclusion criteria: patients suffering from Acute Liver Failure and Acute on Chronic Liver Failure, inherited bleeding disorders and those not consenting for study. To conclude, adipose tissue MSC produces FVIII and should be administrated with caution in prothrombotic conditions. Further MSCs derived from patients differ in their proliferation, senescence and differentiation potential. This fact should be considered during MSC autologous transplantation protocols.

Funding Source: SMG thanks Department of Biotechnology, Ministry of Science and Technology, India (grant #BT/PR15116/MED/31/334/2016) Government of India and Science and Engineering Research Board (grant #ECR/2015/000275).

Keywords: Adipose Tissue MSC, Factor VIII, F8, Liver Cirrhosis, Chronic Liver Disease, Prothrombotic, coagulopathy

TOPIC: MESENCHYMAL STROMAL CELLS, ADIPOSE, AND CONNECTIVE TISSUE

2054

CHARCTERISTICS OF MESENCHYMAL STEM CELLS ENCAPSULATED IN CORE-SHELL HYDROGEL MICROFIBERS AND THEIR THERAPEUTIC EFFECTS ON INFLAMMATORY BOWEL DISEASE MODEL IN RATS

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This study focused on the functional characteristics of mesenchymal stem cells (MSCs) encapsulated in core-shell hydrogel microfibers which are known as "cell fibers" and their therapeutic effects on a colitis model. Inflammatory bowel diseases, especially Crohn's disease, frequently complicates refractory anal fistula. Most anal fistula cases require invasive surgical intervention such as seton drainage or fistula removal surgery. Since existing treatments are not curative for severe inflammatory cases and may lead to anal fistula cancer, establishing a novel treatment with high local efficacy is crucial. MSCs exhibit immunoregulatory functions and tissue regeneration ability by releasing various anti-inflammatory factors and extracellular secretory granules encapsulating nucleic acid and protein components. It is also known that three-dimensional (3D) cultures of MSCs suppress the replicative senescence of cells and enhance their anti-inflammatory effects, angiogenesis, and prolongation of cell survival. Since therapeutic efficacy is associated with cell distribution to the target organ, local cell therapy is expected to be highly effective. However, MSCs administrated directly into an anal fistula could be damaged by intestinal bacteria or aggressive cytokines in vivo. Therefore, we employed a "cell fiber" technology, which is expected to provide the following two advantages. MSCs cultured in cell fibers maintained a 3D state, enhancing cell viability and anti-inflammatory functions for a prolonged time, and MSCs in the fibers could be administered near the anal fistula locally with protection from a cytotoxic environment by the shell and effective secretion of cell-derived factors. In fact, persistent and highly concentrated prostaglandin E2 and TGF-β secretions were observed in the culture supernatant of MSC-fiber compared to those of the two-dimensional cultured MSCs. The expression of tissue regenerative factors, such as VEGF and HGF, was enhanced in the spherical MSCs in the cell fibers. Additionally, MSC-fibers administered directly to the ulcerative lesions of the intestinal mucosa exhibited superior therapeutic effects than vehicle treatment in acute colitis in rats. We will report on the mechanism involved in the therapeutic effects of the MSC-fiber on colitis.

Funding Source: Grants-in-Aid for Scientific Research in Japan CellFiber Co., Ltd.

Keywords: Cell fibers, Mesenchymal stem cells, Inflammatory bowel disease

TOPIC: MUSCULOSKELETAL

2032

IPSC-DERIVED TENOCYTES ON MICROGROOVED SCAFFOLD CONTRIBUTES TO ACHILLES TENDON REGENERATION

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In the US, 50% of musculoskeletal injuries reported annually involve tendons or ligaments. Current treatments for tendon injury include pain management, physical therapy, and in serious cases, surgical repair. Tendons have a poor innate healing capacity and full structural and functional restoration after injury remains an unmet clinical need. The application of a 3D-printed microgrooved scaffold (MGS) provides a customizable matrix for cells to grow while encouraging cellular alignment. We examined the application of induced pluripotent stem cell (iPSC)-derived mesenchymal stem cells (iMSCs) overexpressing the tenogenic transcription factor Scleraxis (SCX, iMSCSCX+) in conjunction with 3D-printed MGS, as a novel method for tendon defect repair. iPSCs were differentiated to iMSCs and transduced with a SCX lentiviral vector to produce iMSCSCX+. iMSCSCX+ were seeded on custom 3D-printed non-patterned scaffolds (NPS), MGS, or regular plates and cultured for 7 days to assess tenogenic gene expression and cell alignment. In vivo, MGS+iMSCSCX+ treatment was tested against MGS-only and suture-only repair for 6 weeks in a rat model of Achilles tendon rupture. The repair was assessed using gait analysis, biomechanical testing, and histological staining. iMSCSCX+-seeded MGS showed significant upregulation of tendon markers compared to NPS and 2D culture after 7 days in vitro. Immunostaining showed more linearly organized cells on the MGS compared to the NPS. Gait analysis found that rats repaired with MGS+iMSCSCX+ had improved stride length compared to the MGS-only group. Sway width and paw spread was also significantly improved in MGS+iMSCSCX+ group the compared to the suture only group. Ex vivo biomechanical testing showed a significant improvement in the biomechanical properties of the MGS+iMSCSCX+ group compared to both the suture and MGS-only controls. Histology and immunostaining demonstrated more regular tissue formation in MGS+iMSCSCX+ group. This study shows the potential of iMSCSCX+-seeded MGS, a combined iPSC and tissue engineering approach, as an alternative for current tendon defect treatments. Further studies of cell-scaffold constructs can potentially revolutionize tendon reconstruction by improving healing and functional outcome at both the cellular and tissue level.

Keywords: Microgrooved Scaffold, iPSC, Microgrooved scaffold

2033

MULTIPASSAGE EXPANSION OF SERUM-FREE ISOLATED EQUINE MESENCHYMAL STEM CELLS WITH ENHANCED SELF-RENEWAL CAPACITY FOR CELL THERAPY APPLICATIONS

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Adult bone marrow derived mesenchymal stem cells (BM-MSCs) have shown promise in treating musculoskeletal diseases. Recently, there has been increasing interest in the veterinary field to use these cells to treat joint injuries in equine athletes. Conventional media for isolating equine BM-MSCs include fetal bovine serum (FBS) to support cell growth, yet FBS is undefined, exhibits batch-to-batch variability, and poses significant concerns about xenogeneic proteins present in the therapeutic fraction. Alternatively, substituting bovine serum with equine serum have shown to result in loss of MSC function. While a few studies have recently used defined media for the short-term culture of equine MSCs, there remains limited knowledge about the impact that removing serum has on these cells at cellular and functional levels during extended culture periods. Here, we present a unique serum-free medium (SFM) that not only supported isolation and expansion of equine BM-MSCs, but also enhanced self-renewal and multipotency properties. We found that serum free isolated equine BM-MSCs differed significantly in morphology, cytokine secretion, growth kinetics, and colony forming capacity from those cells expanded in serum containing medium (SCM). Namely, the cells acquired irregular shapes with multiple cytoplasmatic processes in SFM, while they assumed an elongated uniform spindle shape in SCM. The cells in SFM were 4 times larger in size compared with those in SCM ($p< 0.0001$). Cell doubling level was 1.36 (SD 0.36) in SFM, and 2.42 (SD 0.40) in SCM ($p< 0.0001$). Colonogenic assays revealed the cells in SFM continued to grow in smaller colonial densities for several passages ($p< 0.01$), while those in SCM propagated in a monolayer. While full trilineage differentiation was explored, ongoing work is underway characterizing the proteome and immunomodulatory function of these distinct cell populations. Overall, our findings demonstrate that the defined, serum-free medium can support the efficient isolation and rapid expansion of equine BM-MSCs. We anticipate our systematic characterization of serum free isolated equine BM-MSCs to be a starting point toward developing robust processes for the large-scale production of equine BM-MSCs with enhanced therapeutic effect for preclinical research and cell-based therapies.

Keywords: serum-free medium, bone marrow derived mesenchymal stem cells, preclinical research & cell-based therapies

TOPIC: NO TISSUE SPECIFICITY

2034

ENGINEERED HYPOIMMUNE CAR T CELLS SURVIVE, FUNCTION, AND PERSIST IN IMMUNOCOMPETENT ALLOGENEIC HUMANIZED MICE

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Off-the-shelf CAR T cells potentially offer advantages over autologous strategies such as ease of manufacturing, quality control, off-the-shelf availability, and lack of T cell dysfunction, as well as the ability to generate a more consistent CAR T product from healthy T cells. However, the vigorous host-versus-graft immune response against histoincompatible T cells prevents expansion and persistence of allogeneic CAR T cells and mitigates the efficacy of this approach. A major challenge is that, while HLA deletion can result in adaptive immune evasion, innate reactivity is enhanced with this approach. CD47 overexpression can block both NK cell and macrophage killing (J Exp Med 2021;218(3):e20200839), and we hypothesized that T cells would lose their immunogenicity when human leukocyte antigen (HLA) class I and II genes are disrupted and CD47 is over-expressed. We describe here the engineering of human immune evasive CAR T cells building on our previously described hypoimmune technology (Nat Biotechnol 2019;37(3):252-258 and Proc Natl Acad Sci U S A 2021;118(28):e2022091118). Human T cells from healthy donors were obtained by leukapheresis. CRISPR/Cas12b technology was used to disrupt the B2M, CIITA, and TCR genes, and lentiviral transduction was used to overexpress CD47 and to express a CD19 CAR to generate hypoimmune (HIP) CD19 CAR T cells. Control T cells were unmanipulated except for overexpression of the CD19 CAR (unmodified). For 3 month persistence studies, allogeneic SGM3 humanized mice were injected with 1˙106 Luc+ Nalm6 cells and received 7˙106 unmodified CD19 CAR T cells or HIP CD19 CAR T cells. In the mice treated with either unmodified CD19 CAR T cells and HIP CD19 CAR T cells, tumor control was initially rapidly achieved. However, unmodified CD19 CAR T cells were eventually rejected by the host and the loss of these cells resulted in re-growth of tumor. By contrast, in HIP CD19 CAR T injected mice, tumor control was maintained throughout the study, including following a rechallenge at day 83 with NALM6 cells without further administration of HIP CD19 CAR T cell. Flow cytometry at endpoint from bone marrow and spleen confirmed persistence of HIP CD19 CAR T cells.

Keywords: immune barrier, allogeneic CAR T, persistence

TRACK: CLINICAL APPLICATIONS (CA)

2128

IMPACT OF HYPOXIA ON TISSUE-SPECIFIC MESENCHYMAL STEM CELLS ON IMMUNE CONDITIONING OF ACUTE GRAFT-VERSUS-HOST-DISEASE

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The efficacy of Mesenchymal stem cells (MSCs) in acute Graft-Versus-Host-Disease (aGVHD) is quite variable and unpredictable which needs improvement before their clinical application. Hypoxia is one of the intrinsic factors which promote metabolic programming and stabilize stemness in MSCs. In view of this, the current study explored the influence of hypoxia on MSC-based immune conditioning of aGVHD. MSCs were isolated from two different sources (BM, WJ) followed by their characterization according to the ISCT guidelines. MSCs were preconditioned with an exposure of 1% O2 for 12hr using a tri-gas incubator. At 70-80% confluency, serum-containing media was replenished with serum-free media, and culture-conditioned media (CCM) was collected after 48h from both naive and hypoxia preconditioned MSCs. The impact of hypoxia-conditioned MSCs/MSCs-CCM was evaluated on the phenotypical index of various subsets of NK and T cells from aGVHD patients using 13-color flow cytometry. To assess the direct impact of MSCs, tissue-specific MSCs (naïve, hypoxia preconditioned) and CFSE-labeled activated PBMNCs were co-cultured using the 2D direct co-culture system, and proliferation of CD3+ T cells, CD4+ T cells, CD8+ T cells, and Tregs were assessed using flow cytometry. Phenotypic analysis of MSCs (apoptosis/Efferocytosis) was observed in the co-culture system. Both BM-MSCs and WJ-MSCs enhanced the differentiation of helper T cells towards regulatory phenotype (Tregs) and suppressed the proliferation of CD8+ T cells. WJ–MSCs were more

immunosuppressive (approximately 17%, p-value:< 0.0001) than BM-MSCs, which was due to an increase in late apoptosis of WJ MSCs. Interestingly CD14+ monocyte picked up dying WJ-MSC more effectively (8.5% higher, p:0.0017) over BM-MSC and underwent Efferocytosis. Hypoxia enhanced the immunomodulatory potential of both MSCs but hypoxia-primed BM-MSCs (25%) were more effective than WJ-MSCs (12%) in their immune conditioning capacity. Additionally, responders had more naïve (CD45RA+ CCR7+), effector (CD45RA+ CCR7-) CD8+ T cells, and functional NK cells (CD56+ KIR+) than non-responders. Our results demonstrate that hypoxia-guided BM-derived MSCs bear the potential to mitigate effector immunity and can be used as a palliative cellular therapeutic approach for managing GVHD.

Funding Source: The study was supported by the Indian Council of Medical Research, New Delhi, India.

Keywords: Mesenchymal Stem Cells, Acute Graft-Versus-Host-Disease, Hypoxia

TRACK: ETHICS, POLICY, AND STANDARDS (EPS)

TOPIC: NO TISSUE SPECIFICITY

2035

HOW ARE SURGICAL AND CLINICAL INNOVATION IN MEDICAL PRACTICE DEFINED FOR THE PURPOSE OF DETERMINING REGULATORY JURISDICTION AND OVERSIGHT RESPONSIBILITY: A SCOPING REVIEW

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Regenerative medicine (RM) is a promising field that may ultimately offer improved treatment options for various diseases and conditions. In addition to its scientific complexity, this field raises questions regarding oversight, including whether RM interventions should be characterized and regulated as drugs, whether they fall more appropriately within the practice of medicine and should be regulated accordingly as forms of clinical or surgical innovation, or whether an alternative pathway is most appropriate. Previous work has identified a lack of clarity and consensus regarding how the concepts of clinical and surgical innovation and the practice of medicine are characterized and interpreted. This ambiguity risks uncertainty in some jurisdictions, including Canada, regarding the locus of responsibility for oversight of emerging medical interventions in these categories, potentially including new RMs. Robust oversight with an emphasis on safety and effectiveness is imperative to ensure responsible clinical applications of new therapeutics such as RMs. In this project, we conducted a scoping review to better understand how surgical, clinical, and medical innovations have been defined and characterized for the purpose of determining regulatory jurisdiction and oversight responsibility. We reviewed peer-reviewed literature from various fields using the following databases: PubMed/Medline, Web of Science, Nexis Uni, Embase, HeinOnline, CanLII, and Academic Search Complete. We supplemented our data collection with grey literature searches via Proquest Dissertations and Theses Global, Canadian Electronic Library from Canada Commons, Google, and websites of relevant professional regulatory bodies and healthcare policy organizations. We followed the Preferred Reporting Items for Systematic Reviews and Meta-analysis Protocols (PRISMA) when drafting our scoping protocol. Through this research, we aim to provide greater clarity regarding these concepts and how they have been defined or characterized, systematically map existing research on these issues, and identify relevant gaps that warrant further exploration. Our findings will ideally help inform future policy development regarding regulation and oversight of emerging RMs in Canada and beyond.

Funding Source: This work is funded by the Stem Cell Network.

Keywords: Clinical and Surgical Innovation, Regulations and Oversight Responsibility, Scoping Review

2036

A MACHINE LEARNING AI MODEL AND CLUSTER ANALYSIS TO EXPLORE THE CONTOURS OF ETHICALLY RESPONSIBLE, SCIENTIFICALLY MERITORIOUS INTEGRATED STEM CELL-BASED EMBRYO MODEL RESEARCH BEYOND THE 14-DAY RULE

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In May 2021, following its March 2021 announcement, ISSCR released its updated Guidelines for Stem Cell Research and Clinical Translation. Notably in the March 2021 pre-release announcement, ISSCR telegraphed that the updated Guidelines would "acknowledge the sensitivities surrounding the 14-day rule for in vitro embryo research and call for a public conversation about the scientific significance of in vitro embryo research as well as the associated societal and ethical issues." The "14-day/primitive streak rule" up to the release of ISSCR's May 2021 Guidelines, continues to be, and will continue to be, a sacrosanct component of globally recognized limits to human embryo research. That said, the May 2021 Guidelines explained that the 14-day rule was not written to apply to integrated stem cell-based embryo models ("ISCbEMs") and that, going forward, ISCbEMs would be worthy of pursuit if deemed "highly meritorious by a rigorous review process". The contours of that rigorous review process remain subject to discussion and consensus building, as illustrated by the addition of a track to the 2023 ISSCR for this purpose. To contribute to this process, we have constructed a relevant machine learning model trained on an applicable dataset of the scientific, medical, ethical, and regulatory literature and have also undertaken a natural language processing concept-driven clustering analysis of that literature. The design matrix in the machine learning model identifies various features that attend the important need for the various stakeholders in new stem cell-based research technologies, such as ISCbEMs, to agree upon the policies and standards that will attend the development, implementation, and ongoing use of these technologies, which take stem cell science into the landscape beyond the 14-day/primitive streak border. In addition, we illustrate how the NLP clustering analysis helps to organize the relevant literature for purposes of discovering the lessons it contains that are relevant to navigating and blazing that landscape.

Keywords: Integrated Stem Cell-based Models, Ethically Responsible/Scientific Merit, Machine Language/Clustering Analysis

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META-ANALYSIS FOR REGULATORY SCIENCE ADVOCACY AND CONSENSUS-DRIVEN POLICY-MAKING TO SUPPORT USING ARTIFICIAL INTELLIGENCE (AI) TOOLS TO ADVANCE STEM CELL-BASED REGENERATIVE MEDICINE

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We first undertake a meta-analysis of the impact of various forms of artificial intelligence on advancing stem cell-based regenerative medicine and then apply this meta-analysis to several policy-making areas, viz., regulatory science, science advocacy, patient advocacy, ethics, research funding, health care, and intellectual property. We use two datasets for the meta-analysis: the first derived from the PubMed library; and the second derived from the USPTO library of patent applications and issued patents. For each library, we used the same search string: a conjunction of (A) a list of references to artificial intelligence generally and specific types thereof, (B) a reference to "stem cell", and (C) a reference to "regenerative medicine". The list of specific AI types includes machine learning, deep learning, reinforcement learning, image processing, neural networks, generative AI, and natural language processing. Examination of both datasets lead to constructing a list of stakeholders and stakes relevant to each aforementioned policy-making area. In some cases, conflicts arise between and among the stakeholders; in other cases, virtually all stakeholders are in alignment. The meta-analysis and its policy-making applications help to illuminate both the conflicts and alignments for purposes of building multi-stakeholder consensus building on policies aimed at maximizing the benefits, as well as addressing the challenges, attending continued use and advancement of the various tools within AI to support the advancement of stem cell-based regenerative medicine.

Keywords: Artificial Intelligence, Stem Cell-Based Regenerative Medicine, Regulatory Science

TRACK: MODELING DEVELOPMENT AND DISEASE (MDD)

TOPIC: NO TISSUE SPECIFICITY

2038

CONVERGENCE OF NEURODEVELOPMENTAL SIGNATURES IN PRADER-WILLI SYNDROME FROM AN ALLELIC SERIES OF CRISPR-ENGINEERED NEURONAL MODELS

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Prader-Willi Syndrome (PWS) is a neurodevelopmental genomic disorder characterized by life-threatening childhood hyperphagia and obesity, hypogonadism, muscular hypotonia, and intellectual impairment. Full and partial deletions of the paternally inherited 15q11.2-q13 region, including the loss of nine protein-coding genes and seven noncoding RNAs, are the most common cause of the phenotype. To investigate the underlying molecular pathways, we created an allelic series of CRISPR-engineered Type I (6Mb) and Type II (5Mb) deletions in isogenic human induced pluripotent stem cells (hiPSCs). We also created lines with a critical region deletion (CRD) containing three genes, including the small nucleolar RNA SNORD109A, and individual PWS gene deletions. We differentiated these lines into Ngn2-induced neurons and performed systematic transcriptomic analyses producing 725 libraries (small RNA, mRNA, and total RNA), and functional assays using a multi-electrode array. The differentially expressed and co-expressed genes from Type I and Type II edits were strongly enriched for neurodevelopmental and synaptic-associated pathways, including glutamatergic and cholinergic synaptic pathways, as well as GnRH secretion and oxytocin signaling. Moreover, the

Type I/II edits displayed significantly reduced electrophysiological features. This result was again replicated in the CRD model and some of the individual gene edits, including the deletion of SNORD109A. Preliminary analyses showed amelioration of the electrophysiological deficits by re-introducing exogenous SNORD109A into the Type I and SNORD109A edited lines, suggesting a potential therapeutic target. Our findings demonstrate the functional impact of PWS-associated gene deletions in human-derived neural models and highlight alterations to neuronal networks and pathways associated with PWS pathogenesis.

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Keywords: Prader-Willi Syndrome, CRISPR/Cas9-engineered neuronal model, Transcriptional and functional profiling

2039

SAMPLED AND EBISC: IPSC TOOLS AND SERVICES FOR ACADEMIC AND COMMERCIAL RESEARCHERS WORLDWIDE

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Development of technologies such as iPSC disease modeling and next generation sequencing have ushered in the era of big science, with projects driven by the need for open access, on a global scale, to large, well curated, high quality collections of cells and data. Meeting this need requires biorepositories to move beyond their traditional models to adopt a centralized approach designed to bring together cells and data and make both highly traceable, visible and accessible, enabling re-use and increasing reproducibility. Here we discuss two differing but aligned approaches towards this goal; the Global Integrated Analytical Biorepository (GIAB) by SAMPLED and the European Bank for induced Pluripotent Stem Cells (EBiSC). Both GIAB and EBiSC safeguard, centralise and standardise iPSC lines and associated cell products including pre-differentiated cells and isogenics to increase access to iPSC resources and improve quality of iPSC research. Provision of iPSC services also supports *Research and Development* across the academic and commercial landscapes. GIAB is modeled along the lines of a SMART lab (Storage, Management, Analysis, Research and Transport), offering a comprehensive menu of services ranging from human sample collection, sample processing, cell culture and reprogramming, genomics, analysis and global biosample transport. As part of longstanding grants to operate several NIH biorepositories, SAMPLED hosts the NINDS Cell and Human Data Repository and the NIMH Repository & Genomics Resource. These repositories house iPSC, gene-edited isogenic pairs, NPCs and cryopreserved somatic cells from >1000 subjects, including a GMP grade iPSC line. EBiSC aims to 'open the door' to iPSC research for all, including non-expert Users, providing researchers with a diverse collection of >900 iPSCs across >40 diseases including iPSC neurons as well as protocols, training videos, guidance and comprehensive open and managed access datasets. EBiSC provides a single access point to iPSCs derived within diverse research projects worldwide as well as specialization in iPSC reprogramming, gene editing, differentiation, cryopreservation and scalability. Here we present and compare these differing approaches including mechanisms for obtaining access to cells as well as associated de-identified data in a secure and GDPR compliant manner.

Keywords: Biobank, iPSC, Global Resource

2040

RECURRENT SOMATIC DRIVER MUTATIONS IN HUMAN INDUCED PLURIPOTENT STEM CELLS

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Human Induced Pluripotent Stem Cells (hiPSC) are an established patient-specific model system where opportunities are emerging for cell-based therapies. We studied the mutational landscape of hiPSCs derived from different tissues, at single-nucleotide resolution, across hundreds of cell lines including sub-clones. The majority of fibroblast derived hiPSC (72%) were heavily mutagenized with UV-related damage and there was evidence of genomic heterogeneity amongst hiPSCs derived in the same reprogramming experiment, due to oligoclonal fibroblast populations. We looked for recurrent somatic driver mutations and identified strong selection for mutations in BCOR in approximately 25% of hiPSC lines, predominantly in blood-derived hiPSCs. Our findings suggested the mutations had arisen during in vitro culture. We explored the functional consequences of hiPSCs harbouring BCOR mutations using RNA sequencing and directed differentiation. We observed that BCOR mutations profoundly alter the transcriptome and impair differentiation into the neural lineages. We present the most comprehensive assessment to date of the mutational landscape in hiPSCs and identify the first somatic driver mutation in hiPSCs. Our work demonstrates the importance and utility of high resolution genomic characterisations of hiPSCs prior to research or clinical use.

Keywords: hiPSCs, Mutation, BCOR

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RECONSTRUCTING GOUD POPULATION BASED ON MATTAPALLY SURNAME HISTORY

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In higher vertebrates and some invertebrates, physically different sex chromosomes, which are mainly heterochromatic and rich in highly repetitive DNA, are responsible for the underlying molecular control of primary sex determination. Since they offer a model system that represents all stages in the evolution of sex chromosomes, snakes in particular offer unique opportunity to clarify the mechanisms involved in the evolution and function of these chromosomes. Our understanding of the molecular basis of sex determination began with the identification of highly conserved Bkm (Banded krait minor satellite DNA) DNA sequences associated with the sex chromosomes. Since GATA4 is crucial for embryonic heart development, the goal of this study was to determine whether GATA4 repeats are associated with CHD in south Indian CHD patients and control samples study to examine the genetic diversity of the Goud population in south India. A trustworthy genetic marker for forensic DNA analysis systems is the SE33 locus based on Pakistan study but our studies find M34 sequences are unique to each species. On the Y chromosome, M34 is present in 200–300 copies, mixed together with other sequences. A 1.2 kb segment of M34 contains 32 copies of GATA repeats that are flanked by SAR motifs (ATATTT) that bind to nuclear matrices. Such SAR motifs are firmly bound by histone HI, indicating that histone HI is involved in the condensation of the Y chromosome in somatic cells. The GATA repeat may have functional significance because no other simple repeat is known to be as consistently connected with the sex chromosome. Based on this supposition, the Y chromosome contains GATA repeats all the way down to the region that determines sex. The distribution pattern of GATA repeats on the mouse Y chromosome and the W chromosome of the snake are remarkably comparable. Globally, forensic investigations are using genetic variation, an actual tool of individual discrimination. Even one of the 13–17 indicators with incomplete data results in an equivocal report. To account for this, more trustworthy markers are necessary. such shortcomings. Some nations use forensic investigation techniques and the highly conserved Bkm locus compared SE33 locus, which exhibits considerable genetic variability among populations.

Keywords: GATA4, Goud population, SE33 locus

2042

CERNA NETWORK ANALYSIS OF TURNER SYNDROME IPSC-DERIVED CARDIOMYOCYTES REVEALS DYSREGULATION OF AUTOSOMAL HEART DEVELOPMENT GENES BY ALTERED DOSAGES OF X-INACTIVATION ESCAPING NON-CODING RNAS

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45,X monosomy (Turner syndrome, TS) is the only chromosome haploinsufficiency compatible with life. Nevertheless, the surviving TS patients still suffer from increased morbidity and mortality, with around one third of them subjecting to cardiovascular diseases (CVDs). Although haploinsufficiency of X-inactivation escaping genes could partially explain some of the TS symptoms, the genetic causes that drive the most life-threatening CVDs remain largely unknown, thus requiring more systematic approaches to investigate. Here, we have generated cardiomyocytes (CMs) from wild-type and TS patient-specific induced pluripotent stem cells and profiled the mRNA, lncRNA and circRNA expression in these cells. We observed that the beating frequencies decreased from averagely 78 to 68 beats per minute, and the mitochondrial DNA copies increased from averagely 1.7k to 5.4k per nucleus in TS-CMs vs. WT-CMs. Moreover, by using gene expression microarrays, we have identified a global transcriptome dysregulation of mRNAs, lncRNAs and circRNAs in TS-CMs. Interestingly, there are more differentially expressed (DE) genes, across all mRNA, lncRNA and circRNA classes, of TS-CMs vs. WT-CMs than those of TS-iPSCs vs. WT-iPSCs. The expression patterns are also more distinctive between TS-CMs vs. WT-CMs than those between TS-iPSCs vs. WT-iPSCs. These results implied that loss of one X chromosome exert more genomewide ripple effects in CMs rather than in iPSCs. Gene ontology analysis indicated that the DE mRNAs of TS-CMs vs. WT-CMs were enriched of heart development genes. Further competing endogenous RNA network analysis

revealed putative regulatory circuit of autosomal genes relevant with mitochondrial respiratory chain and heart development, such as COQ10A, RARB and WNT2, mediated by X-inactivation escaping lnc/circRNAs, such as lnc-KDM5C-4:1, hsa_circ_0090421 and hsa_circ_0090392. The aberrant expressions of these genes in TS-CMs were verified by qPCR. In summary, our study has revealed a genomewide ripple effect of X chromosome halpoinsufficiency at post-transcriptional level and provided insights into the molecular mechanisms underlying heart abnormalities in TS patients.

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Keywords: Turner syndrome, induced pluripotent stem cell, noncoding RNA

2043

USING HUMAN INDUCED PLURIPOTENT STEM CELLS TO MODEL OBESITY AND ITS INFLUENCE ON VARIABLE RESPONSE TO PROARRHYTHMIC DRUGS

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Cardiac function is modulated by various autocrine, paracrine, and endocrine signals, many of which are derived from epicardial adipose tissue (EAT). EAT typically has physiological and cardioprotective functions, but under pathological circumstances such as obesity, there are changes in signalling related to cardiac pathophysiology. These can include pro-inflammatory cytokine release and increased levels of fibrosis, all of which play a pivotal role in remodelling of major atrial and ventricular ion channels. This can lead to either faster or delayed repolarisation, and thus, predispose obese patients to higher risk of atrial fibrillation or ventricular arrhythmias, respectively. Additionally, drugs can also contribute to this repolarisation, further increasing risk of cardiac arrhythmias. Although this concept has been demonstrated clinically, the number of in vitro studies conducted has been limited, and the extent to how EAT modifies proarrhythmic risk has not been fully explored. This study will use human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CM) to investigate the extent of change in electrical phenotype and proarrhythmic risk in response to EAT. EAT is collected from swine hearts to produce conditioned media, which is added onto the hiPSC-CMs. Electrical phenotypes are assessed using a multi-electrode array over a 30-hour period, that is split into two phases: the adipose phase and the drug phase. Measurements using a mixed-effects model show minimal changes in field potential duration (FPD), but a significant slowing in beat period (BP) and conduction velocity (CV) over 24-hours in presence of EAT conditioned media. Moreover, conduction maps show EAT-treated cardiomyocytes with a significant slowing in propagation time. The addition of drugs exhibits further alteration in these measurements. Cytokines released from the EAT conditioned media may be responsible for the significant changes observed in BP and CV, as they are known to alter expression level of depolarising and repolarising cardiac ion channels. Furthermore, the continuous decrease in CV is consistent with changes in protein expression such as gap junction remodelling. This study illustrates the importance of EAT influence in both normal and pathological conditions in response to proarrhythmic drugs.

Keywords: Epicardial Adipose Tissue, Obesity, Cardiac Arrhythmias

Abstract Withdrawn

2045

A BRUGADA SYNDROME-ASSOCIATED RARE NON-CODING VARIANT IN SCN5A RESULTS IN DETECTABLE FUNCTIONAL EFFECTS IN HUMAN IPSC-DERIVED CARDIOMYOCYTES

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Brugada syndrome (BrS) is an inherited arrhythmia condition which can cause sudden cardiac death in young and middle-aged individuals. Genetic variation in the Nav1.5 cardiac sodium channel-encoding SCN5A gene is robustly associated with BrS, across both rare coding and common non-coding variants. The prevalence of the disease is several folds higher in Southeast Asia compared to populations of European ancestry which likely reflects population-specific environmental or genetic (founder variant) risk factors. To identify these, we performed genome sequencing in 231 BrS probands from Thailand and 500 population-matched controls. We identified a rare (absent in gnomAD), non-coding variant in a candidate regulatory element (RE) of the SCN5A gene (GRCh37:3-38621871-A-C) which was significantly enriched in cases vs controls (3.9% vs 0.2%, OR=20.2[2.5-160.6], p=2e-04). We further obtained multiple lines of evidence linking this intronic variant to alterations of SCN5A expression and Nav1.5 function. We obtained two isogenic pairs of human induced pluripotent stem cells (hiPSCs) generated by introducing the heterozygous variant in a control hiPSC line and investigated the functional effect of the variant's presence by single cell electrophysiological analysis in the derived cardiomyocytes (hiPSC-CMs) from both pairs. The functional relevance of the variant was confirmed by the detection of a significant reduction of the peak Nav1.5-mediated sodium-current (INa) density (30% decrease at -20mV, p=8e-03), without changes in gating properties, in the variant-carrying hiPSC-CMs compared to the isogenic control-CMs. Additionally, we found that the variant significantly reduced luciferase activity of the 600 bp candidate RE in HEK cells in the presence of the cardiogenic transcription factors Tbx5, Gata4, Mef2A, and Mef2D. These findings demonstrate for the first time that a rare non-coding variant in the SCN5A gene reduces Nav1.5 function and partly explains the increased prevalence of BrS in the Thai population.

Keywords: Non-coding variant, SCN5A, hiPSC-CMs

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THE ROLE OF ROTORS IN ATRIAL ARRHYTHMOGENIC REMODELING: AN IN VITRO STUDY ON HIPSC-DERIVED ATRIAL CARDIOMYOCYTES

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Despite atrial fibrillation (AF) being a common, clinically important, and economically significant public health problem, the initiation mechanisms of atrial remodeling during AF are still unclear. Furthermore, few human atria-specific biological models allow to investigate these mechanisms in vitro. We aim to generate and

characterize human induced pluripotent stem cell (hiPSC)-derived atrial cardiomyocytes to develop a novel human in vitro model of AF able to reproduce and evaluate the arrhythmia initiation and maintenance mechanisms. Two hiPSC lines were differentiated into atrial cardiomyocytes by adding 1 µM retinoic acid (RA) from day 3 to day 8 and compared to the ventricular differentiation. On day 20, cells were seeded into plates with different sizes: 0.32 cm2 and 9.6 cm2, respectively, preventing or allowing rotors formation. After 10 days we performed qPCR and optical mapping experiments to evaluate the remodeling cells undergo during AF initiation and maintenance. The molecular analysis showed higher atrial markers expression in the RA-treated population. Mechanical and electrophysiological properties were analyzed by nanoindentation and patch clamp techniques at 37°C. RA-treated cells had significantly shorter action potential duration at 90% repolarization, 182.3.5 ± 68 ms vs.371.6 ± 82 ms, and higher spontaneous beating frequency, 1.5 Hz vs. 1 Hz (n = 14, ± SD). Optical mapping experiments allowed evaluation of the activation rate depending on the size of the dish: in larger wells activation rate was 1.6 ± 1.9 Hz compared with 0.9 ± 0.5 Hz in smaller wells. The faster activation rate in larger wells was associated with a higher number of reentrant wavefronts: 11.0 ± 8.2 reentries/cm2 vs 0.9 ± 0.5 reentries/cm2, suggesting an AF-like phenotype in the larger wells only. Furthermore, qPCR studies showed that spontaneous initiation of rotors was associated with expression remodeling of the channels SCN5A, KCNJ2, GJA5, ATP2A2 and RYR2. We conclude that AF initiation in hiPSC-derived atrial cardiomyocytes can recapitulate key features of the electrical remodeling observed in atrial myocardium from patients with AF. Such a model, based on human cells and allowing high-throughput approaches, will provide a powerful tool to investigate the initiation of fibrillatory activity and its maintenance.

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Keywords: atrial fibrillation, atrial-like cardiomyocytes, electrophysiology

TOPIC: EPITHELIAL INCLUDES SKIN GUT LUNG

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FIRST MULTI-TISSUE COMPARISON OF SIX CANINE ORGANOID CELL LINES

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Recent legislation, including the FDA Modernization Act 2.0, allows in vitro models to replace live animal testing when seeking approval of therapeutic drug candidates. The longer lifespan of dogs over mice predisposes dogs to develop analogous spontaneous chronic diseases to humans, including diabetes mellitus, inflammatory bowel disease, congestive heart failure, cancers, and cognitive dysfunction. Previously, the use of canine organoids for drug testing and disease modeling was limited to organoids from the intestine, liver, kidney, and urinary bladder. Here, we report the development of additional novel organoid lines from healthy canine endometrium, lung, and pancreas, with the aim to comprehensively compare tissue/organoid pairs from two genetically related healthy canines. Tissues were minced, washed, plated in Matrigel, and cultured using our published media. After expansion, samples were fixed, sequenced, and cryopreserved. Transcriptomic expression comparing all six organoid cell lines identified upregulated tissue-specific genes for the endometrium (SRY-box transcription factor 17; SOX17, Distal-Less Homeobox 6; DLX6), lung (NK2 homeobox 1; NKX2-1, Leucine Rich Repeat Containing 15; LRRC15), pancreas (NK6 homeobox 1; NKX6-1, Maltase-Glucoamylase 2; MGAM2), kidney (SIM bHLH transcription factor 1; SIM1, POU Class 3 Homeobox 3; POU3F3), bladder (Uroplakin 3A; UPK3A, Uroplakin 2; UPK2), and liver (Tripartite Motif Containing 71; TRIM71, NLR Family Pyrin Domain Containing 6; NLRP6). Immunofluorescence staining in tissues and organoids further confirmed tissue-type and localization of protein expression. Importantly, between 78-82% of all expressed genes overlapped between organoid/tissue pairs across all tissues. Taken together, this study constitutes the most comprehensive multi-tissue comparison in canine organoid cell lines to date. Applications range from using urinary bladder organoids to assess the carcino-

genic effects of environmental toxicants, screening drug candidates for liver toxicity, and studying reproductive toxicology using endometrial organoids. Overall, canine organoids can accelerate therapeutic drug screening and broaden the availability of in vitro models from different species, which may decrease the need for in vivo experiments.

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Keywords: canine, organoids, reverse translational medicine

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MESENCHYMAL STEM CELL-DERIVED CONDITIONED MEDIA DOWNREGULATE THE PROTEIN EXPRESSION OF INFLAMMATION-INDUCED CHEMOKINES IN A HUMAN EX VIVO COLONOID MODEL

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Mesenchymal stem cell-derived conditioned media (MSC-CM) have been reported to possess anti-inflammatory and anti-fibrotic properties in in vitro and in vivo experimental models of Idiopathic Pulmonary Fibrosis. In this study, we investigated the effect of MSC-CM on the chemokine responses in human epithelial crypt-derived Colonoids treated with pro-inflammatory cytokines. Human epithelial crypts were isolated from colonic endoscopic biopsies of three different healthy individuals, who had no endoscopic pathological findings. Using a commercially available kit, crypts were differentiated into Colonoids, and then treated with: a) DMEM (control), b) MSC-CM, c) the inflammatory cytokines IL-1α (5ng/ml) and TNF-α (50ng/ml) (2C) and d) MSC-CM and 2C for 12 and 24 hours. At the end of the incubation periods, the mRNA and protein expression of the pro-inflammatory chemokines CXCL10, CXCL11 and CCL20 was examined by reverse transcription RTq-PCR and ELISA, respectively.Untreated Colonoids did not express any of the studied chemokines. Treatment with 2C resulted in a statistically significant upregulation of all studied chemokines both in mRNA (CXCL10: 6.05-fold ± 0.91, p< 0.01; CXCL11: 14.63-fold ± 1.32, p< 0.05; CCL20: 116.8-fold ± 37.0, p< 0.05) and protein levels (CXCL10: 98.28 pg/ml ± 14.06, p< 0.0001; CXCL11: 80.27 pg/ml ± 10.24, p< 0.0001; CCL20: 5,014 pg/ml ± 214.2, p< 0.0001). Treatment of 2C-stimulated Colonoids with MSC-CM led to a statistically significant downregulation of the CXCL10 mRNA (9.63-fold ± 1.77, p< 0.05), but not of CXCL11 or CCL20 mRNA (CXCL11: 11.18-fold ± 1.23, p< 0.05; CCL20: 275.7-fold ± 91.84, p< 0.05). Nonetheless, on the protein level, MSC-CM treatment resulted in a statistically significant reduction of all studied chemokines (CXCL10: 63.12 pg/ml ± 8.27, p< 0.05; CXCL11: 36.01 pg/ml ± 1.59, p< 0.001; CCL20: 3,574 pg/ml ± 481.5, p< 0.01). In conclusion, MSC-CM may have an anti-inflammatory effect, as it strongly downregulated the protein expression of the CXCL10, CXCL11 and CCL20 chemokines, which are known to be involved in intestinal inflammation. Further research is required in order to characterize the MSC-CM's proteomic profile and to elucidate its mechanisms of action.

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Keywords: Mesenchymal stem cells, Conditioned Media, Colonoids

TOPIC: GERMLINE AND EARLY EMBRYO

2049

A 3D SANDWICH CO-CULTURE SYSTEM WITH HPPSCS AND HUVECS SUPPORTS MOUSE EMBRYO DEVELOPMENT FROM E3.5 TO E7.5 IN VITRO

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The implantation of mouse embryos involves intricate signal regulation and key morphological events and is influenced by endometrial secretory factors. To date, a variety of methods for ex utero culture systems have been established, but there remain limitations in terms of in vitro culture platforms used prior to the implantation of mouse embryos, and the ability of mouse blastocysts to develop normally in vitro has not been reported. Here, in order to establish culture conditions for mouse embryos from embryonic day (E) 3.5 to E 7.5 in vitro, we established a three-dimensional (3D) "sandwich" culture system with in vitro culture medium (IVCM) and human placenta perivascular stem cells (hPPSCs) and human umbilical vein endothelial cells (hUVECs), as supportive cells, which were seeded into the bottom layer of Matrigel to boost embryo development. Embryos in the IVCM + Cells group showed higher development rates and greatest diameters at each stage than those in the IVCM group. Embryos in the IVCM + Cells group cultured to E5.5 resembled natural egg cylinders in morphology and expressed specific embryonic cell markers, including Oct4 and Nanog, which were features similar to embryos developed in vivo. After transplantation, the embryos could be re-implanted in the internal uterus and continue to develop to a certain stage. Therefore, the 3D in vitro culture system enabled the development of embryos from E 3.5 to E 7.5, and the vascularization microenvironment constructed by Matrigel, hPPSCs, and hUVECs significantly promoted the development of implanted embryos. This system allowed us to further study the physical and molecular mechanisms of embryo implantation in vitro.

Keywords: hPPSCs and hUVECs, 3D "Sandwich" Co-culture System, Embryo Development, E3.5-E7.5

TOPIC: HEMATOPOIETIC, IMMUNE, AND ENDOTHELIAL

2050

ATF4 PROMOTES ERYTHROID DIFFERENTIATION BY REGULATING RIBOSOME ASSEMBLY VIA FACILITATING THE TRANSCRIPTION OF RPS19BP1

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Erythroid cell production is driven by numerous transcription factors. Investigations into the signals and regulatory networks involved in erythropoiesis have provided significant insights into the biology of erythroid cells and clinical therapy. Previous work reported by our group and others have demonstrated that Atf4, a basic region-leucine zipper transcription factor, plays pivotal roles in fetal liver hematopoiesis, hematopoietic stem cell (HSC) maintenance, bone formation and tumorigenesis. Here, we demonstrated that loss of Atf4 in the hematopoietic system resulted in severe anemia. Atf4 deficiency led to cell cycle arrest in erythroid progenitor cells (EPCs) and impaired their CFU-E and BFU-E colony-forming ability. Single cell RNA-seq (scRNA-seq) analysis of bone marrow (BM) Lin-cKit+ cells confirmed the defects of erythropoiesis after Atf4 depletion. GO analysis of the downregulated genes from RNA-seq, ATAC-seq and H3K4me3 Cut&Tag data of Atf4-deleted MEP cells revealed that several terms related to erythrocyte differentiation and translation process were diminished. O-propargyl-puromycin (OP-Puro) incorporation and SUnSET assays further confirmed the global protein synthesis defect in Atf4-deficient MEP cells. 5-FU administration markedly accelerated the death of the Atf4-deficient mice, showing severe protein synthesis defects of cKit+ cells, thereby causing hematopoietic failure. Subsequent analysis of scRNA-Seq data by using pyScenic predicted Rps19bp1 as the top target gene of Atf4. Luciferase reporter assays validated that Atf4 was a transcriptional activator of Rps19bp1. Downregulation of Rps19bp1 caused by Atf4 deletion led to decreased assembles of 40S proteins, such as RPS3, RPS6 and RPS19. The protein synthesis defect and impaired erythropoiesis were rescued upon Rps19bp1 overexpression in Atf4 knockdown MEL cell line or BM cKit+ cells from Atf4-deficient mice. Taken together, ATF4 promotes erythroid differentiation by regulating ribosome assembly via facilitating the transcription of Rps19bp1. Our current study uncovers an essential role of ATF4/Rps19bp1 in erythropoiesis.

Keywords: ATF4, RPS19BP1, ERYTHROPOIESIS

2051

AML-DERIVED EXOSOMES PROMOTE LEUKEMOGENESIS BY AUTOCRINING MIR-221-3P

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Acute myeloid leukemia (AML) is a malignant disorder characterized with abnormal growth and differentiation of hematopoietic stem cells (HSCs). It has been elucidated that AML-derived exosomes can reprogram bone marrow microenvironment to suppress HSCs function via miRNAs. However, their autocrinal effect remains largely unknown. Here we found that AML-derived exosomes promoted AML cell proliferation and viability. By MiR-seq analysis, we found high expression of miR-221-3p in the AML-derived exosomes, which was validated by qPCR. Then we overexpressed miR-221-3p in HEK293T cells and isolated the exosomes containing high level of miR-221-3p (Exo221) from the cell supernatant by differential ultracentrifugation (dUC). Through co-culturing with AML cells, Exo221 rescued the phenotypes under the treatment of GW4869 (an exosome biogenesis inhibitor), suggesting the secreted exosomes of AML play a role through the delivery of miR-221-3p. Moreover, knockdown of miR-221-3p in AML cells inhibited their proliferation and increased apoptosis, which could be rescued by Exo221. We further validated that miR-221-3p knockdown improved the survival of AML mice. In contrast, overexpression of miR-221-3p in AML cells displayed the opposite phenotypes. To elucidate the mechanisms, we analyzed the differences of gene expressions between WT, miR-221-3p knockdown and miR-221-3p overexpressed AML cells. Combined with miRNA target prediction software, we found several potential targets for miR-221-3p and observed the upregulation of Fzd9 in the miR-221-3p knockdown cells, which was confirmed by luciferase reporter assays. Furthermore, depletion of Fzd9 in AML cells relieved the growth inhibition and apoptosis of AML cells caused by miR-221-3p knockdown. In conclusion, we demonstrate that AML-derived exosomes can promote leukemogenesis through miR-221-3p.

Keywords: AML, Exosome, miRNA

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IDENTIFICATION OF CANCER STEM CELLS IN RICHTERS TRANSFORMATION VIA SINGLE-CELL RNA SEQUENCING LEVERAGING GENE EXPRESSION SIGNATURES AND RNA VELOCITY

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Richter's transformation (RT) is an aggressive lymphoma that occurs upon progression from chronic lymphocytic leukemia (CLL). Transformation has been associated with genetic aberrations in the CLL phase involving TP53, CDKN2A, MYC, and NOTCH1, with recent novel RT genes; CCND3, TNFAIP3, CIITA, EP300, and NRAS being added. However, although recent application of single-cell RNA/DNA-Sequencing has allowed an unparalleled insight into the dormant nature of RT in matched RT and CLL-phase biopsies, a complete assessment of the functional consequences of these changes including the role of the tumor microenvironment (TME) has yet to be determined. Here, we conducted scRNA-Seq in 4 RT lymph node biopsies, retaining >35,000 cells after data pre-processing. Post UMAP clustering we derived the cell types using standard cell type markers, identifying in each RT biopsy at least 1 cluster pertaining to an embryonic stem cell signature via single cell gene set enrichment analysis (ssGSEA) (Figure 1A&B). Grouped gene expression analysis across all 4 RT biopsies identified a shared set of 1656 significant genes (, < 0.05 Q value, >1 Log2 FC; Figure 1C) which upon Gene Ontology analysis unearthed pathways associated with; chromatin segmentation, cell cycle, and focal adhesion, amongst others (Figure 1D). Initial RNA Velocity dynamical modelling of the RT CSCs and main RT clone revealed not only a distinct PAGA cellular trajectory, but also via Latent Time derivation of spliced and unspliced RNA molecules that the earliest cells transcriptionally differentiate from the CSCs to the main RT clone (Figure 1E). We observed a similar finding using previously published scRNA-Seq data of our murine RT mouse model (Eμ-TCL1AKT-C; Figure 1F). Initial flow cytometry observations in murine RT could validate the existence of an expanded Hoechst 33342 negative population (~10%, Figure 1G), including the >80% enrichment of CD321 (F11R/JAM1) in Hoechst negative cells, a significant increase in comparison to the Hoechst positive cells of ~40% (P = 0.0223; Figure 1H). We show here the identification of CSCs in RT for the first time, and in future work we will be looking to validate the CSC phenotype via Colony Forming Unit assay and Hyperion CYTOF imagine in vitro and adoptive transfer of CSCs in NSG mice in vivo.

Funding Source: Jose Carreras (Project Nr. DJCLS 07R/2022)

Keywords: Richters Transformation, scRNA-Seq, Cancer Stem Cells

2053

TARGETED DISRUPTION OF BCL11A BINDING DOMAIN FOR THERAPEUTIC REACTIVATION OF FETAL HEMOGLOBIN IN BETA-HEMOGLOBINOPATHIES

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Disrupting the production or function of the long isoform of the fetal globin repressor BCL11A-XL is a strategy for treating of b-hemoglobinopathies. Multiple clinical trials are focussed on disrupting the erythroid-specific BCL11A enhancer or its binding site in the HBG promoters to reduce its expression or its binding to the fetal globin gene promoters respectively. The complete knockout of BCL11A yields a greater induction of fetal globin (HbF) but affects haematopoietic development. Several inherited mutations in BCL11A result in neuronal disorders together with elevated HbF. In particular missense mutations in the BCL11A-XL Zinc Finger (ZnF) domain exhibit levels of HbF equivalent to those observed with total BCL11A loss although importantly other he-

matological parameters are normal. This suggests that these modifications may have therapeutic potential. We disrupted the BCL11A-XL ZnF domains using CRISPR/Cas9 and observed high HbF induction (~35% HbF vs 12% control) albeit with some defects in Hematopoietic Stem and Progenitor Cells (HSPC) engraftment and erythroid maturation. But we found that base substitutions at the key residues of these ZnF-domains involved in DNA recognition effectively upregulated HbF(60% ZnF4 vs 10% Control) without fewer side effects. A ZF4 mutation resulted in very few changes to erythroid gene expression, no impact on erythroid maturation in vitro (60% ZnF4 vs 10% Control), but some reduction in HSPC engraftment in vivo (60% ZnF4 vs 85% Control). A modification of ZF6 showed a potential elevation of HbF levels, that was unexpected as this region does not directly contact DNA at the HBG promoter. Our strategy of specifically altering residues of BCL11A-XL will potentially identify residues critical for fetal globin repression but less important in the other biological functions of BCL11A-XL.

Keywords: BCL11A, Base Editing, Zinc Finger domain

Abstract Withdrawn

2054

IPSC-DERIVED MACROPHAGES AS A PREVENTATIVE TREATMENT FOR KNEE POST-TRAUMATIC OSTEOARTHRITIS

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Post-traumatic osteoarthritis (PTOA) is a degenerative cartilage disease that occurs in relatively young patients following injury like ACL tear, causes pain, disability, and economic burden. The iPSC-derived macrophages have the potential to prevent the inflammatory insult to the joint and PTOA induction. Non-surgical model of PTOA using a noninvasive ACL tear mimics the human condition as close as possible. Immunogenicity poses challenges for development of human stem cell therapies in animal models. To study the potential iPSC-derived treatments on PTOA, immunocompromised Nude and wild type Sprague Dawley (SD) rats were used to study immune reactivity to human iPSC-derived cells. Since the joint is considered immune privileged site, we hypothesized that SD rats would not have a greater immune reaction compared to Nude rats when human iPSC-derived cells were injected into the injured knee. Human iPSCs were differentiated to macrophages using an optimized protocol, characterized via flow cytometry, and confirmed functionality through phagocytic assay. SD and Nude rats went noninvasive ACL injury and were injected with human cells 2 weeks later. Serum was collected during the day of injury, injection day, day 3, 7 10 and 14 post injection. We observed no significant difference in IgM levels and CD8+ cells between Nude and SD rats, demonstrating no immune reaction in immunocompetent rats after human cell injection to the joint. To further characterize the PTOA model, SD rats underwent non-invasive ACL injury and were followed for 16 weeks. Biobehavioral tests of rats with knee injury demonstrated an increase in left and right stride length, a decrease in sway width and a significant increase in left paw angle and a trend of increasing right paw angle. Rats were sacrificed after 18, 20 or 25 weeks, the knee joints were processed for histology and used for OARSI score, showing development of knee OA as early as week 18 post injury. This confirmed the feasibility of noon-invasive ACL tear to model PTOA and a feasibility of human stem cell therapy development. Future steps of this study will include introducing

differentiated iPSC-derived macrophages into an ACL injured rat model to modify the inflammatory environment and hinder OA onset, progression, and associated pain.

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Keywords: PTOA, iPSC, macrophages

TOPIC: KIDNEY

2055

THE (PRO)RENIN RECEPTOR IS INVOLVED IN KIDNEY ORGANOID DEVELOPMENT

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The (pro)renin receptor [(P)RR], a receptor for prorenin and renin, is widely distributed in the body, including the kidneys. Studies in (P)RR knockout mouse models indicate that (P)RR ablation can be detrimental to the normal development and function of the kidneys. However, the lack of human models hinders investigation into the role of (P)RR in human kidney development. The advent of human induced pluripotent stem cell (iPSC)–derived kidney organoids resembling the first and early second trimester of human fetal kidneys provides a tool to study the role of (P)RR in human kidney development. To investigate the effects of (P)RR knockdown on kidney organoid development, human iPSC–derived kidney organoids were generated according to a previously described protocol. We silenced the (P)RR by introducing (P)RR antisense oligonucleotides (ASOs) by electroporation at the stage of iPSCs and nephron progenitor cells induction, respectively. The development of kidney organoids was monitored morphologically and through protein expression analysis of nephron markers by immunohistochemistry staining which was quantified by Image J. After silencing the (P)RR at the initial stage of differentiation, the size of iPSCs-derived organoids was substantially smaller, but the size of tubular structures was bigger. We observed a decrease by 50% in both WT1 (glomerular cell marker) and PDGFRα (stromal cell marker) expressions in (P)RR-knockdown organoids, a 4-fold and 2-fold increase in expressions of fibrogenic cell markers α-SMA and COL1A1 respectively, and no change in Villin1 (proximal tubular cell marker) and Cadherin-1 (distal tubular cell marker) expression. Additionally, (P)RR knockdown at the stage of nephron progenitor cells induction decreased the expressions of Villin1 and Cadherin-1 in organoids by 50% and 60% separately, while it induced a 4-, 3-, 2- and 2-fold increase in the expressions of CD31 (endothelial cell marker), PDGFRα, α-SMA and COL1A1 respectively. This study shows that (P)RR silencing at the initial stage of differentiation or at the stage of nephron progenitor cells induction impaired the normal development of kidney organoids. Its absence caused a shift into the direction of fibrogenic cells. This study provides new insights into the understanding the role of (P)RR in human kidney development.

Keywords: (pro)renin receptor, human iPSC – derived kidney organoids, human kidney development

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RAAS-DEFICIENT ORGANOIDS REVEAL THAT DELAYED ANGIOGENESIS IS THE PATHOMECHANISM UNDERLYING AUTOSOMAL RECESSIVE RENAL TUBULAR DYSPLASIA

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The renin–angiotensin–aldosterone system (RAAS) regulates blood volume and systemic vascular resistance in humans. Autosomal Recessive Renal Tubular Dysgenesis (AR-RTD) is a fatal genetic disorder of kidney development resulting from pathogenic variants in any of the four genes involved in RAAS (REN, ACE, AGT, AGTR1), characterized by absent or maldeveloped proximal tubules (PT). A similar RTD phenotype is observed as a result of in utero exposure to ACE inhibitors or angiotensin receptor blockers. At present, it is still unclear whether the PT pathology in RTD is caused by a vascular/hemodynamic effect, secondary to the RAAS disruption, or rather by a direct (autonomous) contribution of RAAS to PT development.Human induced Pluripotent Stem Cell (hiPSC)-derived 3D kidney organoids mimic kidney development without effective circulation, providing a system to study the effects of RAAS mutations on PT. To uncover the pathomechanism of AR-RTD we differentiated ACE and AGTR1 deficient pluripotent stem cells and reprogrammed AR-RTD patient cells into kidney organoids. Marker analyses confirmed that all mutant and control organoids generated PT in room air (21% O2) or under hypoxic conditions (2% O2). Thus, PT development did not require RAAS nor high oxygen levels. To ask whether in vitro culture conditions rescued PT development, organoids were implanted under the kidney capsule of immunodeficient mice. AGTR1-/- iPSC organoids were chosen for transplantation, to circumvent confounding effects of host RAAS. When AGTR1-/- and control iPSC-derived organoids were transplanted at a fully developed stage (day 24), or when control organoids were transplanted at the renal vesicle stage (day 14), PT-containing differentiated organoids formed. By contrast, AGTR1-/- organoids transplanted at day 14 failed to engraft due to low levels of pro-angiogenic VEGFA compared to controls. Pre-transplant culture in hypoxia (2% O2) was sufficient to increase VEGFA production and rescue AGTR1-/- organoid engraftment and differentiation. Thus, PT dysgenesis in AR-RTD is a non-autonomous consequence of a developmental delay in

VEGF-A induction, providing a mechanistic link between RAAS and angiogenesis during kidney development.

Keywords: Kidney development, Kidney Organoids, Renal Tubular Dysgenesis

TOPIC: LIVER

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BETA-CATENIN COUPLES SELF-RENEWAL, INDUCTION AND EPITHELIAL MORPHOGENESIS AND PATTERNING AT THE OUTSET OF MAMMALIAN NEPHROGENESIS

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Wnt/β-catenin signaling is a highly conserved molecular pathway that plays a crucial role in many stem/progenitor systems. β-catenin, the main Wnt pathway effector, has two pools within a cell which are required for cell-cell adhesion at the membrane and transcriptional functions in the nucleus. However, how β-catenin mediates both roles remain unknown. The tightly controlled, well characterized system of nephrogenesis is an ideal model to decouple the roles of β-catenin at the membrane and in the nucleus. In kidney development, a delicate balance of NPC self-renewal and differentiation is required for the mesenchymal to epithelial transition (MET) in nephrogenesis and is driven by Wnt/β-catenin signaling. Given an ability to isolate and manipulate large numbers of NPCs in tissue culture, we can dissect the dual nature of β-catenin as a transcriptional activator and component of a cell membrane complex in adhesion. We have pioneered a method using CRISPR/Cas9 gene editing and lipid based mRNA lipofection enabling us to rapidly remove β-catenin,α-catenin and simultaneous cadherin and Tcf/Lef factor gene activity in primary NPCs. We have characterized the effects of modulating β-catenin and integrated RNA-seq results from β-catenin's removal with mouse ChIP-Seq and mouse Single Cell RNA -seq data. Functional analysis of β-catenin removal provides strong evidence for β-catenin regulation of NPC proliferation, independent of a direct Lef/Tcf associated transcriptional program. Our data shows that β-catenin mediates aggregation, the first step in MET, by stabilizing interactions in catenin-cadherin cell adhesion complexes at the membrane by stabilizing cell -cell contacts through interactions with α-catenin and cadherins. Moreover, the findings support a direct transcriptional role for Lef/Tcf/β-catenin complexes in driving differentiation programs, associated with the mesenchymal to epithelial progression of differentiating mammalian NPCs. Our findings have broad significance by gaining a deeper mechanistic understanding of Wnt signaling as Wnt plays a key role directing stem cell/progenitor populations in many organ systems and deregulation of these interactions drives a number of cancers.

Funding Source: F31DK122777-01

Keywords: Wnt Signaling, Kidney Development, Nephron Progenitor

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THE ROLE OF MAT1A MUTATION IN BILIARY ATRESIA DISEASE INITIATION AND PROGRESSION: AN IPSC STUDY

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The pathobiological mechanisms underlying disease initiation and progression in biliary atresia (BA) remain unclear. Limited studies have addressed whether liver deterioration despite surgical treatment represent a primary event affecting hepatocytes or reactive changes secondary to bile duct injuries. A heterozygous de novo G to A mutation in the exon 8 of the MAT1A gene in a BA patient's peripheral blood DNA was identified. This patient has a poor response to Kasai portoenterostomy, requiring liver transplantation shortly after surgery. This G>A mutation created a new splice acceptor site within the exon 8. The aberrant splicing from exon 7 to this new acceptor site within exon 8 generated a mutant transcript with part of the exon 8 deletion, a frameshift leading to a new stretch of 14 amino acids and a stop codon. MAT1A encodes two liver-specific methionine adenosyltransferase isozymes (MATI/III), and the mutant enzymes retained only 14.4% of the wild type enzyme activity. iPSC (BA638C) was generated from this patient's blood and was differentiated into hepatocytes. Morphological/flow cytometric/RT-qPCR analysis was performed to address if MAT1A deficiency impaired hepatocyte development/functioning. Control iPSC (HKUPS-001) derived from a normal person was included for comparison. At iPSC stage, BA638C's proliferation rate was much lower than that of HKUPS-001. After hepatocyte induction, both HKUPS-001 and BA638C iPSCs developed into hepatocyte-like cells showing typical hepatocyte morphology on day 20, expressing high level of hepatic markers. However, by day 22, BA638C-derived hepatocytes gradually lost the hepatocyte features, developed into flat and spindle-shape fibroblast-like cells. Furthermore, BA638C-derived cells expressed not only low level of hepatic makers, but also elevated levels of the epithelial-mesenchymal-transition (EMT) related markers (SMA and FSP1). In conclusion, MAT1A mutation resulted in decreased activity of MATI/III. The disrupted methionine metabolism could lead to reduced iPSC proliferation, a premature loss of differentiated hepatocyte morphology and function, and abnormal mesenchymal transformation, contributing respectively to disease initiation and progression of BA.

Funding Source: Theme-based Research Scheme (T12-712/21-R) RGC Hong Kong SAR Government, Hong Kong SAR, China.

Keywords: Biliary atresia, MAT1A, aberrant splicing

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HIGH-THROUGHPUT CRISPR ACTIVATION SCREENING WITH MOUSE EMBRYONIC HEPATOCYTES IDENTIFIES ADULT HEPATIC METABOLIC GENE ACTIVATOR AND EMBRYONIC GENE SUPPRESSOR

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A deepened understanding of lineage specification mechanisms enabled us to induce various types of organoids from pluripotent stem cells. However, most of the current organoids are still functionally immature and resemble embryonic states, which is partly attributable to our incomplete knowledge of organ maturation mechanisms during the late phase of development. Here we addressed the mouse hepatocyte maturation mechanisms after lineage commitment. Time course RNA-sequencing analysis between embryonic day 11 to adult purified hepatocytes identified about 60 developmentally late-onset transcription regulators (DLTR). We set up a CRISPR-activation and single-cell RNA-seq combined high throughput experimental pipeline, which enabled DLTR-wide perturbation screening with transcriptomic phenotyping on the primary embryonic hepatocytes. Our analysis identified transcription regulators which can boost the expression of a subset of drug-metabolizing enzymes or suppress embryonic genes. Further optimization of signaling molecules additively activated a broader spectrum of metabolic gene expression close to the adult level. Therefore, our study will pave the way toward understanding of transcriptional control of hepatocyte maturation and induction of functionally mature liver organoids from pluripotent stem cells.

Keywords: maturation, CRISPR-activation, transcriptional regulation

2059

MODELING SEPSIS-ASSOCIATED LIVER DYSFUNCTION WITH HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED LIVER ORGANOIDS

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Sepsis associated-liver dysfunction has served as the important prognosis and mortality predictor of sepsis. It is caused by the overwhelming inflammatory response induced by Kupffer cells (KC). Despite intense studies on rodent and nonhuman primate sepsis models, sepsis-targeted drugs or therapies for humans are still not available due to the lack of human models. In this study, we aim to generate a human induced pluripotent stem cell (hiPSC)-based model to mimic the pathological events in sepsis-associated liver dysfunction. For this purpose, we first differentiated hiPSC into erythro-myeloid progenitors (EMP), the embryonic origin of KC. By co-culturing hiPSC-derived EMP (hiPSC-EMP) with liver organoid (LO), generated from hiPSC-derived hepatic endoderm, endothelial progenitors, and mesenchymal progenitors, the proliferation and differentiation of hiPSC-EMP were promoted significantly compared with the cells without co-culture. Furthermore, the supplement of macrophage colony-stimulating factor (M-CSF) induced the directed differentiation of hiPSC-EMP to KC in the co-culture system along with LO maturation. Following liposaccharides (LPS) and interferon-gamma (IFNγ) treatment, we noticed a strong inflammatory response in the LO-KC co-culture system, resulting in significant cell necrosis and decreased hepatic functions. We also observed a significant decrease of endothelial marker CD31 upon stimulation. Furthermore, the RNA sequencing results showed that stimulated organoids shared similar pathological events with septic liver in mice model. More importantly, the impaired hepatic functions could undergo self-recovery after the LPS/IFNγ were removed. Our results showed that the LO-KC co-culture system could recapitulate the onset and resolution of sepsis associated-liver dysfunction, thus have promising prospects for exploring novel therapeutic targets.

Funding Source: This work was supported by the JST Research Centre Network for Realization of Regenerative Medicine (No. 17bm0304002h0105), Grant-in-Aid for Research Activity Start-up (No. 20K22946).

Keywords: Sepsis associated-liver dysfunction, Kupffer cells, Human iPSC

2060

GENERATION OF EXPANDABLE QUIESCENT HEPATIC STELLATE CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS FOR MODELING HUMAN LIVER ORGANOGENESIS

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The biological applications of existing liver organoids have been restricted primarily owing to the lack of relevant cell types like quiescent hepatic stellate cells (qHSCs). Although several protocols have been established to induce qHSCs from human induced pluripotent stem cells (hiPSCs), those low-purity hiPSC-derived cells either exhibited the characteristics of active HSCs or did not have the capacity to expand. To overcome the difficulties in previous research, this study attempted to generate pure qHSCs from hiPSCs (hiPSC-qHSCs) while maintaining their quiescent phenotype during expansion. According to our results, hiPSC-qHSCs reached an extremely high purity without expressing PDGFRα, a surface marker of active HSCs, and displayed signatures of HSCs in a healthy liver. Functional analysis showed that hiPSC-qHSCs can store Vitamin A in lipid droplets and become active after TGFβ stimulation. Additionally, hiPSC-qHSCs can be passaged at least 6 times with an increase of 240 folds while keeping their characteristics and functions. To reveal the role of hiPSC-qHSC in a human liver model, we constructed a functional liver organoid (LO) in a low attachment culture system by co-culturing hiPSC-qHSCs with hiPSC-derived hepatic endoderm and endothelial progenitors. Comparing LOs with or without hiPSC-qHSCs, we found that hiPSC-qHSCs highly promoted the vascularization and maturation of LOs both in vitro and in vivo. Overall, this protocol enabled us to robustly generate qHSCs for the development of vascularized LOs and unveil previous unobservable aspects of human liver development.

Funding Source: This work was supported by the JST Research Centre Network for Realization of Regenerative Medicine (No. 17bm0304002h0105), Grant-in-Aid for Research Activity Start-up (No. 20K22946).

Keywords: Quiescent hepatic stellate cells, Human induced pluripotent stem cells, Liver organoid

TOPIC: MESENCHYMAL STROMAL CELLS, ADIPOSE, AND CONNECTIVE TISSUE

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RUXOLITINIB IMPROVES HEMATOPOIETIC REGENERATION VIA RETRIEVING MESENCHYMAL STROMAL CELL NICHE FUNCTION IN AGVHD

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Acute graft-versus-host disease (aGVHD) is a severe complication of allogeneic hematopoietic stem cell transplantation. Hematopoietic dysfunction, which may be caused by niche impairment, is a long-standing clinical problem. However, how the bone marrow (BM) niche is damaged in aGVHD hosts is poorly defined. To comprehensively address this question, we employed a haplo-MHC-matched transplantation aGVHD mouse model and performed single-cell RNA-sequencing of non-hematopoietic BM cells. Transcriptional analysis showed that BM mesenchymal stromal cells (BMSCs) were severely affected with reduction of cell ratio, obvious abnormalities of metabolism, compromised differentiation potential and defective hematopoietic supportive function, which were validated by in vitro functional assays. Ruxolitinib, a selective JAK1/2 inhibitor, could ameliorate aGVHD when administrated topically and systemically with BMSC function recovery and increase its crosstalk with hematopoietic stem/progenitor cells via mitochondrial transfer. The observations from the mouse model were validated by patients' specimens. Overall, ruxolitinib can directly retrieve BMSC function and in turn improve hematopoietic dysfunction caused by aGVHD

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Keywords: aGVHD, Ruxolitinib, Mesenchymal stromal cell

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ANTIBACTERIAL ACTIVITY OF HUMAN BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS AGAINST STAPHYLOCOCCUS AUREUS IN DIABETIC MICROENVIRONMENT

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Diabetic foot ulcer (DFU) is one of the serious complications of diabetes mellitus. The most common microbe isolated from DFU wound is staphylococcus aureus (S. aureus). Wound healing of foot ulcers in diabetic patients thus remains a real therapeutic challenge. In addition to their regenerative capacities, mesenchymal stem cells (MSCs) were shown to have antimicrobial activities against various microbes. These properties are mediated by secretion of antimicrobial peptides (AMPs). In this project, we investigate the capacity of bone marrow MSCs and their conditioned medium (CM) to ameliorate infection in the diabetic microenvironment. BM-MSCs were cultured in serum collected from normal subjects and diabetic patients over six days, and their antimicrobial effect against S. aureus was evaluated. Our results demonstrate that both MSCs and their CM caused marked inhibition of S. aureus bacterial growth in both normal and diabetic conditions. MSCs cultured in diabetic serum showed no significant difference in the expression of AMPs genes beta-defensins, cathelicidin and hepcidin compared with normal. Bacterial colony forming units (CFU) were markedly reduced when cultured with MSCs from both normal and diabetic group, but inhibition was observed only in the normal CM but not the diabetic CM. Bacterial biofilm formation was also significantly inhibited when cultured with MSCs from both groups, and when bacteria were cultured in normal CM, but not diabetic CM. Proteomics analysis were undertaken to confirm these findings. Our data support that BM-MSCs have direct antimicrobial activity against S. aureus in diabetic microenvironment.

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Keywords: MSCs, antibacterial peptide, s aureus.

TOPIC: NEURAL

2063

CYTOSKELETAL REARRANGEMENT IN UBE2A-KO IPSC DERIVATIVES PROMOTES ENHANCED CELL SPREADING AND MIGRATION

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The Nascimento syndrome, or X-linked intellectual developmental disorder type Nascimento, was first described in 2006. It is characterized by mental retardation, developmental delay, skin anomalies, and seizures. The Nascimento syndrome is caused by loss-of-function mutations in the UBE2A gene, which encodes the E2 enzyme UBE2A related to the ubiquitin-proteasome system. The role of UBE2A in normal brain development and the molecular mechanisms underlying the Nascimento syndrome are

still poorly understood. At the same time, neurodevelopmental disorders are often associated with defects in the cytoskeleton and abnormal neuronal migration. Thus, our study focused on cytoskeletal alterations in cells with UBE2A knockout (KO). We derived UBE2A-KO iPSC clones from the healthy male iPSCs using CRISPR/Cas9 genome editing. The pluripotent state of iPSC UBE2A-KO lines was confirmed by expressions of stem cell markers, typical stem cell morphology and the ability to differentiate into three germ layers. The iPSCs were differentiated into fibroblast-like cells (CD105+, CD73+, CD90+) and glial cells (s100β+). Using live-cell imaging, we demonstrated that UBE2A-KO iPSC derivatives were characterized by enlarged focal adhesions, enhanced cell spreading and polarization dynamics. The random cell migration assay showed a dramatic increase of migration velocity of UBE2A-KO glial cells, compared to WT glial cells ($p < 0.0001$). In contrast, the migration velocity of UBE2A-KO fibroblast-like cells was slightly increased ($p = 0.04$). Similarly, in the wound-healing assay, we observed increased velocity of UBE2A-KO derivatives, more significant for glial cells. Further immunostaining experiments performed with super-resolution microscopy showed that the actin cytoskeleton was drastically altered in the lamellipodia of UBE2A-deficient derivatives. The actin cytoskeleton is involved in the fine-tune regulation of dendritic spines formation, and dysregulated dendritic spine dynamics play a crucial role in the neurodevelopmental disorder. The UBE2A protein might regulate the dynamics of actin networks, thereby influencing cell migration. Further investigations are required to verify if altered actin cytoskeleton dynamics results in dendritic spines anomalies in patients with Nascimento syndrome.

Funding Source: The project is supported by RSF grant #21-65-00017

Keywords: UBE2A, X-linked intellectual developmental disorder type Nascimento, Actin cytoskeleton

2064

KDM5B TRUNCATION ALTERS ECTODERM PATTERNING AND CORTICAL NEURAL DIFFERENTIATION

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KDM5B, a lysine-specific demethylase, plays an important role in stem cell differentiation and embryonic development. Loss of function mutations in KDM5B have implicated the gene as a risk factor for developmental disorders, including autism spectrum disorder, attention-deficit/hyperactivity disorder, congenital heart diseases, facial dysmorphism, and agenesis of the corpus callosum. The specific alterations in the human brain resulting from mutations in KDM5B remain unclear. To investigate the effect of KDM5B deficiency on early embryonic brain development, we used human induced pluripotent stem cells (iPSC) and 3D neural differentiation systems to identify cell type specific developmental abnormalities that result from KDM5B truncation created by CRISPR gene editing. By performing single-cell RNA sequencing analysis of more than 30,000 cells and immunochemistry analysis of individual organoids, we identify that a deficiency of KDM5B results in an increased ratio of cortical neural progenitor cells (PAX6+) to cortical neural cells (TBR1+ and SLC17A6+). This increase is associated with increased glial cell (S100B+) counts, suggesting impairment of cortical neural cell maturation and skewing toward glial cell linage. Furthermore, loss of KDM5B disrupts patterning of sensory placodes and the neural crest around neural progenitor cells during neurulation. Finally, depletion of KDM5B leads to agenesis of choroid-plexus-like cystic structures in brain organoids. Our results indicate KDM5B is essential for neural cell fate and self-organizing neurodevelopmental patterning. This study demonstrates the effects of KDM5B truncation with relevance for neurodevelopmental disorders and provides a path for exploring the function of KDM5B in early neurodevelopment.

Funding Source: Yale Child Study Center Pilot Research Award

Keywords: Induced Pluripotent Stem Cells (iPSCs), Neurodevelopmental Disorder (DD), KDM5B

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POTENTIAL ROLE OF BRIDGING INTEGRATOR PROTEIN 3 (BIN3) IN PARKINSONS DISEASE

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Genome Wide Association Studies (GWAS) in Parkinson's Disease (PD) patients have identified numerous non-coding variants. Using expression Quantitative Trait Locus (eQTL) analyses of hundreds of thousands of single brain cells in the Parkinson Brain Atlas initiative and in 1,170 human brains, we identified BIN3

(Bridging Integrator Protein 3) as an effect gene regulated by a GWAS peak on Chromosome 8. This raises the possibility of BIN3 involvement in PD pathogenesis. By using CRISPR cas9 gene editing technology, we generated BIN3 Knockout (KO) and Over Expression (OE) human pluripotent stem cell (hPSC) lines. In the BIN3 KO hPSC-derived midbrain dopamine neurons, there is a substantial increase in alpha-synuclein and serine 129 phosphorylated alpha-synuclein. Western blotting showed decreased levels of proteins related to the autophagosomal-lysosomal system, including P62, LAMP1 and LC3BII, suggesting that accumulation of alpha-synuclein may be due to impaired degradation. Indeed, the BIN3 KO cells display enlarged lysosomes, as indicated by lysotracker red. Furthermore, BIN3 KO neurons show reduced endocytosis, especially when the neurons were stimulated, indicated by the uptake of a fluorescent dye. These results suggest that BIN3 plays a role in regulating vesicular trafficking and alteration of BIN3 (e.g., KO) impairs the vesicular trafficking, which may impair dopamine neuron function and result in degeneration.

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Keywords: Parkinson's Disease, Midbrain Dopaminergic neurons, Vesicular trafficking

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DISEASE MODELING OF IMPAIRED BRAIN GLUCOSE METABOLISM USING PATIENT-SPECIFIC IPSC-DERIVED MICROPHYSIOLOGICAL MODELS OF THE NEUROVASCULAR UNIT

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Neurological conditions conquer the world; they are the leading cause of disability and second leading cause of death worldwide. Although there is growing evidence for the immense impact of disturbances in neurometabolism for overall brain function, only little is known about the underlying mechanisms. Especially human insights are sparse due to a paucity of physiologically relevant model systems. To address these challenges, we are developing a novel, human iPSC-based organ-on-chip model of the neurovascular unit (NVU) that integrates all neurometabolically active NVU cell types and specifically enables the inspection of neurometabolic coupling mechanisms. To categorically cast light onto the mechanisms behind impaired metabolism of glucose, the brain's principal energy supplier, we are focusing on an NVU-on-Chip disease model of glucose transporter 1 deficiency syndrome (GLUT1-DS). Since GLUT1-DS is monogenic, it presents an excellent paradigm to study cellular and molecular consequences of disturbed neuroenergetics, even beyond the disease itself. We have successfully generated induced pluripotent stem cells (iPSCs) derived from fibroblasts of two GLUT1-DS patients carrying different mutations in the solute carrier family 2 member 1 (SLC2A1) gene. Moreover, we have differentiated neurometabolically relevant NVU cell types (endothelial cells, perivascular cells, astrocytes) as well as cerebral organoids from these GLUT1-DS-iPSCs and investigated (i) key cell type-specific characteristics as well as (ii) changes in energy metabolism compared to neural cells/organoids derived from apparently healthy iPSCs. To address shortcomings of existing NVU-on-Chip systems, we developed a novel microfluidic platform allowing for advanced neurometabolic coupling while at the same time retaining accessibility of individual compartments. Thereby, we can specifically study perturbations in energy metabolism, blood-brain barrier integrity and neuroinflammation as a consequence of GLUT1-DS in vitro. Our GLUT1-DS-NVU-on-Chip model holds great promise to provide novel knowledge on the underlying mechanisms and pathophysiology of GLUT1-DS, and thereby not only benefit those afflicted by the orphan disease but impact our understanding of a variety of other CNS and metabolically linked disorders.

Keywords: neurovascular unit-on-chip, CNS energy metabolism, glucose transporter 1 deficiency syndrome

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ENHANCEMENT OF NEURONAL DIFFERENTIATION OF HUMAN DENTAL PULP STEM CELLS INDUCED BY ERGOTHIONEINE

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Neurodegenerative diseases were characterized by the loss of neurons. Stem cell-based therapy is a promising treatment to generate functional neurons for neurodegenerative disease. Human dental pulp stem cells (hDPSCs) are alternative candidates due to their neuronal differentiation. However, the differentiation ability still presented a low percentage of differentiated cells. Interestingly, Ergothioneine (ERGO), a mushroom-extracted compound consisting of sulfur-containing amino acid, can be specifically triggered neuronal differentiation via organic cation transporter N1 uptake. Therefore, this study aims to demonstrate the potential of ERGO as an enhancer of neuronal differentiation of hDPSCs. The hDPSCs were characterized by cell morphology, plastic adherent, self-renewal, and multipotential differentiation. Following, neuronal induction treatment with/without 500 µM ERGO was performed. The characterization of differentiated hDPSCs was evaluated by cell morphology and β-III tubulin immunofluorescence

staining. The hDPSCs exhibited fibroblast-like shape morphology with plastic adherent and can be performed self-renewal, and multipotential differentiation including osteogenic, adipogenic, and neurogenic lineage which is characterized as mesenchymal stem cells. Importantly, under neuronal induction medium, the hDPSCs were differentiated into neuronal-like morphology which positively expressed β-III tubulin (neurogenic-associated protein, immature neuron marker) while the hDPSCs presented the fibroblast morphology. Interestingly, synergistic treatment with 500 μM ERGO significantly increased the percentage of neuronal-like cells, which enhanced 1.5 times and 7.6 times neuronal differentiation when compared to the neuronal induction medium and negative control medium, respectively. Taken together, these findings could be suggested that using ERGO as the neuronal differentiation enhancer to increase the number of neuron for neurodegenerative disease.

Keywords: Human dental pulp stem cells, Neuronal differentiation, Ergothioneine

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TRANSCRIPTOMIC PROFILING OF PSEN1-MUTANT CEREBRAL ORGANOIDS REVEALS DYSREGULATED MITOCHONDRIA FUNCTIONS IN A SUBPOPULATION OF ASTROCYTES

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Alzheimer's disease (AD) is the primary form of dementia with at least 50 million people suffering from it. Our understanding of AD pathophysiology is still rudimentary, due to the complexity of the brain and disease, and limitations with animal models. Cerebral organoids (COs) are self-organizing and offer an unprecedented model with better structural and functional complexity resembling the human brain. Our work aims to model Presenilin1 (PS1)-mutant AD pathogenesis and study the effects of astrocytes on Aβ clearance using patient iPSCs-derived COs. In this study, COs were differentiated from patient-derived iPSCs that harbour PS1 mutation and their isogenic controls were corrected via CRISPR/Cas9. Subsequently, we conducted single-cell RNA sequencing (scRNAseq) on mutant and corrected COs and studied transcriptomic differences in a subpopulation of astrocytes. We observed typical neurodevelopment patterning and spontaneous differentiation of astrocytes in COs with accumulation AD disease hallmarks such as Aβ aggregates and p-Tau in PS1-mutant COs which recapitulates AD progression. scRNAseq revealed genes implicated in mitochondria process were significantly upregulated in a subpopulation of astrocytes present in PS1-mutant COs. Interestingly these genes are respectively and have also been reported to be implicated in other diseases such as Parkinson's Disease. To our knowledge, the role of these genes in astrocytes involved AD pathology have not been studied extensively. Studying these genes in the context of AD could help us unravel new mechanisms leading up to AD neuropathology and provide an opportunity to develop drugs to intervene in AD progression. Ultimately, unravelling deregulation of mitochondria processes in astrocytes and its effects on Aβ clearance will shed light on their role in AD progression.

Keywords: cerebral organoid, Alzheimer's disease, scRNAseq

2069

C9ORF72 MUTATION INDUCES HUMAN ENDOGENOUS RETROVIRUS-K EXPRESSION IN MOTOR NEURONS

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Integrated retroviral elements in human genome play an important role in human brain development and in the pathogenesis of neurological disorders. HERV-K components have been found reactivated in brain and cerebrospinal fluid of individuals with Amyotrophic Lateral Sclerosis (ALS) which can cause motor neurotoxicity. C9orf72 repeat expansion is known to occur in a subset of ALS patients. It introduces DNA damage and increases KU80 activity, a protein responsible for recruiting DNA repair elements. Whether the C9orf72 repeat expansion itself results in HERV-K activation is unknown. We generated iPSCs from CD34 cells purified from PBMCs of ALS patients with C9orf72 expansion. iPSCs were further differentiated into motor neurons and monitored for HERV-K env expression using RT-PCR. We found that HERV-K env expression was increased in C9orf72 motor neurons compared to those derived from healthy controls. The abnormal activation of HERV-K env was specific for motor neurons, and was not noted in iPSCs. An antisense oligonucleotide (ASO) targeting C9orf72 mutations, also decreased HERV-K env activation and KU80 expression. Inhibition of KU80 using siRNAs resulted in an attenuation of HERV-K expression as well. The results indicate that C9of72 expansion causes HERV-K activation specifically in motor neurons. This activation may be mediated by KU80-associated DNA repair process. As HERV-K activation has been shown to cause detrimental effect on motor neurons, ASOs targeting C9orf72 or HERV-K env could provide the therapeutic benefit in ALS patients with C9orf72 expansion.

Funding Source: Funded by the NIH/NINDS intramural fund

Keywords: C9orf72, ALS, iPSC

2070

DEFECTIVE OXPHOS PERTURBS THE NEURONAL DIFFERENTIATION OF HUMAN ENTERIC NEURAL CREST CELLS AS REVEALED BY THE IPSC MODEL OF HIRSCHSPRUNG DISEASE AND SINGLE-CELL TRANSCRIPTOMIC ANALYSIS

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Hirschsprung disease (HSCR) is characterized by the absence of enteric neurons caused by the defects of enteric neural crest cells (ENCCs), leading to intestinal obstruction. Here, using iPSC-based models of HSCR and single-cell transcriptomic analysis, we identified a gene set of 118 genes commonly dysregulated in all HSCR-ENCCs, with HDAC1 found to be a key regulator of these genes. Furthermore, upregulation of RNA splicing mediators and enhanced alternative splicing events were associated with severe form of HSCR. In particular, the higher inclusion rate of exon 9 in PTBP1 and the perturbed expression of a PTBP1-target, PKM, were significantly enriched in these HSCR-ENCCs, and associated with the defective oxidative-phosphorylation (OXPHOS) and impaired neurogenesis. Inhibition of HDAC1 and up-regulation of PKM1 significantly enhanced the differentiation capacity of HSCR-ENCCs. In sum, we have defined the core factors underpinning HSCR disease and demonstrated the implications of OXPHOS in ENCC development and HSCR pathogenesis.

Funding Source: This work was substantially supported by the HMRF grants (Project no.: 06173306, 08192786) and the General Research Fund (GRF: HKU 17108019, 17101320) Hong Kong

Keywords: neural crest, neuronal differentiation, Hirschsprung disease

2071

DEVELOPING A SCALEABLE, 3D, BIOREACTOR BASED PROTOCOL FOR THE GENERATION OF EBISC-NEUR1, HUMAN INDUCIBLE NGN2 NEURONS

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Human induced pluripotent stem cells (hiPSCs) represent a valuable and versatile tool for research and drug development. To meet the increasing demand in high-quality hiPSCs and their derivatives, the non-profit European Bank for induced pluripotent Stem Cells (EBiSC) was founded in 2014. By integrating state-of-the-art upscaling and automation, EBiSC provides a central platform for standardized cells and operating procedures. Here we applied this approach for a fast and efficient protocol to differentiate genetically modified, doxycycline-inducible Neurogenin 2 (iNGN2) expressing hiPSCs into EBiSC-NEUR1 neurons using a benchtop 3D cell culture incubator. In brief, single cell suspensions of hiPSCs were allowed to form aggregates first, followed by the addition of doxycycline to drive neuronal lineage commitment. After two days of transgene induction, aggregates were dissociated and cells either cryopreserved or replated for terminal maturation. BIONi010-C-13 iNGN2 neurons are generated in high yield and are also available as the hiPSC-derived product NEUR1 via EBiSC (www.EBiSC.org). Besides the expression of classical neuronal markers such as beta-III-tubulin and microtubule associated protein 2 early on, iNGN2 neurons form complex and dense neuritic networks within one week after replating indicating an increasing maturity of the cultures. In summary, we demonstrate the bulk generation of iNGN2 neurons in a 3D environment, with potential applications in disease modeling, phenotypic high-throughput drug screenings and large-scale toxicity testing.

Funding Source: This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under

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Keywords: iPSC, Neuron, 3D

2072

ALTERED GLUTAMATERGIC DEVELOPMENT IN HIPPOCAMPAL-CORTICAL CIRCUITRY IN 3D MODELS OF SCHIZOPHRENIA

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SCZ has neurodevelopmental origins. Difficulties in recapitulating human brain development has slowed our understanding of its pathophysiology. Here we propose to use, single region Hippocampal Organoids (HOs) and double region hippocampal+-cortical organoids (DOs or double organoids) to investigate key neurodevelopmental alterations in the brains of individuals diagnosed with SCZ. While much remains to be clarified, it is accepted that patients with SCZ have altered brain structure and function. Post-mortem studies indicate that the disorder is characterized by reduced brain volume, cell size, spine density and abnormal neural distribution in the prefrontal cortex (PFC) and hippocampus (Hc). At the same time iPSC studies show deficits in migration, synaptic maturation, and activity of SCZ neurons suggesting that abnormal neural development culminates in the failure of neural circuits to form and function appropriately. The PFC and the Hc are central to architectural and circuit abnormalities in SCZ. The Hc projects profusely to the entire PFC via a strong monosynaptic glutamatergic projection in the Hippocampal-prefrontal pathway (H-PFC); but the interplay between these regions is deficient in SCZ. We hypothesize that the H-PFC is a primary source of glutamatergic induced dysfunction of the PFC. The prominence of the H-PFC pathway in executive function and emotional regulation underscores its potential role in SCZ's cognitive symptoms and, thereby, it may be a promising therapeutic target. We will utilize Brain Organoids as models to investigate the neurodevelopmental and circuitry alterations in SCZ. We posit that i) reduced progenitor pool and impaired migration are responsible for smaller Hc size in SCZ and ii) defects in glutamatergic neural transmission will produce imprecise temporal coordination of neural activity manifesting in abnormal H-PFC circuitry synchronization. We will address this through the study of i) HOs and ii) DOs, respectively, to understand how disturbed neural synchrony can contribute to failures of effective connectivity and neural integration in SCZ, and how these are modulated by a glutamatergic dysfunction. This study will contribute to our knowledge of SCZ's neurodevelopmental roots by creating an early Hc and H-PFC model system.

Funding Source: T32 NIMH Fellow

Keywords: Organoid, Neural, Schizophrenia

2073

TET1-EXPRESSING IPS CELLS REPRESENT HIGHER SOMATIC DIFFERENTIATION CAPABILITY BY SUPPRESSION OF EXTRAEMBRYONIC LINEAGE

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Human pluripotent stem cells such as human induced pluripotent stem cells (hiPSCs) would meet promising clinical and pharmaceutical needs if these cells robustly are induced to the desired cell types in vitro. Currently, numerous aberrant regulations of the signaling pathways and epigenetic statuses have been proposed to be causative of the observed differentiation defectiveness, but no consensus has been attained. Therefore, we screened in-house large isogenic male and female hiPSCs and unveiled a subpopulation of extraembryonic-biased cells within most hiPSC clones, coinciding with TET1-low cells. The extraembryonic propensity of these hiPSC clones was mainly driven by high BMP4 expressivities, solely produced by the TET1-low subpopulation. Our TET1-aided hiPSCs (T-iPSCs), where the conventional reprogramming cocktail (OSKM+LIN28A+p53shRNA) was supplemented with the DNA demethylase TET1, showed enhanced epithelialized morphology, a trait attributable to TET1 activity during reprogramming, but also were devoid of TET1-low subpopulation and exhibited minimal extraembryonic gene noises. Furthermore, we enhanced the neural inductivity of not only the dual SMAD inhibition (inhibiting BMP and NODAL signaling) but also WNT inhibition (triple inhibition). Notably, the triple inhibition of T-iPSCs, but no other iPSC clones, led to the in vitro differentiation of ventral midbrain dopaminergic neurons with unprecedented fidelity and efficiency by responding to the embryonic axes' morphogens robustly. We propose the T-iPSC production pipeline as a de facto standard for human iPSC reprogramming.

Funding Source: This work was supported by AMED under Grant Number JP19bk0104090h0001.

Keywords: TET-1, Differentiation, Extraembryonic propensity

2074

MAPPING THE STRUCTURE AND FUNCTION OF WHOLE-MOUNT HUMAN RETINAL ORGANOIDS

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Retinal organoids derived from human pluripotent stem cells are highly similar to the human retinal structure. They have many potential applications in research, including the study of retinal development and disease, the testing of new therapies for retinal diseases, and the development of new models for drug screening. For that, it is essential to understand how they work from a 4D perspective in order to test their reproducibility. In this study, we first characterized the structure of whole-mount mature retinal organoids in their natural 3D architecture by optimizing several optical clearing methods (fructose/glycerol, ECi, and FluoClear BABB) in combination with improved antibody permeabilization. We identified the distinct retinal cell types and their spatial-temporal interaction within the organoid using specific antibodies. We described the morphology and population of the three neuron-path that provide the direct route for visual information transmission: cone and rod photoreceptors, bipolar and ganglion cells. Then, we used light-sheet fluorescence microscopy (LSFM) to define the functional window of the retinal organoid's responsiveness to light. LSFM uses a thin sheet of light to irradiate the sample, and the fluorescence generated is captured with a camera. In this manner, we can track fast live dynamics (15 Z-planes at 5 Hz; 200 ms/volume) occurring on a volumetric scale (600x830x150 µm) with minor (or none) photo-toxic or photo-damage effects. To functionally characterize the organoids, we studied calcium dynamics by delivering genetically encoded calcium indicators (GCaMP6s) with viral vectors. We imaged in high-resolution the retinal organoid response to light stimuli in 3D and at a cellular scale. The changes in fluorescence intensity were quantified to determine the pattern and frequency of calcium signals. These findings shed more light on the temporal, spatial, and functional organization of retinal cells within the organoid and are highly promising as an in vitro model for retinal diseases due to their recapitulation of the structure and physiology of the human retina.

Keywords: retinal organoids, in vivo imaging, Calcium imaging

2075

HUMAN PLURIPOTENT STEM CELLS-DERIVED INNER EAR ORGANOIDS RECAPITULATE OTIC DEVELOPMENT IN VITRO

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Our molecular understanding of the early stages of human inner ear development has been limited by the difficulty in accessing fetal samples at early gestational stages. As an alternative, previous studies have shown that inner ear morphogenesis can be partially recapitulated using pluripotent stem cells (iPSCs) directed to differentiate into Inner Ear Organoids (IEOs). Once validated and benchmarked, these systems could represent unique tools to complement and refine our understanding of human otic differentiation and model developmental defects. Here, we provide one of the first direct comparisons of the early human embryonic otocyst and iPSC-derived IEOs. We use multiplexed immunostaining, and single-cell RNA sequencing to characterize IEOs at three key developmental steps, providing a new and unique signature of in vitro derived otic -placode, -epithelium, -neuroblasts, and -sensory epithelia. In parallel, we evaluated the expression and localization of critical markers at these equivalent stages in human embryos. We show that the otic placodes derived in vitro (day 8-12) match marker expression of Carnegie Stage (CS) 11 embryos and subsequently (day 20-40) give rise to otic epithelia and neuroblasts comparable to the CS13 embryonic stage. Differentiation of sensory epithelia, including supporting cells and hair cells, starts in vitro at day 50-60 of culture. The maturity of these cells is equivalent to vestibular sensory epithelia at week 10 or cochlear tissue at week 12 of development, before functional onset. Together, our data indicate that the current state-of-the-art protocol enables the specification of bona fide otic tissue, sup-

porting the further application of IEOs to inform inner ear biology and disease.

Keywords: Human Otic Development, Inner Ear Organoids, scRNASEQ profiling otic epithelia, neuroblasts and sensory hair cells

2076

CTIP2-DRIVEN MONOSYNAPTIC TRACING TO CHARACTERIZE DEVELOPMENT OF CORTICAL MICROCIRCUITRY IN LONG-TERM ORGANOID CULTURES

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Recent advances in brain organoid (Org) technology have enabled expansion of laminar structure in long-term cultures. However, it is still unknown to what extent circuit-level architecture in developing Orgs reflects the “canonical” cortical microcircuit. Obtaining a stronger understanding of Org neural circuity is important to their translational use as a platform to study neurodevelopmental disorders as well as a substrate for use in neural repair. To explore circuit structure in forebrain Orgs, we used the layer V (L5)-specific CTIP2 promoter to drive rabies virus-based monosynaptic retrograde tracing and characterize the network of inputs to L5 cells. A “helper” virus was used to co-express rabies glycoprotein and TVA in CTIP2+ starter cells, which are required genes for spread of subsequently added ΔG rabies viral vector expressing GFP. This system was used to assess the sources of L5 inputs at 3 time points: differentiation day (dd) 70, 100 and 130. Orgs were grown from an iPSC line derived from a healthy volunteer. Transduction with LV-CTIP2-DsRedExpress2-G-TVA was performed 7 days before addition of EnvA-RVΔG-GFP. Orgs were fixed 7 days after rabies infection for histology. Images were processed in CellProfiler and segmented for DAPI, cortical layer markers and GFP. Percent abundance of each layer marker was compared between total cells (TC) and inputs to L5 cells (L5-Inp) to determine preferential connectivity. Statistical analyses were performed using a Student’s t-test (sig. = $P < 0.05$). Orgs were selected randomly from cultures in 2 independent experiments. The fraction of CTIP2+ (L5) L5-Inp was significantly higher than TC at all time points, while fractions of Cux1+ (L2-3) and Satb2+ (L4) L5-Inp were significantly higher only at later time points. Foxp2 (L6) was lower in L5-Inp than TC (NS). Overall expression of Cux1, Ctip2 and Satb2 increased significantly from dd100 to dd130. Total number of GFP+ cells was comparable over time, indicating stable network size of L5-Inp in maturing Orgs. Our data broadly suggests development of an organized circuit in maturing Orgs, with distribution of layers in L5-Inp clearly differing from that in TC. Next steps to investigate micro-circuitry of Orgs include analyzing later developmental time points and performing tracing driven by other layer-specific promoters.

Funding Source: National Institutes of Health (R01NS119472 to H.-C.I.C.)

Keywords: Cortical organoids, Retrograde tracing, Tissue engineering

TOPIC: NO TISSUE SPECIFICITY

2077

POST GASTRULATION SYNTHETIC EMBRYOS GENERATED EX UTERO FROM MOUSE NAIVE ESCS

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In vitro cultured stem cells with distinct developmental capacities can contribute to embryonic or extraembryonic tissues after microinjection into pre-implantation mammalian embryos. However, whether cultured stem cells can independently give rise to entire gastrulating embryo-like structures with embryonic and extraembryonic compartments remains unknown. Here, we adapt a recently established platform for prolonged ex utero growth of natural embryos to generate mouse post-gastrulation synthetic whole embryo models (sEmbryos), with both embryonic and extraembryonic compartments, starting solely from naive ESCs. This was achieved by co-aggregating non-transduced ESCs, with naive ESCs transiently expressing Cdx2 or Gata4 to promote their priming toward trophectoderm and primitive endoderm lineages, respectively. sEmbryos adequately accomplish gastrulation, advance through key developmental milestones, and develop organ progenitors within complex extraembryonic compartments similar to E8.5 stage mouse embryos. Our findings highlight the plastic potential of naive pluripotent cells to self-organize and functionally reconstitute and model the entire mammalian embryo beyond gastrulation.

Keywords: synthetic embryos., naive pluripotency, ex utero

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EPIGENETIC REGULATOR PTIP GOVERNS SKELETAL STEM CELL QUIESCENCE VIA REGULATING GLYCOLYSIS FOR SKELETAL HOMEOSTASIS

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Skeletal homeostasis depends on the stringent governance of quiescent state of stem cells. Skeletal stem cells (SSCs), with hierarchical cell populations similar to hematopoietic stem cell lineage and specific cell surface markers, have been identified in mouse and human growth plates. A series of studies have found that SSCs play an important role in fracture repair, distraction osteogenesis, articular cartilage regeneration and craniosynostosis. However, the underlying mechanisms in controlling the SSCs quiescence remain largely unclear. Chromatin states exhibiting various epigenetic landscapes are crucial for defining cellular identify and differentiation, but how it acts in determining SSC fate is totally unknown. Here, we profiled histone modifications by focusing on H3K4me1, H3K4me3 and H3K27ac in SSCs and subpopulations including pBCSPs and BCSPs, and found epigenetic alterations play a crucial role in governing the developmental trajectory of SSCs. To further identify the key epigenetic regulator in SSCs, firstly, we identified the super-enhancer in SSCs, pBCSPs and BCSPs by ranking H3K27ac signal. Surprisingly, we found that Ptip was the gene with the highest H3K27ac modification in SSCs. Then, we first crossed Ptipfloxp/floxp mice with Ubc-CreERT mice and R26-confetti mice to trace clonal activity of SSCs in the long bone and found loss of Ptip disturbed SSC quiescence. Ptip-deficient mice showed skeletal dysplasia and disturbed SSCs quiescence. By analyzing of RNA-seq, we found that the expression levels of metabolic genes were markedly increased. As expected, glucose consumption, lactic acid production and glycolytic capacity were markedly increased. Integrated analysis of RNA-seq and ChIP-seq data showed Pgk1 was the direct target of Ptip. When inhibiting glycolysis using 2-DG, the level of ECAR, the cell number and percentage, chondrogenic capacity were normalized. Taken together, our results reveal that Ptip is a critical glycolytic regulator in controlling SSCs quiescence for skeletal homeostasis.

Keywords: Epigenetic, skeletal stem cell, bone development

2078

DISCOVERY OF NOVEL CANCER T CELL ANTIGENS THROUGH MULTI-OMICS ANALYSIS OF PLURIPOTENT STEM CELLS

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In recent studies, pluripotent stem cells (PSCs) have been shown to boost antigen-specific immunity in cancer treatment. It is hypothesized that non-mutant antigens presented by PSCs can prime T cells against cancer, thereby reducing tumour burden and preventing cancer growth. To investigate this hypothesis, we initiated a mass spectrometry-based epitope discovery program. Our program focuses on interrogating the HLA-I immunopeptidome of both embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) to determine the mechanisms that enable PSCs to generate anticancer responses. Our data show that the inflammatory cytokine IFN-γ upregulates HLA-I mediated antigen presentation in undifferentiated PSCs. This upregulation results in increased expression of key antigen presentation machinery components TAP1 and Tapasin, which are normally poorly expressed in PSCs. These findings suggest that undifferentiated PSCs can express high levels of antigen presentation machinery components and present antigens for T cell recognition. In support of this, our HLA-I immunoprecipitation and mass spectrometric analysis of eluted peptide ligands revealed the presentation of a wide array of novel and previously reported cancer T cell epitopes. Taken together, our data provide a mechanism for the observed anticancer effects induced by PSCs and identify PSCs as a novel antigen source with potential to induce T cell-mediated immune response against diverse cancer types. Furthermore, we identified a subset of HLA-I bound peptides derived from common PSC and cancer-derived antigens that are marginally or not at all expressed by somatic tissues. These findings underscore the potential of PSCs as a novel and powerful tool in cancer immunotherapy.

Keywords: induced pluripotent stem cells, tumor-specific antigens, cancer immunotherapy

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CHARACTERİZATION OF HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED PARATHYROID ORGANOIDS IN VITRO AND IN VIVO

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The parathyroid gland consists of four small glands located at the posterior of the thyroid gland which are originated from the endoderm layer. Parathyroid cells secrete parathyroid hormone (PTH) which acts on the bone, kidney, and small intestine to maintain the calcium/phosphorus balance. Due to the limited proliferation capacity of parathyroid tissue cells in culture, alternative cell and tissue sources are required for cell therapy. The microanatomy of the parathyroid gland might enable modelling of the organ structure in vitro via organoids. In the current study, we demonstrated for the first time that parathyroid organoids derived from induced pluripotent stem cells (iPSCs) are capable of efficient hormone release. Parathyroid organoids were derived from fibroblast derived iPSCs in 3D Matrigel dome structures by induction of endoderm generation as the initial step of a 20 days of differentiation protocol. Parathyroid organoids were characterized by the morphological, physiological, and molecular analyses. The PTH secretion by the parathyroid organoids was evaluated in a time dependent manner. Protein expression and RNA-Seq analyses confirmed the expression of parathyroid related markers such as Foxn1, Gcm2, CasR, PTH, and CxCR4 in organoids. Organoid condition medium induced osteogenic differentiation of the preosteoblast cell line indicating active and functional PTH release. The rise in ERK phosphorylation in CasR+ cells in response to varying calcium doses, provided evidence that organoids can functionally respond to calcium via CasR. Moreover, parathyroid organoids can be sub-cultured up to 4 passages without losing their morphological, physiological, or functional integrity. In a parathyroidectomy animal model, the in vivo functional activity of organoids was controlled by active PTH secretion, blood calcium/phosphorus levels, and bone mineralization. The parathyroid organoid model generated in this study is a promising candidate for parathyroid disease modelling and reflects tissue anatomy and physiology in vitro and in vivo. This model might be a potential organoid source for transplantation in the near future.

Funding Source: This study is supported by Tübitak (Project no: 219S665). This study was also supported by Yeditepe University.

Keywords: Organoid, Pluripotent stem cell, Parathyroid

2080

PRIORITIZATION OF GENETIC CONTRIBUTORS TO NEOCORTICAL ALTERATIONS IN 2Q37 DELETION SYNDROME USING GENOMIC ANALYSIS OF NEURAL STEM CELLS AND HUMAN CEREBRAL ORGANOIDS

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2q37 microdeletion syndrome is a rare syndrome characterized by neurodevelopmental delay, bone, cardiovascular, and neurological alterations including autism spectrum disorder, and microcephaly. This syndrome is typically associated with loss of genetic material of approximately 100 genes in the 2q37 band. However, the genes and cells most associated with the neurodevelopmental phenotype in this syndrome are still unknown. Previously, we identified a deleted region of 496 kb in the distal region of 2q37, in a patient with developmental delay, microcephaly and hypoplasia of the corpus callosum. This segment contains genes that are highly expressed in the developing human cortex, the subventricular zone (SVZ) in vivo and human neural progenitors in vitro. To further elucidate the genes involved in the neurodevelopmental phenotype, we used genomics analysis of fetal brain and single cell RNAseq of normal cerebral organoids, to prioritize genes with a developmental pattern most consistent with neural stem cell function. In addition. to genetically define the brain cell types underlying the syndrome based on genes enriched in human cerebral organoids. We found PTMA is highly expressed in multiple populations of radial glia, neurons and cycling cells as a potential candidate. In addition to SEPT2 that is expressed in NSCs and radial glia. Our study narrows not only potential segment of novel neuronal genes but the cells that may be involved in 2q37 microdeletion syndrome. Although, a rare syndrome, our data may suggest novel pathways for the development of the human cortex and corpus callosum.

Keywords: GENOMICS, BIOINFORMATICS, 2q37 deletion syndrome

2081

KNOCKOUT OF THE NADSYN1 GENE IN HUMAN INDUCED PLURIPOTENT STEM CELLS

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VACTERL association is a specific set of developmental defects that can impact multiple organs. VACTERL stands for vertebral defects, anal atresia, cardiac defects, trachea-esophageal fistula, renal anomalies, and limb abnormalities. The exact cause of VACTERL association has yet to be identified, although it appears to be a combination of genetic and environmental factors. Whole exome sequencing of the 2 patients who presented at the Children's Hospital of Western Ontario and their parents identified a novel mutation in the gene NADSYN1, the final enzyme in the NAD synthesis pathway. NAD is a vital molecule found in all living cells which regulates multiple biological processes, including metabolism and DNA repair. Given that NAD is central to cell metabolism and is a co-factor for many essential enzymes, it is surprising that humans and mice with severe deficiencies sur-

vive past birth and that the resulting VACTERL association is a specific set of defects. Our hypothesis for the specificity in the defects seen in the VACTERL patients is that mesodermal progenitors are under-represented given that the affected tissues are predominantly mesodermal in origin. To test this hypothesis, induced pluripotent stem cells with the same novel patient mutation in NADYSYN1 were generated at the Centre for Commercialization of Regenerative Medicine (Toronto). NADSYN1 mutant cells grew in compact colonies similar to their parental line and maintained expression of pluripotency-associated markers. We then examined the differentiation potential of NADSYN1 mutant cells via embryoid body (EB) formation. NADSYN1 mutant cells formed similar sized EBs while in suspension as the parental line. After reattachment to Geltrex-coated dishes, NADSYN1 mutant EBs were significantly smaller in size and morphologically distinct from the parental line, although they displayed expression of differentiation markers as assayed through RT-PCR and immunolabeling. This work is part of a highly collaborative effort to uncover the novel role of NADSYN1 in VACTERL association. Our overall aim is to contribute to the identification and understanding of the genetic variants that may be associated with VACTERL association in hopes of developing better treatment options.

Keywords: induced pluripotent stem cells, Embryoid bodies, NADSYN1 gene

2082

HYPOIMMUNE RHESUS MACAQUE INDUCED PLURIPOTENT STEM CELLS ACHIEVE LONG-TERM SURVIVAL IN FULLY IMMUNOCOMPETENT ALLOGENEIC RECIPIENTS

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Transplantation of cells that are allogeneic—derived from a donor who is not genetically identical to the recipient—induces an immune response that subsequently leads to rejection of the transplant. However, survival, engraftment, and long-term functionality of transplanted cells is crucial for the success of cell replacement therapy. Therefore, the generation of allogeneic, immune-evasive cells might better facilitate large-scale manufacturing and maximize patient access to such therapies. To avoid rejection absent immunosuppression (IS), rhesus macaque (NHP) iPSCs were engineered to knock out function of MHC class I and II and overexpress CD47 (HIP iPSCs). HIP iPSCs and wild type iPSCs (wt iPSCs) were transplanted into allogeneic, immunocompetent NHPs without IS and followed over time (n=4/group). At all time points tested up to 10 weeks, peripheral blood mononuclear cells killed wt iPSCs via direct cytotoxicity and antibody-mediated cellular cytotoxicity. There was a strong IFN-g ELISpot T cell response one week after wt iPSC transplantation, accompanied by a surge in wt iPSC specific IgM antibodies and, with delayed kinetics, also IgG antibodies. Furthermore, wt iPSCs were killed via complement-dependent and antibody-dependent cellular cytotoxicity. In contrast, NHPs that received HIP iPSCs showed no measurable immune response against HIP iPSCs at all time points. After 6 weeks, NHPs initially receiving wt iPSCs were injected with HIP iPSCs. Although they maintained their strong immune response against wt iPSCs, they did not mount any response against HIP iPSCs, indicating that HIP iPSCs evade immune recognition even in sensitized recipients. NHPs receiving HIP iPSCs first developed a strong cellular and antibody-response against the subsequently injected wt iPSCs but continued to have no reactivity against HIP iPSCs, demonstrating that the HIP iPSCs do not alter the recipients immune system. In vivo bioluminescence imaging up to 16 weeks revealed rejection of all wt iPSC grafts in both groups within 2-3 weeks after transplantation, while all HIP iPSC grafts survived the study period. HIP engineered cells hold promise to achieve long-term graft survival in patients leading to a new class of cell-based medicines.

Keywords: Immune barrier, iPSC, rejection

TOPIC: PANCREAS

2083

LAWSONE PRECONDITIONED MESENCHYMAL STEM CELLS EXHIBIT PANCREATIC BETA CELL REGENERATION IN RAT MODEL OF TYPE 1 DIABETES

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Diabetes is one of the major health issues globally. Mesenchymal stem cells (MSCs) having remarkable self-renewal and differentiation potential, can regenerate beta cells. MSCs preconditioned with bioactive small molecules including naphthoquinones have been shown to possess better therapeutic potential under in vivo environment. The current study analyzed the effect of lawsone (2-hydroxy-1,4-naphthoquinone) preconditioned human umbilical cord MSCs (hUMSCs) on the regeneration of pancreatic beta cells in the STZ-induced type 1 diabetic rat model. hUMSCs were preconditioned with optimized concentration of lawsone, as assessed by MTT assay. Type 1 diabetic rat model was established by injecting 50 mg/kg of streptozotocin intraperitoneally. Normal and

lawsone preconditioned MSCs were transplanted via tail vein in the diabetic rats. Fasting blood sugar and body weight were monitored regularly for 4 weeks. Beta cell regeneration was evaluated by H&E staining and gene expression analysis of the pancreatic tissue, while immunohistochemical analysis was performed to assess insulin expression by the regenerated beta cells. Lawsone preconditioned MSCs showed better anti-hyperglycemic effect compared to the untreated MSCs. Histological analysis showed regeneration of pancreatic islets with higher expression of INS1, PDX1, MafA and NGN3 genes and reduced expression of inflammatory markers. Immunohistochemical analysis showed strong insulin expression in the preconditioned MSCs as compared to the untreated MSCs. The present study demonstrates that the lawsone preconditioned MSCs exhibit pronounced anti-hyperglycemic effect in vivo compared to MSCs alone. Lawsone treated MSCs not only expressed pancreatic beta cell markers, but also decreased inflammation in the STZ-induced diabetic rats.

Funding Source: Higher Education Commission - Pakistan

Keywords: TYPE 1 DIABETES, Lawsone, PANCREATIC BETA CELL REGENERATION

TRACK: NEW TECHNOLOGIES (NT)

TOPIC: CARDIAC

2084

BATCH ACTION POTENTIAL ANALYSER (BAPTA): AN OPEN SOURCE TOOL FOR AUTOMATED HIGH THROUGHPUT ANALYSIS OF CARDIAC ACTION POTENTIALS

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The cardiac action potential (AP) is a key species-specific feature of cardiomyocytes that occurs in response to coordinated actions of ion channels. It represents the first step of the cardiac excitation-contraction coupling and it is crucial for cardiomyocyte (CM) physiology. Changes in the cardiac AP may primarily occur as a consequence of diseases or as a direct or unwanted response to drugs. Our ability to quantify these changes defines the reliability of our measurements and its throughput. Cardiac AP parameters are often quantified through manual time-consuming data analysis protocols or custom-made and proprietary data analysis pipelines; to the best of our knowledge, no tools are currently available for automated cardiac AP analysis and AP parameter quantification. Here we introduce a free and open source software tool named Batch Action PoTential Analyser (BAPTA), written in the R language, designed to i) overcome the inherent operator-dependent bias on trace selection affecting reproducibility, ii) vastly improve the throughput of the analyses of large datasets and iii) analyse both spontaneous and triggered APs from CMs of multiple species and origin. We present here four use-cases in which BAPTA can be used at high throughput to investigate the effects of: 1) metabolic electrophysiological maturation in human stem-cell-derived CMs; 2) a disease (cardiomyopathy) on rat CMs, 3) drugs on mouse pacemaker cells and 4) rate-dependency of AP duration in guinea pig CMs. BAPTA was effective in the analysis of APs from some of the most widely used species and platforms in both academia and industry. The reliability of BAPTA was assessed on a broad range of experimental conditions and on a wide range of AP shapes. Overall, BAPTA consistently provides faster, more reproducible and scalable readouts which excellently correlate with manual analyses performed by experienced electrophysiologists.

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Keywords: Electrophysiology, Cardiac Arrhythmias, Drug Screening

2085

A LIVE-CELL IMAGE-BASED MACHINE LEARNING STRATEGY FOR REDUCING VARIABILITY IN PSC DIFFERENTIATION SYSTEMS

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The differentiation of pluripotent stem cells (PSCs) into diverse functional cell types provides a promising solution to support drug discovery, disease modeling, and regenerative medicine. However, functional cell differentiation is currently limited by the substantial line-to-line and batch-to-batch variabilities, which severely impede the progress of scientific research and the manufacturing of cell products. For instance, PSC-to-cardiomyocyte (CM) differentiation is vulnerable to inappropriate doses of CHIR99021 (CHIR) that are applied in the initial stage of mesoderm differentiation. Here, by harnessing live-cell bright-field imaging and machine learning (ML), we realize real-time cell recognition in the entire differentiation process, e.g., CMs, cardiac progenitor cells (CPCs), PSC clones, and even misdifferentiated cells. This enables non-invasive prediction of differentiation efficiency, purification of ML-recognized CMs and CPCs for reducing cell contamination, early assessment of the CHIR dose for correcting the misdifferentiation trajectory, and evaluation of initial PSC colonies for controlling the start point of differentiation, all of which provide a more invulnerable differentiation method with resistance to variability. Moreover, with the established ML models as a readout for the chemical screen, we identify a CDK8 inhibitor that can further improve the cell resistance to the overdose

of CHIR. Together, this study indicates that artificial intelligence is able to guide and iteratively optimize PSC differentiation to achieve consistently high efficiency across cell lines and batches, providing a better understanding and rational modulation of the differentiation process for functional cell manufacturing in biomedical applications.

Keywords: Pluripotent stem cell-derived cardiomyocyte, Variability, Image-based machine learning

TOPIC: GERMLINE AND EARLY EMBRYO

2086

CELL GROWTH AND MAINTENANCE OF THE UNDIFFERENTIATED STATE IN A MICROCARRIER CULTURE SYSTEM FOR HUMAN INDUCED PLURIPOTENT STEM CELLS USING THE LAMININ 511E8 FRAGMENT

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Human pluripotent stem cells (hPSCs) hold a great promise for regenerative medicine. However, a large number of cells is required for a clinical study, where conventional monolayer and 3D suspension culture systems might pose problems in the scale-up and maintenance of critical cell functionality. The microcarrier culture system permits hPSC growth as monolayer on the microcarrier surface with the benefits of a homogeneous suspension culture. Nevertheless, this culture system with xeno-free matrix to support the strong hPSC adhesion and expansion has not yet been fully established. Recombinant laminin (LM) 511E8 fragment (= iMatrix-TM-511) is a xeno-free truncated form of laminin-511 containing the binding site for the integrin α6β1 predominantly expressed on hPSCs. The strong interaction of LM511E8 with integrin α6β1 enables hPSCs to preserve not only an undifferentiated state and pluripotency, but also long-term single cell passaging. Previously, we introduced a series of workflows in a small-scale microcarrier culture system using LM511E8, which is scalable for therapeutic application. We presented the acquisition of multiple-layer human induced pluripotent stem cell (iPSC) expansion on microcarriers after 6 days in culture with the protocol. Moreover, we showed the dissociation of cells from microcarriers with 5 mM EDTA/D-PBS(-). In the current study, we continuously aim to establish the microcarrier culture system in the hiPSCs with LM511E8, particularly focusing on cell growth and maintenance of undifferentiated state. We cultured hiPSCs with 11.2 mg of microcarriers and a seeding density of 3.0x105 cells with a working volume of 5 mL bioreactor stirring at 40 rpm for up to 6 days. We first quantified the number of cells at days 3 to 6 and found a linear correlation between cell expansion and culture period. The average growth rate at day 6 reached approximately 14-folds. We next investigated the preservation of undifferentiated state by performing flow cytometry and immunostaining analyses. Both analyses revealed the expression of an undifferentiation marker, OCT3/4, in almost all cells up to day 4, while cells not expressing the marker started observing at day 5 and longer. In conclusion, hiPSCs cultured with our current protocol expanded linearly and could maintain undifferentiated state up to day 4.

Keywords: Microcarriers, Recombinant laminin 511E8 fragment, Human pluripotent stem cells

TOPIC: HEMATOPOIETIC, IMMUNE, AND ENDOTHELIAL

2087

PROSPECTIVE ANALYSIS OF INNER SYDNEY GERIATRIC OUTPATIENTS FOR ANAEMIA PREVALENCE AND CLONAL HAEMATOPOIESIS OF INDETERMINATE POTENTIAL

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Anaemia affects more than 10% of adults over 60 years of age, and incidence increases by 5% per decade. Anaemia is an independent risk factor for frailty, increased hospitalisations and mortality, and decreased quality of life. Unexplained anaemia (UA) accounts for approximately one third of anaemia. Additional data is needed to compare worldwide data to the general aging community in Australia, and to identify factors potentially driving UA. We undertook the first prospective study in Australia to evaluate incidence and causes of incidental anaemia. In addition, we utilised high-depth next generation sequencing, identified aging-acquired mutations in blood cells, and correlated these with anaemia type. Standardised clinical and anaemia-associated blood test data were collected from consented participants referred to geriatric clinics for frailty assessment (St Vincent's and War Memorial Hospitals, Sept 2017 to Oct 2019, HREC approval 2019/ETH03362). Clonal haematopoiesis of indeterminate potential (CHIP) mutations were detected by ultra-high depth NGS (limit of detection variant allele frequency, VAF = 0.1%) using the Archer VariantPlex 75 Myeloid gene panel (Invitae), analysed with Archer Analysis 4.01. CHIP is currently defined by VAF ≥ 2%. Of the 216 participants (mean age 82.5, range 64-100), 40% were male and 18% (n=38) were anaemic. The main causes were chronic kidney disease and iron deficiency. Seventeen patients (8%) were diagnosed with UA, which is slightly greater than expected. The mean numbers of CHIP mutations at VAF ≥ 2% was 5.8 per participant, and correlated positively to age and male gender, similar to worldwide published studies. The most prevalent somatic mutations were EZH2 (60%), ATRX (55.6%), and U2AF2 (44.4%), which were 1.4–1.7 fold more frequent in UA compared to non-anaemic participants. This suggests these mutations may be drivers of the disease in some cases, which warrants further investigation.

Keywords: Anaemia, Aging, Next Generation Sequencing

TOPIC: LIVER

2129

LONG-TERM MAINTENANCE OF SELF-RENEWAL AND METABOLIC FUNCTION IN CULTURED HUMAN HEPATOCYTES IN VITRO

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In vitro long-term culture of human hepatocytes (HHs) has been limited by intrinsic effects of the preservation process that either block proliferation or require dedifferentiation and loss of function to induce proliferation. Here, two small molecule cocktails are described that can revitalize hepatocytes from an injured state that enable in vitro culture of fresh or cryopreserved HHs (CHHs) for up to 100 days. These treatments maintain metabolic function and stable expression of hepatocyte-specific secreted proteins, metabolic enzymes, and polarized proteins in CHHs, without limiting their self-renewal capacity. Long-term cultured CHHs could potentially be used for modeling mild (chronic) hepatitis, cholestasis, and toxic drug-induced liver injury in vitro. Further, the proliferative capacity of these chemically maintained CHHs was boosted by the addition of hepatocyte growth factor (HGF) in vitro, in a manner similar to hepatic regeneration in vivo. Overall, our findings provide a method for HH culture that recapitulates healthy hepatocyte phenotypes in vivo, facilitating liver disease modeling, drug toxicity evaluation, and production of HHs for transplantation in regenerative medicine therapies.

Funding Source: This work is supported by Plastech Pharmaceutical Technology Co., Ltd. Nanjing, China.

Keywords: self-renewal, hepatocyte culture, in vitro model

TOPIC: MESENCHYMAL STROMAL CELLS, ADIPOSE, AND CONNECTIVE TISSUE

2088

CHARACTERIZING MORPHOLOGICAL VARIATION OF MONOCLONAL AND POLYCLONAL HMSC POPULATIONS IN CULTURE OVER TIME

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Culture expanded human mesenchymal stromal cells (hMSCs) have been explored for use in cell-based therapies. However, the repeatability and stability of hMSC populations varies between donors and batches. This study explores the variation in hMSC populations with respect to biological performance and morphology. Bone marrow-derived cells were obtained from 6 consented patient donors and were cultured in 6-well plates in established conditions. Two population types were assessed: 1) Polyclonal MSCs isolated using competitive expansion (CE) and 2) Clonal MSCs derived from progeny of a single founding cell (SFC). The Cell X Technologies automated cell imaging and processing platform was used to characterize clonal colonies and to “pick” clones with defined colony metrics (i.e. “performance based selection”). High-resolution whole well phase contrast images were captured at passages 3 (P3) and P5 and a fixed pattern of 9 distinct regions was defined. Thirteen morphological parameters were scored by consensus of 3 reviewers. Morphometric changes between P3 and P5 were assessed using a paired Wilcoxon test. Nine CE and 5 SFC populations achieving expansion to P5 were assessed. MSC populations varied widely in morphology. Individual MSC clones demonstrated significant differences and patterns of morphological change between P3 and P5, reflecting differences in performance and stability. Two patterns observed were: a) retention or increase in fibroblastic features over time or, b) manifestation of features suggesting cell senescence (e.g. “ganglion” cells). Correlation with performance data (e.g. differentiation, gene expression, secretome composition) is in progress. CE and SFC populations exhibit wide variation in morphological attributes and patterns of change over time. While morphometric scoring of observable features has inherent face validity, it is time consuming and inherently subjective limiting the value of morphological scoring. However, the same 252 standardized high-resolution images used for scoring can now be subjected to quantitative radiomic analysis using a broad range of computational approaches. Radiomic analysis may provide more repeatable, reproducible, and unbiased metrics of morphological differences between MSC populations and stability as populations are expanded.

Keywords: hMSC, Morphology, Characterization

2089

SPECIFIC BIOMOLECULAR TRAJECTORIES OF HUMAN REPROGRAMMING BASED ON OOCYTE FACTORS

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The reprogramming of human somatic cells to induced pluripotent cells (iPSCs) has become a milestone and a paradigm shift in the field of regenerative medicine and human disease modeling including drug testing and genome editing. However, the molecular processes occurring during human reprogramming and affecting the pluripotent state acquired remain largely unknown. Of interest, different pluripotent states have been described depending on the reprogramming factors used and the oocyte has emerged as a valuable source of information for candidate factors. The present study investigates the molecular changes occurring in human somatic cells during reprogramming with either canonical or oocyte-based combinations using synchrotron-radiation Fourier transform infrared (SR FTIR) spectroscopy. The data acquired by SR FTIR indicates different representation and conformation of biological relevant macromolecules (lipids, nucleic acids, carbohydrates and proteins) depending on the reprogramming combination used and at different stages during the reprogramming process. Association analysis based on cells spectra suggest that pluripotency acquisition trajectories converge at late intermediate stages while they diverge at early stages. Our results suggest that canonical and oocyte-based reprogramming operates through differential mechanisms affecting nucleic acids reorganization and day 10 comes out as a candidate hinge point to further study the molecular pathways involved in the reprogramming process. This study indicates that SR FTIR approach contribute unpaired information to distinguish pluripotent states and to decipher pluripotency acquisition roadmaps and landmarks that will enable advanced biomedical applications of iPSCs.

Funding Source: Ministerio de Ciencia y Tecnologia Gobierno de España ALBA CELL SYNCHROTRON

Keywords: Oocyte based reprogramming, FTIR Fourier transformed infrared microscopy, iPSCs

TOPIC: MUSCULOSKELETAL

2090

IN VIVO LONGITUDINAL TRACKING OF STEM CELL IMPLANTS IN PIG CARTILAGE DEFECTS BY MEGAPRO, A CLINICALLY TRANSLATABLE NANOPARTICLE

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Stem cell therapies have shown great promise in cartilage regeneration. However, current techniques do not allow for the tracking the efficacy of treatment over time. The use of nanoparticles enables in vivo imaging of stem cells with magnetic resonance imaging (MRI). However, ferumoxytol nanoparticles used for stem cell tracking with MRI have short-time signals and cause adverse effects, limiting their ability to be used for longitudinal efficacy tracking. Here, we investigated MegaPro nanoparticles as a new MRI contrast agent for in vivo tracking of stem cells in a large animal model. We labeled stem cells isolated form the bone marrow by mechanoporation method, which provides time-efficient stem cell labelling. Mesenchymal stromal cells (MSC) were labeled with MegaPro or ferumoxytol nanoparticles, implanted in twenty-six cartilage defects in ten pig knee joints, and then longitudinally imaged by MRI over 12 weeks. MSC showed high cellular uptake of MegaPro nanoparticles without affecting cell viability. Histological analyses demonstrated that MegaPro nanoparticles are initially retained in implanted cells and then fully metabolized by the cells by 12 weeks of implantation. The T2* relaxation times of the MegaPro-labeled MSC were significantly shorter than those of the ferumoxytol-labeled cells, control cells and surrounding cartilage. Notably, the MegaPro signal persisted up to 4 weeks (compared with less than 2 weeks for ferumoxytol). Therefore, MegaPro shows great promise as a safe and long-lasting contrast agent for the longitudinal tracking of engrafted MSC in cartilage defects of arthritic joints. MegaPro labeling enabled visualization of MSC in cartilage defects on clinical-translational MRI scans and

correlations of the MSC engraftment with cartilage regeneration outcomes.

Keywords: Megapro, Nanoparticles, MRI

TOPIC: NEURAL

2091

CHARACTERISTICS OF ELECTRICAL ACTIVITIES IN BRAIN ORGANOIDS USING MEA AND HD-CMOS-MEA

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Brain organoids with three-dimensional structure are highly demanded for brain disease research and drug evaluation, and expected as samples approached for in vitro to in vivo extrapolation (IVIVE). We investigated electrical activity of healthy and Dravet Syndrome(DS) organoids and compared the frequency characteristics of spontaneous activity using micro-electrode array (MEA) and the response to phenytoin which is known as a contraindicated drug. Furthermore, we evaluated the firing patterns using a high-density (HD)- complementary metal–oxide semiconductor (CMOS)-MEA that can measure electrical activity at the single-neuron level in order to clarify the generation mechanism of the frequency characteristics. In spontaneous activities, the signals between 35 to 500Hz was particularly strong in healthy organoids. On the other hand, DS had strong signals between 4 to 50Hz and very low content above 80Hz. In response to phenytoin, the signals between 30 to 500Hz decreased concentration-dependently in healthy organoids, although the oscillation frequency increased at 30uM. Whereas, for DS, the frequency of oscillation increased in a concentration-dependent manner, and there was no change in signal strength for each frequency component. It is suggested that this is the result of reproducing seizure exacerbation by phenytoin in clinical DS. Moreover, by using a 236,880-electrodes CMOS-MEA, we succeeded in detecting the responses inside organoids at the single-neuron level. The frequency characteristics and response to phenytoin suggested that the DS organoids might reproduce the characteristics of the disease. MEA and HD-CMOS-MEA measurement of brain organoids will be useful for drug safety assessment and drug discovery in brain disease patient.

Keywords: Brain Organoid, Electrical activity, MEA

TOPIC: NO TISSUE SPECIFICITY

2092

INNATE IMMUNE CELL ACTIVATION CAUSES LUNG FIBROSIS IN A HUMANIZED MODEL OF LONG COVID

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COVID-19 remains a global pandemic of an unprecedented magnitude with millions of people now developing “COVID lung fibrosis” Single cell transcriptomics of lungs of patients with long COVID revealed a unique immune signature demonstrating the upregulation of key proinflammatory and innate immune effector genes CD47, IL-6 and JUN. We modeled the transition to lung fibrosis after COVID and profiled the immune response with single cell mass cytometry in JUN mice. These studies revealed that COVID mediated chronic immune activation reminiscent to long COVID in humans. It was characterized by increased CD47, IL-6 and pJUN expression which correlated with disease severity and pathogenic fibroblast populations. When we subsequently treated a humanized COVID lung fibrosis model by combined blockade of inflammation and fibrosis we not only ameliorated fibrosis but restored innate immune equilibrium indicating possible implications for clinical management of COVID lung fibrosis in patients. Significance Statement Post COVID-19 induced pulmonary fibrosis is the most severe of a number of clinical symptoms associated with “long COVID” a condition which is currently incompletely understood but affects millions given the ongoing COVID-19 pandemic. Using systems biology and mechanistic studies in a humanized mouse model of COVID lung fibrosis, we identify the essential genetic and immunologic perturbations occurring in patients with long COVID lung fibrosis and reveal promising therapeutic targets.

Funding Source: Boehringer-Ingelheim, Stinehart-Reed Foundation, Scleroderma Research Foundation, Ludwig Institute at Stanford University

Keywords: Long COVID pulmonary fibrosis, innate immunity, immune checkpoint therapy

2093

ESTABLISHMENT OF 3D-IPS CELLS USING CLOSED CULTURE SYSTEM

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Human induced pluripotent stem cells (hiPSCs) have great potential as source cells for cell therapy. We are investigating the use of a closed-type device for the establishment of hiPSCs in a low-cost and convenient operation. Until now, hiPSCs have generally been established under 2D (attached) conditions, but we have succeeded in establishing hiPSCs from whole blood under 3D (suspension) conditions. In addition, the closed device that we have focused was Thermo Fisher Scientific's Gibco™ CTS™ Rotea™ System, which is used for cell processing automation, but our process development allowed it to not only separate white blood cells from whole blood but also infect them with Sendai Virus vector carrying Yamanaka factors in the single-use flow path and establish hiPSCs from heterogeneous reprogramming after harvesting the processed cells. These results are expected to enable automation of cell production using hiPSCs in a closed culture system, and to realize low-cost cellular medicine. In this presentation, the characteristics of 3D-iPS cells and the 3D culture process including induction of cardiac differentiation will be discussed.

Keywords: 3D-iPS cells, Gibco™ CTS™ Rotea™ System, automation of cell production using hiPSCs

2094

PHDS-SEQ: A LARGE-SCALE PHENOTYPIC SCREENING METHOD FOR DRUG DISCOVERY THROUGH PARALLEL MULTI-READOUT QUANTIFICATION

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High-throughput phenotypic screening is a cornerstone of drug development and the main technical approach for stem cell research. However, simultaneous detection of activated core factors responsible for cell fate determination and accurate assessment of directional cell transition are difficult using conventional screening methods that focus on changes in only a few biomarkers. The PHDs-seq (Probe Hybridization based Drug screening by sequencing) platform was developed to evaluate compound function based on their transcriptional effects in a wide range of signature biomarkers. In this proof-of-concept demonstration, several sets of markers related to cell fate determination were profiled in adipocyte reprogramming from dermal fibroblasts. After validating the accuracy, sensitivity, and reproducibility of PHDs-seq data in molecular and cellular assays, a panel of 128 signaling-related compounds was screened for the ability to induce the reprogramming of keloid fibroblasts (KF) into adipocytes. Notably, the potent ATP-competitive VEGFR/PDGFR inhibitor compound, ABT869, was found to promote the transition from fibroblasts to adipocytes. This study highlights the power and accuracy of the PHDs-seq platform for high-throughput drug screening in stem cell research and supports its use in basic explorations of the molecular mechanisms underlying disease development.

Funding Source: the National Key Research and Development Program of China (2019YFA0110000, 2018YFA0800504), the National Natural Science Foundation of China (31922020), and funding provided by Plastech Pharmaceutical Technology Co., LTD.

Keywords: high-throughput screening, PHDs-seq, ABT869

TRACK: TISSUE STEM CELLS AND REGENERATION (TSC)

TOPIC: CARDIAC

2095

CARDIAC ADIPOSE TISSUE-DERIVED STEM CELLS FROM PATIENTS UNDERGOING CORONARY ARTERY BYPASS GRAFTING AMELIORATE CARDIAC FUNCTION AFTER ISCHEMIA-REPERFUSION INJURY IN MICE.

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Pluripotent stem cells in adipose tissue have attracted attention as a source of stem cells because of their easy accessibility. In patients undergoing coronary artery bypass surgery, it is common to remove fatty tissue around the aortic root. we investigate whether adipose tissue-derived stem cells collected from peri-cardiac (PA) fat tissue have better properties than those from subcutaneous (SC) fat. Following informed consent from cardiac surgery candidates, a small amount of fat tissue was harvested from PA and SC fat tissue beneath the skin incision at the coronary artery bypass grafting (6 males and 1 female; mean age 70.9 years). Adipose tissue-derived stem cells (AdSCs) were cultured for 3 passages to study in vitro cell properties, including the cell surface markers and gene expressions of tissue factors. To further investigate patients' PA- and SC-AdSCs of beneficial effects on ischemic myocardium, the ischemia-reperfusion injury (IR) was induced in nude mice, and AdSCs were injected into tail veins 3 days after IR; PA-and SC-AdSCs from a male patient of 70's with angina pectoris with plasma BNP level 166 pg/mL, and PA- and SC- AdSCs from another patient with same sex and similar age with old infarction with plasma BNP level 80 pg/mL. Four weeks after IR, echocardiography and LV-catheter measurements were recorded. PA fat (0.88±0.37 g) and SC fat (0.79±0.16 g) were collected. The cell density was significantly higher in SC fat, while AdSCs were more abundant in PA fat. Proliferative capacity was on average 1.3 times higher for PA- than SC-AdSCs. Anti-cytotoxic resistance was significantly higher in PA-AdSCs ($p < 0.01$), and migration capacity assessment showed no significant differences. Cell surface markers of both PA- and SC-AdSCs were positive for CD44, CD29, CD90, and CD105, and negative for CD45;

however, CD34 was varied in patients and tissues. In vivo studies showed that PA-AdSCs were more effective than SC-AdSCs in preserving LVEF and FS and preventing LVDd and Ds dilatation in the heart after IR. PA adipose tissue that can be easily harvested during cardiac surgery contains more stem cells than SC tissue. PA-AdSC has superior proliferative capacity and anti-cytotoxicity than SC-AdSC. PA-AdSC injection might attenuate cardiac dysfunction induced by ischemia.

Keywords: Adipose stem cell, Cardiac surgery, stem cell transplantation

TOPIC: EPITHELIAL INCLUDES SKIN GUT LUNG

2096

TREATMENT OF SALIVARY GLAND ORGANOTYPIC TISSUE CULTURE SLICES WITH MESENCHYMAL STEM CELL CONDITIONED MEDIA AMELIORATES RADIATION INJURY

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Background: Dry mouth, a consequence of radiation therapy, decreases the quality of life of cancer patients undergoing treatment. A decrease in the quality and quantity of saliva increases the chances of oral and systemic infections; causes difficulty in chewing and swallowing, leading to malnutrition. This morbidity lasts for the remainder of the patient's life. Due to patient discontent with the available therapies, stem cells are being explored to reduce this side effect. Previously, Mesenchymal stem cell-derived proteins were shown to reduce radiation damage in salivary glands in-vivo. Aim: To identify the mechanism of action of mesenchymal stem cell conditioned media (MSC-CM) in in-vitro and organotypic slice cultures. Methods: MSC-CM was obtained from minor salivary gland-derived MSCs after 24 hours of serum-free media culture. Primary salivary cells were cultured after enzymatic digestion of human salivary gland biopsies. 100–150-micron thick slices were sectioned separately for organotypic tissue culture. The cells and tissue slices were radiated and treated after an appropriate culture period. The following variables were investigated: DNA damage marker expression to assess recovery from radiation-induced damage, superoxide dismutase enzyme activity to measure oxidative stress, and viability to determine the action of the MSC-CM on cell survival. Additionally, salivary gland-specific protein expression was assessed using immunofluorescence. Outcome: An increase in salivary gland cell viability and proliferation was observed after treatment of the radiated cells with the conditioned media ($P \leq 0.05$). DNA damage (Gamma H2AX) and apoptotic genes (p53), which were increased after radiation, were decreased significantly after treatment ($P \leq 0.05$, and $P \leq 0.01$, respectively). SOX2 gene expression was higher in the treated tissues ($P \leq 0.05$). Moreover, the expression of AQP5(a crucial water channel protein) was maintained and localized towards the apical side of the saliva-secreting (acinar) cells. Conclusion: MSC-CM can reduce radiation-induced damage in salivary gland models in-vitro. Further studies to confirm the mechanism and the active proteins in MSC-CM can accelerate its translation as a treatment into the clinics, where oral dryness after radiation therapy remains a challenge.

Funding Source: Canadian Institutes of Health Research

Keywords: Mesenchymal stem cells, radiation injury, salivary glands

2097

REGENERATIVE POTENTIAL OF PRECONDITIONED HUMAN MESENCHYMAL STEM CELLS IN FULL THICKNESS ACID BURN WOUNDS

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Regeneration, a synchronized response to tissue injury, involves the interactions between multiple cell types, damaged vascular components, and extracellular matrix of the injured skin. In full thickness burn wounds, complete regeneration is not possible, and healing leaves hypertrophic scars. The direct transplantation of stem cells has promising effect in scar-free wound regeneration. However, the survival of stem cells at the wound bed is critical. Preconditioning of stem cells with the cytoprotective compounds can improve efficiency of stem cells for effective regeneration. This study aims to evaluate the effect of human mesenchymal stem cells (hMSCs) after preconditioning with alpha-terpineol (10µM) in efficient healing of full thickness acid burn wounds. hMSCs were isolated from the human umbilical cord tissue and characterized. Scratch assay was performed to analyze in vitro cell migration. Full thickness acid burn wound was induced in Wistar rats using sulphuric acid. Normal and preconditioned hMSCs were transplanted after 48 h of burn wound induction. Wound healing pattern was examined till day 40 and tissues were harvested at early (day 10) and late (day 40) phases of wound healing. Harvested tissues were evaluated by histological grading, gene and protein expression analysis, and immunohistochemical analysis. In scratch assay, preconditioned hMSCs showed enhanced wound closure compared to the control. In vivo results showed significantly improved healing potential in the preconditioned hMSCs group than that of normal MSCs and burn control. Similarly, histological analysis revealed intact collagen with regenerated skin layers and appendages, while enhanced angiogenesis in the late

phase of healing was also confirmed by immunohistochemistry and Western blotting. Gene expression of treated burn wounds showed significant reduction in the inflammatory and oxidative cytokines, and upregulation of pro/angiogenic and remodeling cytokines. It is concluded that preconditioning of hMSCs with alpha-terpineol enhances wound healing via reducing inflammation, and oxidative stress, and enhancing neovascularization with complete remodeling. Based on these results, an improved cell based therapeutic strategy can be designed for full thickness skin burn wounds in future.

Keywords: Full thickness acid burn wound, Human Mesenchymal Stem Cells, Alpha-terpineol

2098

HYPOXIC HUCMSC-DERIVED EXOSOMES MIR-542-3P PROMOTE CUTANEOUS WOUND HEALING BY ACTIVATION OF LATS1/YAP PATHWAY

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Chronic skin wound healing is an intractable, urgent clinical need. It can cause persistent inflammation and delayed healing due to hypoxia. The hypoxic microenvironment makes it difficult for the exogenous cells, such as stem cells, to live there and for therapy. Our previous study demonstrated that the Exosomes of human umbilical cord mesenchymal stem cells (hucMSC-Exo) can promote diabetic mouse skin wound healing. We also found that it significantly enhanced the survival and proliferation of hucMSC after culture in hypoxia environments compared to the normal oxygen. In this study, we aimed to investigate if hypoxic hucMSC-Exo (Hyp-Exo) participates in hypoxia adaptability and accelerates diabetic wound healing. We use a gelatin microbeads system to culture the hucMSCs under 21% and 1% oxygen to expansion cells in a short time. We demonstrate that hypoxia enhances the paracrine function of hucMSCs secret exosomes and increases HaCaT cell proliferation, and migration, and inhibits cell apoptosis. In a mouse full-thickness skin injury model, Hyp-Exo can accelerate wound healing better than normoxia hucMSC-Exo (Nor-Exo). The miRNA sequencing showed that there are 77 miRNAs were upregulated and 49 miRNAs downregulated in Hyp-Exo compared to Nor-Exo. According to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Gnomes (KEGG), miRNA may be involved in regulating cell metabolism, and differentiation through the Hippo signaling pathway. microRNA-542-3p (miR-542-3p) transcription is increased by Hyp-Exo and gene screening predicted that large tumor suppressor kinase 1 (LATS1) may be the target gene of miR-542-3p. It is demonstrated by a double luciferase assay. After being packaged into hypoxic exosomes and transported to HaCaT cells, miR-542-3p targets and suppresses the expression LATS1 while the expression of YAP and downstream target genes CTGF and CYR61 was increased. Overexpression of miR-542-3p in HaCaT cells can alleviate LPS-induced HaCaT cell inflammation and enhances cell proliferation and migration while knockdown miR-542-3p has the opposite results. Our findings reveal communication between hucMSCs and HaCaT cells via exosomal miR-542-3p/LATS1/YAP signaling in the hypoxic microenvironment and present hypoxic exosomes as a promising therapeutic strategy to enhance skin wound healing.

Keywords: Hypoxia, Exosomes, miR-542-3p, LAST1, YAP, skin wound healing

2099

OPTIMIZING LIMBAL STEM CELL DIFFERENTIATION THROUGH MANIPULATION OF MICROENVIRONMENT

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Limbal stem cells (LSCs) are the adult stem cells (i.e., unipotent) of the corneal epithelium residing in an area known as limbus, which borders the circumference of the cornea. The LSCs are responsible for maintaining homeostasis of the corneal epithelium by differentiating and migrating to corneal epithelium. Limbal stem cell deficiency (LSCD) is a debilitating ocular surface disorder occurring due to the loss or dysfunction of LSCs. However, treatment of LSCD is exacerbated by a limited supply of healthy LSCs, especially in the case of bilateral LCSD. LSCs derived from induced pluripotent stem cells (iPSCs) have the advantage of being an unlimited and non-immunogenic source. We aim to develop a xeno-free and chemically defined protocol for differentiating iPSCs to LSCs. Through inhibition of Wnt signaling and TGFβ with activating FGF signaling for 4 days we were able to direct the differentiation toward corneal epithelium using different synthetic matrices. During day 10 of the differentiation protocol, putative makers (p63α) of LSCs were expressed. After day 25, makers associated with maturation (CK 12) were expressed. We further aim to optimize the differentiation protocol by recapitulating the 3D microenvironment synthetically as a graft for treatment of LSCD. This will lead to the development of a simpler and well-defined protocol to develop clinically relevant iPSC-LSC compared to existing established protocols.

Funding Source: Wilson College of Textiles, North Carolina State University

Keywords: Cornea, Differentiation, limbal stem cell deficiency

2100

MESENCHYMAL STEM CELLS REGENERATE SKIN TISSUE IN COLD INDUCED RAT BURN WOUNDS

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Burn wounds remain one of the most common injuries worldwide. Wound healing phases and associated mediators are crucial for skin regeneration. Mesenchymal stem cells (MSCs) secrete various paracrine factors which aid in wound healing via acceleration of cell migration, angiogenesis, tissue granulation, and modulation of inflammation at the wound site. This study aims to investigate the therapeutic effect of human umbilical cord MSCs (hUMSCs) in the skin tissue regeneration following a cold induced rat burn wound model. hUMSCs were isolated, and characterized by immunocytochemistry and flow cytometry. An in vivo cold burn wound model was developed and hUMSCs were locally transplanted. Macroscopic analysis of wound closure was done at days 1, 3, 7 and 14 corresponding to wound healing phases. Gene expression, histological and immunohistochemical analyses were performed to evaluate wound repair and regeneration. Wound area was remarkably reduced in the burn wound model transplanted with hUMSCs well before the end of the experimental period (day 14). Histological analysis showed intact collagen with regenerated epidermis, dermis and hair follicles, while immunohistochemistry displayed enhanced angiogenesis in the last phase of healing in the transplanted group. Temporal gene expression analysis showed significant downregulation of inflammatory cytokines and upregulation of pro/angiogenic and remodeling cytokines at particular time points. It is concluded from this study that hUMSCs accelerate wound closure with enhanced neovascularization and reduced inflammation, leading to effective skin tissue regeneration.

Funding Source: Higher Education Commission, Pakistan

Keywords: Skin Regeneration, Dermal, Repair

2101

LINEAGE PLASTICITY OF AIRWAY SECRETORY CELLS IS REGULATED BY THE TISSUE MICROENVIRONMENT

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Secretory Club cells (CCs) of the lower airways are highly plastic multifunctional cells that metabolise xenobiotics, regulate inflammation and generate other cell types during homeostasis and post-injury repair. Understanding the signals and mechanisms regulating the fate of CCs is of vital importance due to their versatility. Notch signalling has been shown to specify CC fate during development but how the fate of CCs is regulated in the adult lung remains ambiguous. Using pharmacological and genetic approaches to perturb canonical Notch signalling in CCs, we probed the dependence of CC fate on Notch in adult mice lungs. Our findings show that the Notch pathway actively controls the fate of all CCs throughout the airways. Interestingly, different CCs respond differently to Notch inhibition. While bulk CCs transdifferentiate into multiciliated cells, variant-CCs (v-CCs), a subpopulation corralling Neuroepithelial Bodies and at Bronchioalveolar Duct Junctions do not. v-CCs resist transdifferentiation and transition into lineage ambiguous states instead. Ablation of the ligand-expressing ciliated cells also led to v-CCs adopting similar lineage trajectories. Lineage analysis of v-CCs post-acute Notch inhibition shows that these cells revert to a CC fate and generate v-CCs in these microenvironments. They also generate CCs and ciliated cells further away in the long term. The v-CC-derived progeny that moves away from the tissue microenvironment do not retain the characteristics of the parent v-CCs and transdifferentiate into ciliated cells upon subsequent Notch inhibition. We hypothesise that the differential behaviour of v-CCs results from interactions/signals with the extracellular matrix and/or other cell types in these tissue microenvironments. Taken together, our findings show that CC fate is actively controlled by canonical Notch signalling and that the tissue microenvironment regulates the lineage plasticity of CCs. Finally, we propose that v-CCs constitute a progenitor pool that in conditions of severe perturbation to CCs and ciliated cells, generates and replaces both cell types and restores the balance in the long term.

Keywords: Club cells, variant-Club cells, Ciliated cells, Lung, Neuroepithelial Bodies, Bronchioalveolar Duct Junctions, Tissue microenvironment, Notch, Active control of cell fate, Plasticity

TOPIC: HEMATOPOIETIC, IMMUNE, AND ENDOTHELIAL

2102

SINGLE-CELL ANALYSIS REVEALS DISTINCT TRANSCRIPTOME SIGNATURE OF HSPCS UNDER CHEMOTHERAPY STRESS IN B-ALL

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The hematopoietic system is a highly organized system in which self-renewing hematopoietic stem cells (HSCs) divide and differentiate into all blood cell types. During leukemia progression, hematopoietic populations undergo stress while competing in the BM environment against the highly proliferating leukemic clones. Despite the major advances made in our understanding of the molecular mechanisms governing the phenotypes and function of normal HSPCs and leukemic cells, little is known about the in vivo dynamics of HSPCs in response to chemotherapy and during recovery in leukemic patients. This information barrier is mainly due to the lack of a proper disease model that fully resembles the leukemic niche in vivo and a lack of advanced technology that can accurately decipher the molecular features of heterogeneous and rare HSPC populations in leukemic patients. In this study, we applied scRNA-seq to investigate the transcriptomic signature of normal HSPCs in B-cell acute lymphoblastic leukemia (B-ALL). This condition is an ideal model, as the recurring genetically driven alterations originate from B cell progenitors, while normal HSCs are unaffected by these intrinsic genetic alterations. We obtained BM samples from a group of healthy donors and B-ALL patients with good and poor recovery profiles at matched diagnosis, treatment, and recovery timepoints. More than 156,000 high-quality transcriptomes of human HSPCs from B-ALL patients were acquired for scRNA-seq analysis. Our data revealed that, although all 22 identified cell types could be found in all sequenced samples, the transcriptomic features of HSPCs differed between B-ALL patient and healthy donor samples. HSCs in newly diagnostic patients had activated interferon and immune response pathways and increased cell differentiation potential. Additionally, chemotherapy treatment reversed the increased proportion of HSCs observed at diagnosis, causing them to be less differentiated and have inactive interferon and immune response pathways. We also revealed the poor and good recovery-related-phenotypic and mo-

lecular signatures of HSCs in B-ALL. These findings suggest that therapies targeting interferon signaling might help improve the treatment effects in B-ALL patients who are unresponsive to chemotherapy.

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Keywords: Hematopoietic stem and progenitor cells, B-cell acute lymphoblastic leukemia, Single-cell transcriptomic profiling

2103

RNA BINDING PROTEIN HNRNPF REGULATES HEMATOPOIETIC STEM CELL QUIESCENCE AT POST-TRANSCRIPTION LEVEL

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RNA binding proteins are key regulators of transcripts throughout their life cycle, and thus they may play pivotal roles in regulating the function of hematopoietic stem and progenitor cells (HSPCs). Here we identified an RNA binding protein, hnRNPF, which is highly expressed in HSPCs compared to differentiated hematopoietic cells. Conditional knockout (cKO) of hnRNPF in adult hematopoietic cells promoted the proliferation of HSPCs in vivo. Bone marrow transplantation assay revealed that deletion of hnRNPF compromised the reconstitution capacity of hematopoietic stem cells (HSCs) in primary and secondary recipient mice. RNA-seq analysis showed that very few genes were changed in HSCs after hnRNPF depletion, implying that the hnRNPF is not important for the regulation of HSCs at transcription level. Moreover, hnRNPF depletion led to the abnormal splicing of Cdk2 and Cdk4, resulting in increased level of short isoform of CDK2. The abnormal splicing of cell cycle genes maybe the cause for HSC exiting the resting state after hnRNPF depletion. HNRNPF protein was more localized in the cytoplasm in HSPCs, which suggests that HNRNPF has other functions besides regulating splicing. Cytoplasmic protein interaction analysis showed that hnRNPF could directly bind to translation elongation factors EEF2 and EEF1A1, but not affect their expression. We isolated the ribosomes of HSPCs and found that HNRNPF affected EEF2 and EEF1A1 loading to the ribosome, leading to a decrease in translation efficiency of HSPCs. In addition, we found the translation of key cell cycle and lysosome genes was decreased via Ribo-seq. Taken together, our current study demonstrates that hnRNPF regulates the quiescence and function of HSCs at post-transcription level.

Keywords: RNA binding protein, Haemopoietic stem cell, Quiescence

TOPIC: KIDNEY

2104

GENERATION OF EPO-OVEREXPRESSING KIDNEY ORGANOIDS BY DNA VECTORS

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The kidney plays an important endocrine role, including the secretion of erythropoietin (EPO) under hypoxia. EPO production is impaired in kidney disease patients, leading to anemia. Kidney organoids derived from human induced pluripotent stem cells (iPSC) are a model to study restoration of EPO production. We aimed to generate stable EPO-overexpressing (EPO-oe) kidney organoids using DNA vectors to study the effects of EPO on kidney development and examine the physiological effects of EPO-oe organoids. A scaffold/matrix attachment region (S/MAR) DNA vector was used to genetically modify iPSC for EPO overexpression. The EPO-oe organoids were characterized using markers including PODXL for podocytes, Villin-1 for proximal tubular cells and E-cadherin for distal tubular cells. To study whether EPO-oe organoids had physiological effects, EPO-oe kidney organoids were subcutaneously implanted in immuno-deficient mice for one month. In control organoids, EPO was undetectable at transcript and protein levels. After 24 hours in hypoxia, EPO was detected at 0.43 (interquartile range [IQR] 0.34-0.59) mIU/ml in the supernatant. This low level of EPO was inadequate for functional studies. EPO-oe kidney organoids produced constitutive high levels of EPO mRNA expression throughout their differentiation. EPO levels in the supernatant were 968 mIU/ day per EPO-oe organoid (IQR 908-1318). Immunohistochemistry staining showed that EPO-oe kidney organoids formed similar kidney structures compared to control organoids and the expression

of the markers PODXL, Villin-1 and E-cadherin was not changed. One-month after implantation, EPOoe organoids expressed EPO at mRNA level. Hematocrit levels were significantly increased in mice that received EPO-oe organoids (0.48, IQR 0.44-0.55) compared with mice that received control organoids (0.40, IQR 0.40-0.41) (p=0.0092, n=9). In conclusion, S/MAR DNA vectors can be used to generate robust and stable transgene expression in iPSC throughout their differentiation into kidney organoids. EPO-oe human kidney organoids showed high levels of sustained and functional EPO release in vitro and in vivo. The implantation of functional EPO-secreting organoids envisions the application of gene-edited kidney organoids for regenerative therapy.

Keywords: Kidney organoids, DNA vector, EPO

2105

KINASE B INHIBITION PROMOTES KIDNEY REPAIR THROUGH HNF1B-MEDIATED RENAL TUBULAR EPITHELIAL CELL REVITALIZATION

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Renal tubular epithelial cells (RTECs) can lose fatty acid metabolism and transport functions while acquiring pro-fibrosis and pro-inflammatory phenotypes in acute kidney injury (AKI), which results in maladaptive repair and AKI transition to chronic kidney disease (CKD). Here, using high-throughput chemical screening based on morphological and transcriptional profiles, we identified a kinase inhibitor, Compound EC, which can reverse these injury-associated phenotypes, re-establish a pre-injury transcriptional profile, and restore fatty acid metabolism and transport functions in injured RTECs. Mechanistically, Compound EC inhibits kinase B, which binds the RTEC identity determinant, HNF1B, reducing HNF1B protein level. Kinase B inhibition by Compound EC or knockdown restores HNF1B expression, leading to RTEC revitalization. In ischemic AKI mice, Compound EC increases survival rate, improves renal function, and alleviates kidney damage, ultimately attenuating AKI to CKD transition. Summarily, this study establishes an RTEC revitalization strategy and suggests a chemical-based regenerative medicine approach targeting kinase B/HNF1B for kidney repair through RTEC regeneration.

Keywords: Renal Tubular Epithelial Cells, Chemical-induced Revitalization, HNF1B

TOPIC: LIVER

2106

LACTATE PROMOTES MORE EFFICIENT HEPATOCYTE DIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELLS IN COMAPRISON TO SODIUM LACTATE AND CALCIUM LACTATE

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Glucose is an essential nutrient that maintains cellular energy homeostasis. Only hepatocytes can metabolize galactose to glucose through gluconeogenesis. These cells when co-cultured with induced pluripotent stem (iPS) cells in a medium containing only galactose and not glucose (hepatocyte selection medium, HSM), become isolated and enriched. Furthermore, when calcium lactate is added to HSM, iPS cells survive for seven days, and their hepatocyte differentiation property is enhanced. In order to delineate which of the three forms of lactate, namely lactate, sodium lactate, and calcium lactate, would be best suitable for hepatocyte differentiation, they were added to HSM (1mM), and cultured for four days. The media were changed for maintaining the undifferentiated state, and cultured for seven days. Following RNA isolation, cDNA was synthesized and subjected to real-time PCR to quantify the expression of alpha-feto protein, albumin, SOX17, and NANOG. Higher scores were allocated to each form of lactate based on higher levels of expression of alpha-feto protein, albumin, SOX17 and lower levels of NANOG expression. Scores of alpha-feto protein and albumin were added for each form of lactate and was found to be highest for lactate followed by sodium lactate and calcium lactate. Therefore, it could be suggested that lactate was most suitable for hepatocyte differentiation of iPS cells. Future studies should involve culturing iPS cells in spheroids under HSM containing lactate for more efficient hepatocyte differentiation.

Keywords: hepatocyte, glucose, lactate

TOPIC: MESENCHYMAL STROMAL CELLS, ADIPOSE, AND CONNECTIVE TISSUE

2107

A COMPARISON OF PHENOTYPIC AND FUNCTIONAL PROPERTIES OF PRECONDITIONED MESENCHYMAL STEM CELLS

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Cell therapy in regenerative medicine is largely determined by the use of mesenchymal stem cells (MSCs), known to affect regenerative processes due to the presence of a wide range of membrane receptors and secreted factors. Despite the fact that MSCs are distinguished by the ease of isolation and pro-regenerative properties, the high heterogeneity of these cells is a major obstacle to their effective use in clinical practice. In the present study, we attempted to modulate the phenotypic properties of MSCs and their regulatory activity using a controlled inflammatory and anti-inflammatory microenvironment. We divided MSCs into groups that were treated separately with proinflammatory factors, - lipopolysaccharide (LPS), tumor necrosis factor alpha (TNF-α), interleukin-17 (IL-17), and conditionally anti-inflammatory factors, polyriboinosinic:polyribocytidylic acid (p(I:C)) and interleukin 4 (IL-4). MSCs treated with proinflammatory agents demonstrated increased expression levels of IL1, IL6, IL8 and intercellular adhesion molecule 1 (ICAM1). TNF-α treatment of MSCs upregulated the expression of plasminogen activator inhibitor 2 (PAI2), transcription factor STAT1 and superoxide dismutase-2 (SOD2). Significant difference in the regulation of secretion between LPS and p(I:C) treated MSCs were noted for IP10, GM-CSF, RANTES, TNF-α, IL8, MCP3, GRO1 and PDGF-AB/BB. The results of mitochondrial activity assessment by seahorse-assay showed that MSCs tended to increase spare respiratory capacity in the presence of p(I:C) and IL4, while tended to decrease it when treated with pro-inflammatory agents. Treatment of MSCs with inflammatory factors results in enhanced autophagy as assessed by increased light chain protein 3 (LC3II/I) forms ratio and upregulated sequestosome 1 (SQSTM1) expression as well as by dansylcadaverine imaging. After inflammatory exposure MSCs also demonstrated an increased ability to stimulate angiogenesis. This data indicates that proinflammatory stimulation of adipose-derived MSCs mediates the modulation of their therapeutic properties, which is accompanied by the acquisition of a new phenotype by these cells, as evidenced by the specific expression profile and the alteration of autophagy processes in preconditioned MSCs.

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Keywords: mesenchymal stem cells, autophagy, inflammation

2108

THE INFLUENCE OF BIOMIMETIC CONDITIONS ON NEUROGENIC AND NEUROPROTECTIVE PROPERTIES OF DEDIFFERENTIATED FAT CELLS (DFAT CELLS)

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In the era of a constantly growing number of reports on mesenchymal stem/stromal cell (MSC) therapeutic properties and their use in the treatment of neurological diseases, the optimization of their derivation and long-term culture methods seems to be crucial. The solution is seen in the use of dedifferentiated fat cells (DFAT cells), which are characterized by a greater homogeneity. Moreover, these cells seem to possess a higher expression of transcriptional factors necessary to maintain pluripotency (STRFs), greater ability to differentiate in vitro into three embryonic germ layers, and higher proliferative potential in comparison to adipose stem/stromal cells (ADSCs). However, the neurogenic and neuroprotective potential of DFAT cells is still insufficiently understood, thus, it became the subject of our research. To recreate the brain's physiological (biomimetic) conditions, the cells were cultured at 5% oxygen concentration. The neural differentiation capacity of DFAT cells was assessed in the presence of the N21 supplement (containing factors found in the natural environment of the neural cell niche) or the presence of cerebrospinal fluid (CSF) and under various spatial conditions (microprinting). The neuroprotective properties of DFAT cells were assessed by performing the co-culture with ischemically damaged organotypic hippocampal slices.

Funding Source: Medical Research Agency grant no. 2020/ABM/01/00014

Keywords: DFAT cells, cell niche, cerebrospinal fluid

2109

CHANGES IN EXPRESSION OF NEURAL, ANGIOGENIC, AND STRF GENES IN DIRECT AND INDIRECT CO-CULTURE OF MESENCHYMAL STEM/STROMAL CELLS AND ENDOTHELIAL CELLS

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The interactions between neural, endothelial, and supportive/stromal cells have an impact on the fate of stem cells in the neural niche. Both, direct cell-cell contact and indirect paracrine

signals regulate niche homeostasis. The supportive cells may play a crucial role in neuro- and vasculoprotection, the integrity of the neurovascular unit, and the quiescent state of stem/progenitor cells. Mesenchymal stem/stromal cells (MSCs) have the capacity to interact and support angiogenesis and regeneration. Additionally, MSCs derived from Wharton's jelly spontaneously express neural genes, neural markers and secrete neurotrophic factors. Hence MSCs are proposed as a therapeutic approach to promote neuroregeneration. Understanding the interactions between the different components of a cell niche may help in its proper maintenance. Moreover, the 3D culture environment is important to normal cell physiology and biomimetic conditions. Cell-to-cell adhesion and cytoskeletal arrangement are critical factors that drive the formation of 3D cellular spheroids. The aim of the present study was to analyze the changes in neural, angiogenic, and stemness-related transcriptional factors (SRTF) genes expression in two types of interaction models of supportive cells (WJ-MSCs- MSCs derived from Wharton's jelly) and endothelial cells (HUVEC). In this research, we used quantitative real-time PCR (qPCR) as a method for quantitative analysis of targeted gene expression. We compared three groups of genes into 2D trans-well and 3D spheroid co-cultures. In our study, we observed spontaneous formulation of spheres (WJ-MSC) and co-spheres (WJMSC-HUVEC) on the non-adhesive surface. During the formation of the co-spheres, HUVECs were located inside the structure. Monoculture of HUVECs did not show the ability to self-organize into spheres. These cells formed irregular aggregates. In genes expression analysis, we observed higher neural and SRTF genes express levels in spatial model of culture (3D) than in 2D culture, while the increased expression of angiogenic genes was insignificant. The effect was observed in both mono- and co-cultures. Concluding, spatial method of culture increase neural and SRTF gene expression.

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Keywords: WJMSC-HUVEC co-spheres, mesenchymal stem/stromal cells, WJMSC-HUVEC co-culture

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OVEREXPRESSION OF FIBROBLAST GROWTH FACTOR RECEPTOR 2 IN BONE MARROW STEM CELLS ENHANCES OSTEOGENESIS AND PROMOTES CRITICAL CRANIAL DEFECT REGENERATION

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Reconstruction of cranial bone defects is one of the most challenging problems in reconstructive surgery. Availability of bone is often limited, thus critical-size bone defect often need substitutes to reconstruct. Fibroblast growth factor receptor 2 (Fgfr2) is an important regulator of bone construction. However, its role in osteogenesis of bone marrow stem cells (BMSCs) at different stages remains unclear. This article clarifies the function of Fgfr2 in BMSCs and explores the role of Fgfr2-overexpressed BMSCs combining photocured GelMA in the repair of cranial bone defects. Lenti-virus was used to overexpress Fgfr2 in BMSCs. Cell counting kit 8, transwell and flow cytometry were conducted to investigate the proliferation, migration and stemness. After 0,3,7,10 ,21days of osteogenic induction, the changes of osteogenic ability were detected by real-time quantitative PCR, westernblot, alkaline phosphatase and alizarin red staining. To investigate the viability of BMSCs after mixed with photocured GelMA, calcein and propyl iodide staining was performed. Finally, critical cranial bone defect model was established and micro-computerized tomography, masson staining, immunohistochemistry were constructed to explore the promoting effect of Fgfr2-overexpressed BMSCs with photocured GelMA. In none-osteogenic environment, overexpression of Fgfr2 in BMSCs significantly promoted proliferation and migration, and increased the percentage of CD200&CD105 double-positive cells. Under an osteogenic induction, Fgfr2 overexpression enhanced the osteogenic ability of BMSCs, with higher expression of Runx2, Alp, and Col1a1. BMSCs mixed with photocured GelMA showed favourable viability. Compared with normal BMSCs, Fgfr2-overexpressed BMSCs could better promote the repair of critical cranial defects, accompanied by more new bone formation. Fgfr2 promotes proliferation and migration of BMSCs and maintains cell stemness during the non-osteogenic state and promotes osteogenesis and mineralization after osteogenic induction. BMSCs with Fgfr2 overexpression combined with photocured GelMA is a good choice for the treatment of critical cranial bone defects.

Keywords: fibroblast growth factor receptor 2, mesenchymal stem cells, bone regeneration

2111

NESTIN-GFP+ PROGENITORS OF THE BONE MARROW ARE RETAINED IN ECTOPIC FOCI OF HEMATOPOIESIS FORMED IN RECIPIENTS IMMUNIZED AGAINST GFP

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Immune privileges (IPs) were demonstrated for stem cells of different tissues origins, such as hair follicle stem cells, muscle stem cells, hematopoietic stem cells, and mesenchymal stem cells (MSCs). In the latter case, we showed IPs by means of Nestin-GFP mouse strain, in which several types of stem cells are marked with GFP expression including MSCs. GFP is a well-established immunogeneic protein, which makes this strain a convenient tool for assessing IPs of different stem and progenitor cells. We implanted bone marrow (BM) of Nestin-GFP under the renal capsule of isogenic C57Black/6J mice lacking transgene. The ectopic foci of hematopoiesis were formed containing alive GFP+ cells 6 weeks after implantation procedure. The long-standing presence of GFP+ cells allowed us to conclude about their IPs in vivo. Demonstration of IPs for Nestin-GFP expressing cells in BM in addition to literature data contributes to idea that IPs are a common property of stem cells from different origins and even cancer stem cells. We have noted that such immune privileged stem cells may also be associated with nestin expression in adult mammals. However, the power of MSCs privileges was an open question.

In this study we conducted a similar experiment, but immunized the recipients before the implantation of the BM. The foci were obtained, and GFP+ cells were registered in them. We connected the success of our approach with three key aspects: a) we concentrated our attention on small compartment of stem cells rather than considering all mesenchymal stromal cells to be immune privileged; b) the immunogenic marker was presented only on a small subpopulation of transplanted cells, while most cells were immunologically equivalent; c) minimally disturbed BM fragments were used for transplantation. Our new results strengthen previous data showing that the observed GFP+ progenitors including MSCs are immune privileged against full-fledged and activated immune system. Summarizing we contribute to a concept that full-fledged IPs are a property of quiescent stem cells. That fundamental conception provides a new perspective not only for understanding stem cells regulation in health, but for oncogenes as well.

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Keywords: nestin-GFP, immune privileges, ectopic foci of hematopoiesis

2112

THE NOVEL 3D TISSUE-LIKE HUMAN ENDOMETRIUM MODEL IN VITRO

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The challenges of restoring and to cure women's reproductive health issues draw a lot of attention of the world scientific society. One of the most important factors in the pathogenesis of female reproductive disorders is an endometrial dysfunction. Three-dimensional (3D) endometrial mesenchymal stromal/stem cells (eMSC) culture systems are promising tools to study the endometrial function in vitro. The 3D cultivation of the eMSCs is possible in the form of simple systems such as cell sheets and spheroids, as well as of more complex scaffold-based systems. These tissue-like structural eMSC platforms provide the good models to study the endometrium intercellular interactions alongside with the microenvironment similar to those observed in vivo. In this study, we developed a hybrid 3D cultivation system to create a functional 3D model of the human endometrium. For this purpose, the human eMSCs (obtained from the desquamated endometrium from menstrual blood) and HUVECs were co-cultivated in spheroids and incorporated into an apple-derived decellularized porous scaffold coated with type I collagen. Then the derived epithelial endometrial cells were seeded on top of the scaffold. One of the advantages of this 3D hybrid model is that it includes all main cellular components as well as the extracellular matrix of the human endometrium. The other advantage is the possibility to achieve the cell density inside of the scaffold comparable to the native tissue, while the cells retained their proliferation activity and viability during a long-term cultivation. The functionality of this model was confirmed by the ability of the eMSCs within the 3D culture system to respond to hormonal stimulation by differentiation into endometrial tissue-specific decidual cells. Moreover, our preliminary data shows that the developed model can be considered as physiologically relevant to mimic the human menstrual cycle. To summarize, this 3D tissue engineering construct is valued as a tool for both fundamental study of normal and pathological conditions of the endometrium as well as for translational medicine approaches to cure reproductive diseases.

Funding Source: Russian Science Foundation grant #22-74-10126

Keywords: Endometrium, Mesenchymal Stromal Cells, 3D Cell Culture

2113

CONDITIONED MEDIUM OF MESENCHYMAL STROMAL/STEM CELLS CULTURED ON DECELLULARIZED MATRIX IMPROVES SCRATCH WOUND HEALING IN VITRO

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The mesenchymal stromal/stem cells (MSCs) are considered to be promising therapeutic tools for various clinical applications due to the secretion of paracrine factors promoting tissue regeneration. There are several preconditioning methods such as hypoxic culture conditions or pharmacological treatment were developed to improve MSCs secretory activity. The number of studies demonstrate that the decellularized extracellular matrix (dECM), the complex meshwork of proteins, have a comprehensive impact on cell functions, mimics the natural cellular environment and provides a more physiologically relevant substrate compared to routinely used culture plastic. However, the issue of whether dECM modulates MSCs secretome remains unknown. Using the publicly available transcriptomic dataset (Ragelle et al., 2017), we found that cultivation of the MSCs on the dECM leads to a significant upregulation of several genes coding secretome constituents, such as MMP-1, HGF, PGF and chemokines of CXCL family. We hypothesized that the MSCs cultivation on the dECM can modulate MSCs secretome. In order to test the hypothesis, we cultivated human MSCs of various origin (derived from endometrium, fetal bone marrow, Wharton's jelly and dental pulp) on the dECMs produced by previously cultured MSCs. Strong upregulation of IL8, CXCL5 and CXCL6 chemokines in MSCs cultured on dECM was validated by means of RT-qPCR. We found that conditioned media from the MSCs cultured on dECM significantly improved 3T3 fibroblasts scratch wound healing compared to conditioned media from the MSCs cultured on plastic. It suggests that the cultivation of the MSCs of the different origin on the dECM may lead to enrichment of the conditioned medium in some biologically active components that correlates with chemokine upregulation. These results allow us to suggest that MSCs cultivation on dECM may be considered as a novel preconditioning approach. It could be suggested that integrin receptors transmitting signals from extracellular matrix may be responsible for upregulation of the above-mentioned pro-inflammatory and angiogenic chemokines.

It could be a tentative explanation of how integrins contribute to inflammation and angiogenesis.

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Keywords: extracellular matrix, MSCs preconditioning, secretome

2114

MESENCHYMAL STROMAL CELLS AFFECT CD8 NAIVE TO MEMORY SUBSET POLARIZATION BY DOWN-MODULATING IL12Rβ1 AND IL2Rα SIGNALING PATHWAYS

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Mesenchymal stromal cells (MSCs) have been investigated for their potential therapeutic applications in a variety of diseases in which a dysregulated immune response plays a central role in pathogenesis or progression. MSCs have been shown to contribute to therapeutic effects due to their immunomodulatory activity on various subsets of the immune system belonging to both innate and adaptive immunity. Notably, for adaptive immunity, MSCs can reduce the activation and cytotoxic activity of CD8 T lymphocytes and the polarization of CD4 T lymphocytes toward inflammatory subsets and instead favor polarization toward the immunoregulatory Treg subset. Although MSCs have been widely reported to impact T cell proliferation and polarization, there are no studies that report how MSCs affect T-cell commitment toward memory subsets. Here, we report for the first time that MSCs isolated from the amniotic membrane of human term placenta (hAMSC) modulate naïve CD8 cell activation and differentiation by downregulating mTOR pathway activation and modulating the expression of Tbet and Eomes, two of the major transcription factors whose balance is involved in polarizing naïve CD8 cells toward memory precursor effector cells (MPEC). This effect is due to the ability of hAMSC to reduce the phosphorylation of STAT4 and STAT5, two transcriptional factors downstream the IL -12Rβ1, IL -2Rα receptors. Our results contribute to the understanding of one of the possible mechanisms of action of MSCs, and may provide new interpretations on the effects of MSCs when used for the treatment of diseases in which the memory response plays a central role, such as autoimmune diseases and graft versus host disease.

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Keywords: Mesenchymal stromal cells, CD8 T lymphocyte immunology, T Memory subset polarization

2115

MESENCHYMAL STEM/STROMAL CELLS-DERIVED EXOSOMES CONTAIN HIGHER LEVELS OF IMMUNOMODULATORY AND NEUROPROTECTIVE FACTORS THAN THE SOLUBLE SECRETOME OF MSCS

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Mesenchymal stem/stromal cells (MSCs) have regenerative properties - through secretion of different immunomodulatory and neuroprotective factors they can modulate the process of inflammation and regeneration in situ. These factors are either released directly into the environment (described as 'soluble secretome') or are firstly encapsulated within exosomes. Exosomes are small structures, spherical in shape and surrounded by two-layer lipid film. They are the smallest of all extracellular vesicles (EV) and play a key role in cell to cell communication. Exosomes originate from endosomal compartments, known as multi-vesicular bodies (MVBs) and are involved in intercellular communication via the transfer of numerous membrane receptors, proteins, lipids, RNA and miRNA between cells. As exosomes contain many different factors, they can have a great therapeutic potential as MSCs themselves. There are several potential advantages of exosomes when compared to MSCs alone, which are: 1) inability to self-replicate suggesting no risk of uncontrolled growth; 2) limited potential to trigger the immune system and 3) easier transport and storage, which makes cellular-free treatment more promising when compared to standard MSC-based therapies. In order to investigate the secretory profile of adipose-derived mesenchymal stem/stromal cells (ASCs) a Luminex Assay was performed. Secretory profile analysis revealed that most of the investigated factors were encapsulated inside the exosomes, rather than secreted directly into the microenvironment. The ASCs-derived exosomes contained higher levels of BDNF, FGF, beta-NGF, EGF, LIF than the soluble secretome of ASCs. Although, when it comes to VEGF, higher levels of this factor were detected to be the part of soluble secretome rather than encapsulated within the exosomes. The obtained results provide important knowledge regarding the secretory profile of ASCs – it seems that therapeutic properties of ASCs rely mostly on the released exosomes as they contain a vast majority of the secreted factors and therefore should be considered for therapy rather than ASCs themselves.

Funding Source: This research was funded by 2020/ABM/01/00014-00 grant.

Keywords: adipose-derived mesenchymal stem/stromal cells, extracellular vesicles, exosomes

2116

PRIMING WITH NEUROTROPHIC FACTORS ENHANCES THE NEURO-REGENERATIVE PROPERTIES OF MESENCHYMAL STROMAL CELLS DERIVED SECRETOME

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Neurodegenerative diseases (ND) are characterized by the progressive and irreversible loss of neurons. The lack of in vitro model systems that mimic the in vivo situations of chronic insults is a major hurdle for developing effective drugs against ND. Therefore, the primary aim of this study was to establish a physiologically relevant in vitro ND model system by inducing oxidative stress (OS) and endoplasmic reticulum stress (ERS) in such a way that the cells remain viable but lose their structural and functional characteristics. Using Neuro-2a cells, we have shown that induction of OS and ERS significantly affects the neurite outgrowth parameters (NOPs) and also reduces the expression of neuronal markers such as βIII tubulin and Map2 in them. Although induction of OS led to a decreased expression of autophagy markers - Lc3a, Lc3b, Becn1, and Atg7 - at an mRNA and protein level, the ERS model did not depict so. Nevertheless, ERS induction elevated the expression of inflammatory markers NF-κB and Cox2 and stress kinases p38-MAPK, SAPK, and JNK in them. The role of mesenchymal stromal cells (MSCs) derived secretome in neuronal regeneration is well documented. Using OS and ERS model systems, we have shown that as compared to the unprimed MSC secretome, priming of MSCs with neurotrophic factors boosts the regenerative potential of their secretome. Here we further show that the salutary effects of the primed MSC secretome are exerted by their soluble factors (SF) and extracellular vesicles (EVs). Both these fractions of the MSC secretome exhibit a significant improvement in NOPs and in the expression of neuronal markers as compared to their unprimed counterparts. Interestingly, here the effect of primed EVs (pEVs) was superior as compared to the primed SF (pSF). In addition, pSF and pEVs significantly restored the autophagy markers in the OS model. Furthermore, both the primed fractions also alleviated the expression of the inflammatory markers and stress kinases in the ERS model system. Overall, our findings suggest that priming with neurotrophic factors enhances the neuro-regenerative potential of both the fractions (SF and EVs) of the MSC secretome against stress-induced neurodegeneration, thereby providing hope for developing competent cell-free therapies against ND.

Funding Source: Symbiosis Centre for Research & Innovation, Symbiosis International (Deemed University), Pune, India, provided Junior Research Fellowship (JRF) and financial support under Major Research Project: SIU/SCRI/MJRP/19-20/1516-C.

Keywords: Extracellular vesicles, Priming, Neuro-regeneration

TOPIC: NEURAL

2117

REGENERATIVE POTENTIAL OF INTRANASALLY DELIVERED NEURAL STEM CELLS (LMNSC008) IN THE MODELS OF CONTROLLED CORTICAL IMPACT INJURY IN MALE AND FEMALE RATS

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Effective stem cell-based therapies for traumatic brain injury (TBI) depend on efficient delivery to the affected areas. Immortalized L-myc expressing human neural stem cells (hNSCs; LM-NSC008) display an inherent tropism to sites of damage after intranasal (IN) administration, and thus can potentially be exploited for cell replacement and regeneration strategies. The effectiveness of hNSC-mediated therapies requires successful engraftment, which in turn depends in part on the number of hNSCs reaching the target site. We hypothesize that IN hNSCs will migrate toward injury sites in an organized manner and contribute to cell replacement, regeneration, as well as neuroprotection via secretion of neurotrophins and induction of host neurogenesis. Defining the processes underlying migration of hNSCs to injury sites thus becomes critical to achieving optimally effective therapies. Here we demonstrate visualization of the distribution of LM-NSC008 cells in optically cleared tissue after multiple IN injections beginning 7 days post-TBI in an immunocompetent rat model of controlled cortical impact [CCI] injury. eGFP-expressing LM-NSC008 cells were administered in six IN injections of 1x106 cells in 24ml2% HSA in PBS every other day. Daily cyclosporin A (10 mg/kg; s.c.) injections started on day 5 post-TBI prevented immune rejection of the hNSCs. 5-8 weeks after TBI, and 4-7 weeks after initiating administration of LM-NSC008 cells, rat brains were harvested, and 1mm-thick coronal sections of fixed brain were cleared with CLARITY, immunostained, and imaged at TBI and contralateral sites. LM-NSC008 cells were quantified across 7 sections obtained from male and female rat brains. We observed NSC migration along white matter tracts towards TBI sites and hippocampal areas, contributing to the recovery via downregulation of GFAP after TBI. We also demonstrated modulation of cytokines and chemokines at the TBI site as compared in CCI+ Veh and CCI + NSC groups via ELISA and change in gene expression by Nanostring assays. Our work serves as proof-of-concept for the use of cell-mediated therapies for the treatment of TBI and provides a strong rationale for using LM-NSC008 cells and IN delivery for translation to patients post TBI.

Funding Source: NINDS

Keywords: NEURAL STEM CELLS LMNSC008, TRAUMATIC BRAIN INJURY, CONTROLLED CORTICAL IMPACT INJURY

2118

ANTITUMORIGENIC EFFECT OF THE HUMAN AMNIOTIC MEMBRANE EXTRACT ON NEUROBLASTOMA CELL LINE

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Neuroblastoma (NB) is a common sympathoadrenal solid malignant tumor in newborns. Current chemotherapeutic approaches provide moderate outcome and substantial side effects. Adjuvant therapy promises to enhance treatment efficacy, alleviate the side effects, and improve the therapeutic outcome. The human amniotic membrane (hAM) is a transparent embryonic envelope surrounding the fetus and includes mesenchymal stromal cells, epithelial cells, and extracellular matrix proteins. The hAM was found to offer potent natural antimicrobial, anticancer and antiangiogenic properties. Herein, we investigate the antitumorigenic activity of hAM extract on SH-SY5Y neuroblastoma cell line. The freshly collected hAM was homogenized and stained with Calcein AM to confirm the viability of the stem cells within the membrane. Treatment of SH-SY5Y neuroblastoma cell line with 1 mg/ml hAM extract (hAME) for 4 days showed a significant cytotoxic effect compared to the untreated group. Treated cells showed a significant upregulation in apoptotic markers Bcl-2, Caspase-3, and P53, and downregulation of anti-apoptosis regulator Bax along with a significant increase in Annexin V positive population, showing priming of the apoptosis pathway. Flow cytometry analysis showed G2/M phase cell cycle arrest, and RT-qPCR showed upregulation in cyclin-dependent kinase inhibitor (p21) and downregulation of cyclin-dependent kinase 4 (CDK-4) genotypic expression. Treated cells also showed downregulation of proliferation-inducing proteins and inhibitors of cell fate commitment differentiation proteins (ID) -1, -2, -3, -4, along with upregulation in TOB1 anti-proliferation marker, and a concomitant decrease in the protein expression of proliferation markers cMYC, HDAC1 and telomerase. Epithelial to mesenchymal transition markers Snail and Slug were repressed in the treated group as measured by Western blot. These data provide evidence to the antitumor activity of hAME and suggest its useful utility as a biomimetic possible adjuvant therapy for NB.

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Keywords: Neuroblastoma, Tumorigenesis, Amniotic membrane

2119

GABA RECEPTOR-MEDIATED PURKINJE NEURON DIFFERENIATION IN JDP2-DEPLETED CEREBELLAR GRANULE PROGENITOR CELLS

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The Jun dimerization protein 2 (Jdp2) gene is active in mouse cerebellar granule cells and plays a crucial role in the formation of the cerebellum lobes through programmed cell death. However, the role of Jdp2 in cellular differentiation and pluripotency in the cerebellum and the effect of the antioxidation reagents remain unknown. Thus, studies of the molecular mechanism underlying the effects of Jdp2 and N-acetyl-L-cysteine (NAC) in terms of the redox control are crucial for the understanding of the differentiation of granule cell progenitors (GCPs) into neurons and of the neural circuits of redox control during development. In vitro, treatment with NAC decreased the oxidative stress levels (i.e., reactive oxygen species [ROS] production and aryl hydrocarbon receptor levels) and increased the nuclear factor-erythroid-2-related factor 2 (Nrf2) levels and antioxidative response (ARE) activities. These activities were more significant in Jdp2-depleted mouse GCPs. NAC addition induced the early differentiation of GCPs to neurons, including Purkinje cells, through the enhanced expression of the GABA receptor a6 and calcium uptake. An inhibitor of the GABA receptor a6 reduced calcium uptake and the differentiation of GCPs to Purkinje cells. The depletion of Jdp2 promoted neuronal differentiation more significantly. NAC induced the early commitment of the differentiation of GCPs to neurons, especially to Purkinje cells, through the GABA receptor axis, and Jdp2 depletion enhanced this differentiation program of GCPs. The antioxidative effect of NAC was the main driving force of this decision for neural differentiation in vitro. This implies that antioxidative drugs may be an effective target for rescuing the oxidative-stress-induced GCP damages in the cerebellum.

Keywords: Calcium uptake, GABAR, Granule cell progenitor, Jun dimerization protein 2, N-acetyl-L-cysteine, Neural differentiation, Purkinje cells

2120

CUSTOM MICROFLUIDIC DEVICE FOR ASSESSMENT OF DORSOVENTRAL PATTERNING OF HUMAN IPSC-DERIVED OTOCYST BY KEY MODULATORS SONIC HEDGEHOG (SHH) AND WNT3A: IMPLICATIONS FOR REGENERATING VESTIBULAR NEURONS

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Vestibular dysfunction (VD) affects a significant portion of the US population. Unfortunately, current rehabilitation therapies are ineffective, with many patients experiencing lifelong debilitating symptoms. Potential treatments for VD include vestibular implants (VI), which have shown promise in clinical studies. However, the unique anatomical challenge presented by the location of vestibular cell bodies inside the internal auditory canal necessitates the in vitro regeneration of vestibular neurons (VN) to bridge the spatial gap. In recent decades, research on regenerating SGNs has been the primary focus, with little attention paid to VN regeneration. We developed a step-wise in vitro protocol to address this gap and generate VN phenotypes from human induced pluripotent stem cells (hiPSCs). Our protocol was designed to preferentially select for VNs rather than SGNs by shortening the otic neural differentiation period. To assess the impact of sonic hedgehog (SHH) and wingless-type MMTV integration site family member 3A (Wnt3a) on dorsoventral patterning in hiPSCs otocyst development, we used a two-by-two factorial design. We then characterized and validated hiPSC-derived 3D otic spheroids for vestibular neuron lineages using immunocytochemistry (ICC) and real-time RT-qPCR. We also found that 3D otic spheroids had similar percentages of PAX8(+)/β-III tubulin (+) cells when cultured in either a nanofibrillar cellulose hydrogel (GrowDex-T) or a peptide amphiphile hydrogel. In addition, we compared the efficacy of a slow-release human brain-derived neurotrophic factor (BDNF) system called the Polyhedrin Delivery System (PODS) to traditional recombinant hBDNF in promoting cell growth and differentiation. Our findings indicated that the PODS system supported longer neurites and more neurite-bearing cells. Based on PCR data, the high Wnt3a/low SHH culture condition promoted dorsal otocyst gene expression from Day 18-25 and early VN gene expression after Day 20. In summary, these cells can potentially improve VI outcomes and identify genetic signatures of non-functional vestibular neurons in VD patients. This may contribute to developing more effective treatments for this debilitating condition.

Keywords: Vestibular neuron, Regeneration, human induced pluripotent stem cells

2121

BRIDGING THE ELECTRODE-NEURON GAP: FINITE ELEMENT MODELING OF IN VITRO NEUROTROPHIN GRADIENTS TO OPTIMIZE NEUROELECTRONIC INTERFACES IN THE INNER EAR

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Although cochlear implant (CI) technology has allowed for the partial restoration of hearing over the last few decades, persistent challenges (e.g., poor performance in noisy environments and limited ability to decode intonation and music) remain. The “electrode–neuron gap” is inherent to these challenges and poses the most significant obstacle to advancing past the current plateau in CI performance. We propose the development of a “neuro-regenerative nexus”—a biological interface that doubly preserves native spi- ral ganglion neurons (SGNs) while precisely directing the growth of neurites arising from transplanted human pluripotent stem cell (hPSC)-derived otic neuronal progenitors (ONPs) toward the native SGN population. We hypothesized that the Polyhedrin Delivery System (PODS-recombinant human brain- derived neurotrophic factor [rhBDNF]) could stably provide the adequate BDNF concentration gradient to hPSC-derived late-stage ONPs to facilitate otic neuronal differentiation and directional neurite outgrowth. To test this hypothesis, a finite element model (FEM) was constructed to simulate BDNF concentration profiles generated by PODS-rhBDNF based on initial concentration and culture device geometry. For bi- ological validation of the FEM, cell culture experiments assessing survival, differentiation, neurite growth direction, and synaptic connections were conducted using a multi-chamber microfluidic device. We were able to successfully generate the optimal BDNF concentration gradient to enable survival, neuronal differentiation toward SGNs, directed neurite extension of hPSC-derived SGNs, and synaptogenesis between two hPSC-derived SGN populations. This proof-of-concept study provides a step toward the next generation of CI technology.

Funding Source: The Office of the Assistant Secretary of Defense of Health Af- fairs through the Hearing Restoration Research Program (Award #: RH170013:WU81XWUH-18-0712).
Imaging

Keywords: Auditory neurons, Regeneration, Neurotrophins

2122

KINETIC STEM CELL (KSC) COUNTING: A FIRST METHOD FOR ROUTINE QUANTIFICATION OF THE SPECIFIC FRACTION OR DOSAGE OF TISSUE STEM CELLS

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One of the most challenging research problems in the nearly six-decade history of stem cell science and stem cell medicine has been developing a method to quantify the specific fraction or dosage of tissue stem cells (TSCs). This counting problem was present from the beginning with hematopoietic stem cells (HSCs) and has continued with other important TSCs like mesenchymal stem cells (MSCs). The only previous method with the capability of estimating the specific TSC fraction of samples, the SCID mouse repopulation cell (SRC) assay, works only for HSCs; and it has proven impractical for routine use in both research and clinical practice. To address this longstanding problem, we recently developed a method, called kinetic stem cell (KSC) counting, that provides the ability to conveniently and routinely quantify the specific fraction of any TSC type in complex research, biomanufacturing, and clinical tissue cell preparations. KSC counting is a computational simulation method that defines the TSC-specific fraction (SCF) of tissue cell samples based on their cell proliferation kinetics. KSC counting has been validated for accuracy and specificity by several orthogonal tests, including comparison to HSC-specific fraction determinations by the SRC assay. We will describe the underlying stem cell kinetics principles of KSC counting and show how it can be used to specifically and accurately quantify diverse TSCs and monitor changes in their SCF in response to different culture factors and culture conditions. Future general implementation of KSC counting will improve the quality of stem cell research and stem cell medicine, accelerating progress in the development of more effective treatments for patients.

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Keywords: tissue stem cell, stem cell fraction, stem cell dosage

2123

PROPERTIES OF A NOVEL BIOMATERIAL SCAFFOLD IN REGULATING STEM CELL PROLIFERATION AND SELF-RENEWAL

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Our peripheral nervous system has the capacity for injured neurons to regenerate axons and rebuild functional connections. This unique potential relies on Schwann cells (SC) that support axon regeneration. Current treatment methods are insufficient to ensure a full functional nerve recovery. Toward this issue, nerve guide conduits (NGC) are used to guide regenerating axons. Stem cells also play a critical role in tissue repair throughout life. They can undergo self-renewal and give rise to several specialized cell types to repair the tissue surrounding their niche. In the bioengineering field, stem cells are being considered the primary research target as they can be incorporated into the generation of new NGC. Pre-seeding of a biomaterial scaffold with patient-derived induced pluripotent stem cells (iPSC) can promote neuronal regeneration through their differentiation into non-neuronal support cells i.e., growth-permissive SC. Here we reported the development of a novel and tunable NGC featuring interconnected microchannels with a composition analogous to the extracellular matrix. We assessed the matrix and physical properties of this biomaterial scaffold to regulate iPSC proliferation and self-renewal by conducting a transcriptomic analysis of iPSC growing on a biomaterial scaffold and in a 3D environment. We evaluated the impact of biomaterial scaffolds on iPSC survival, self-differentiation, and proliferation. Our finding gives insights into the biological mechanisms by which stem cells sense and interact with their surrounding mechanical environment and highlights how physical stimulation play important role in regulating stem cell behavior. Our results will help develop more effective regenerative medicine approaches for peripheral nerve injuries.

Keywords: induced pluripotent stem cells, nerve guide conduits, transcriptomics

2124

THERAPEUTIC ROLES OF HUMAN CORNEAL STROMAL STEM CELL-DERIVED EXTRACELLULAR VESICLES IN A SCAR-FREE CORNEAL WOUND HEALING

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Corneal blindness due to scarring/opacities is a leading cause of visual impairment. The standard of care is donor corneal transplantation, but the global shortage of donor tissue limits the outcome. Different reports have shown that direct application of corneal stromal stem cells (CSSCs) to corneal wounds in animal models suppressed opacities and recovered clear corneas. The underlying mechanism associated to this anti-scarring effect is not fully elucidated. Our recent work has revealed the production

of regenerative cytokine, transforming growth factor β3 (TGFβ3), by human CSSCs. This study investigated the CSSC-derived extracellular vesicles (EVs) and examined various cell stress effects on TGFb3 expression. Primary human CSSCs isolated from donor corneas were cultured under different conditions: (1) with M1 mouse macrophages (RAW264.7), (2) serum deprivation, (3) H2O2-mediated oxidative stress, (4) transient heat shock, and (5) metabolic stress with tunicamycin-induced unfolded protein response. The cellular and EV expression of TGFb3 and other regenerative cytokines (HGF, SDF1) were examined by qPCR. We detected full-length TGF^{2}3 mRNA in CSSC-derived EVs. The inflammatory stress induced TGF^{2}3 upregulation in CSSCs and their EV fractions, whereas there was no change in HGF and SDF1 expression. More importantly, the stimulated CSSCs produced EVs with increased TGF^{2}3 transcripts. To validate the therapeutic effect, human CSSCs with or without TGF^{2}3 knockdown and CSSC-derived secretome were applied to a mouse model of anterior corneal stromal injury caused by mechanical ablation. At day 1 and 3 post-injury and cell treatment, TGF^{2}3 was significantly upregulated in mouse corneal samples. At day 14, the treated corneas remained clear and had significantly reduced expression of fibrosis markers (fibronectin, hyaluronan synthase 2, SPARC, tenascin C, collagen 3, and ±-smooth muscle actin) when compared with untreated wound controls. CSSCs with siRNA-mediated TGFβ3 knockdown lost these therapeutic effects. In conclusion, we identified the paracrine action of CSSCs on corneal scar prevention through EV-mediated delivery of TGFβ3 mRNA. High TGFb3 expression could redirect the tissue fibrosis during corneal wound healing to a scar-reducing regeneration.

Funding Source: Hillman Foundation Grant

Keywords: Corneal opacities; Corneal stromal stem cells, TGFβ3; Extracellular vesicles, Corneal regeneration

2126

INVESTIGATION OF THE EFFSCTS OF CRANIAL BONE-DERIVED MESENCHYMAL STEM CELL TRANSPLANTATION IN A BRAIN INJURY RAT MODEL USING LONGITUDINAL ELECTROPHYSIOLOGICAL EVALUATION

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Hiroshima University has independently established cranial bone-derived mesenchymal stem cells (cMSCs) and compared their neurotrophic effects such as neurotrophic factor expression and effects of transplantation with those of other MSCs in a central nervous system injury model. In this study, we investigated the effects of cMSCs in a brain injury rat model using our previously established longitudinal electrophysiological evaluation method. Adult female Sprague-Dawley rats were used to construct a brain injury model. The study included rats that were transplanted with cMSCs from the tail vein on the first day after injury (n=8) and a control group (rats with brain injury but without cMSCs transplantation) (n=7). The Basso Beattie Bresnahan (BBB) scale scores and inclined plate test measurements were recorded for motor function evaluation. Transcranial electrically stimulated motor-evoked potentials were recorded in the rats' forelimbs for electrophysiological evaluation, and the amplitude recovery rate was measured. Histopathological evaluation of rat brains was performed 4 weeks after the injury. Compared with the control group, the transplanted cMSCs group showed significant recovery of motor function in the inclined plate test the day following transplantation and in the BBB score 5 days post-transplantation. With regard to electrophysiological evaluation, we observed significant recovery of amplitude 14 days post-transplantation. Histopathological evaluation confirmed cerebral contusions in both groups. We established the effects of cMSCs transplantation on motor function and electrophysiological activity in a rat model of brain injury. We intend to perform further research to investigate the mechanism underlying electrophysiological recovery associated with cMSCs transplantation.

Keywords: brain injury, mesenchymal stem cells, motor-evoked potential

2127

CANCER STEM CELLS AS THERAPEUTIC TARGET FOR COLORECTAL CANCER

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There has been an arousing interest in cancer stem cells (CSCs) ever since it was discovered few decades ago. CSCs are well-known by not only their ability to undergo self-renewal and differentiate into more mature cancer cells but also by their tumour-initiating ability from relatively very small number of cells. Only little investigation into the exact role of isolated populations of (CSCs) has been undertaken and the prevalence of CSCs in malignancies is still a matter of some debate and controversy. Here, we aim to identify specific CSC markers and isolate CSC sub-populations from colon cancer in order to force them from dormancy into active division, which will potentially make them more susceptible to chemotherapy. Expression levels of several colorectal CSCs markers including CD271, SSEA1, EPCAM, Cript-1, or ABCG2 were validated under both hypoxic and normoxic conditions in SW480 and CSC480, colorectal cancer cell lines, using Flow cytometry and immunofluorescence. The relationship between hypoxia and cellular expression of Brn2, which is a transcription factor that could be a CSC marker, was explored via flow cytometry an immunofluorescence. Furthermore, correlation between CSC markers expression in primary and metastasis tissues in human colorectal cancer was examined by immunofluorescence. Results: ABCG2 and Cripto-1 were expressed in low levels on cell-subpopulations compared to CD271, EPCAM or SSEA1. Interestingly, all the markers expression levels were increased in a subpopulation by 72 hours under hypoxia compared to normoxia conditions. However, comparison over the time course of hypoxia; EPCAM, Cripto-1, or ABCG2 expression were decreased at 48 hours and then increased again at 72 hours. The SW480 Brn2-EGFP cell line showed a significant decreased in Brn2 positive cells between the normoxia and hypoxia samples at 24, 48, or 72 hours.

We found that all markers were highly expressed in metastasis compared to primary sections in human tissues. ABCG2 and Cripto-1 are potentially suitable markers for studying colon CSCs. Furthermore, CSC markers were found to be more highly expressed in the metastatic colorectal cancer samples compared primary sections.

Keywords: Cancer stem cells, Colorectal cancer, Markers

TOPIC: MESENCHYMAL STROMAL CELLS, ADIPOSE, AND CONNECTIVE TISSUE

2130

ESTABLISHMENT OF ADIPOSE-DERIVED MESENCHYMAL STEM CELL THERAPY FOR NOVEL MODALITY TOWARDS OCULAR SURFACE INTRACTABLE DISEASES

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Human adipose-derived mesenchymal stem cells (ADSCs) have immunomodulatory and anti-inflammatory functions and are expected to be a new modality to treat immune disorders resistant to existing therapies. We will evaluate the efficacy and safety of ADSCs in ophthalmology using a chronic graft-versus-host disease (cGVHD) model. A mouse cGVHD model was created, and a single subconjunctival dose of 2×10^5 ADSCs was administered. Three weeks after ADSC transplantation, corneal epithelial damage and corneal opacity scores were quantified. The ADSC transplant group showed significant improvement in epithelial damage and suppression of corneal opacity compared to the negative control group. ($p < 0.05$) To evaluate the pharmacokinetics, the luciferase gene was introduced by the lentiviral vector, and the luminescence signal was detected by in vivo luminescence imaging. Human-derived DNA in the samples was detected by real-time PCR of human-specific Alu sequences. The results showed that ADSCs were distributed in the eye and ocular tissues after administration but disappeared within seven days. In an ocular local irritation test conducted on domestic rabbits, no obvious irritation was observed after administering 1×10^7 ADSCs. Subconjunctival administration of ADSC has shown efficacy against ocular surface disorders of cGVHD. The results of pharmacokinetic and topical irritation studies indicate that it is safe and can be expected as a new modality for new ocular surface diseases.

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