INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

STEM CELLS IN HUMAN DEVELOPMENT AND DISEASE

An ISSCR International Symposium

4-5 APRIL 2024
CINCINNATI, USA

IN PARTNERSHIP WITH

Cincinnati Children's®

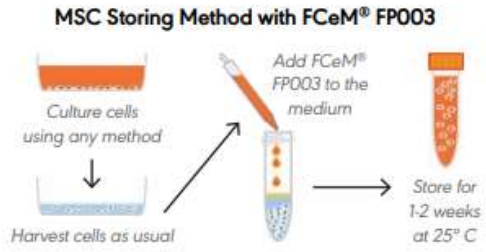
PROGRAM BOOK

Nissan Chemical
CORPORATION

FCeM® Advance-CR Preparation kit

Secure cell storage and transportation at room temperature

FCeM® FP003B creates scaffolds in cell culture media and allows for cells and cell spheroids to be stored in suspension condition. Cells and spheroids kept in the FP003B medium are viable for over 2 weeks at room or cold temperature (4-25 degree C).



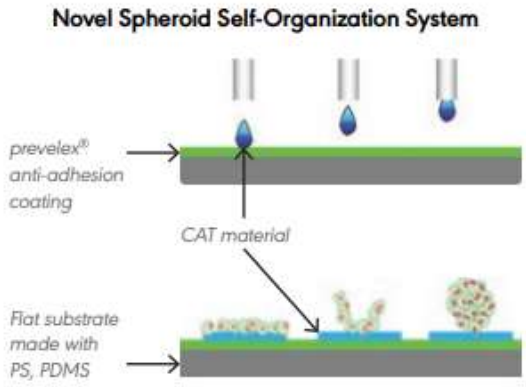
Advantage of FCeM®

- Cells can be stored at room temperature (Cryopreservation-free storage)
- Same viscosity as water
- Allows for performance of pipetting mix or dispensing medium freely
- Prevents unwanted aggregation between cell spheroids or organoids

prevelex® CAT system

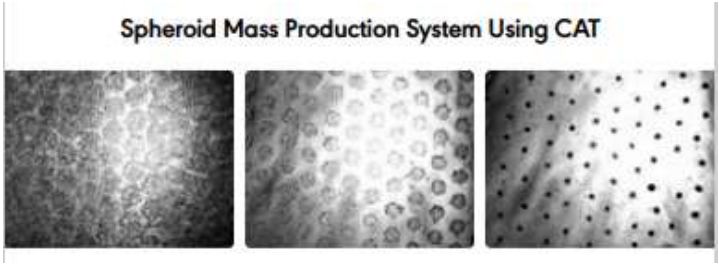
Highly efficient and precise size-controlled spheroid production

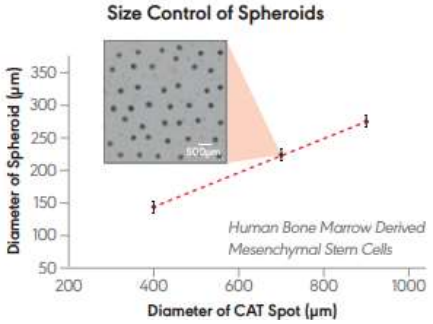
prevelex® CAT is a combination of adhesive materials (CAT material) and anti-adhesion coatings (prevelex® CC1), which allows for self-organized, size controlled and massive scale spheroid production. In the prevelex® CAT system, cells gather on CAT materials and form spheroids spontaneously in direct correlation to the diameter of the CAT material spot.



Advantage of CAT

- Size of CAT material spots control spheroid size precisely
- Applicable to large scale manufacturing





Nissan Chemical America Corporation 101 Metro Drive, San Jose, CA 95110 info@nissanchem-usa.com

WELCOME

Dear Colleagues,

On behalf of the International Society for Stem Cell Research (ISSCR) and the Center for Stem Cell & Organoid Medicine (CuSTOM) at Cincinnati Children's Hospital, welcome to Cincinnati, Ohio. The ISSCR and CuSTOM are proud to partner to bring you the 2024 International Symposium "*Stem Cells in Human Development and Disease*."

The discovery of signaling pathways, gene regulatory networks, and morphological changes during development have been fundamental for understanding and harnessing the functionality of stem cells. These principles and insights have led to significant advancement in the application of stem cells and stem cell-derived in vitro models in studying human development and disease, which pave the way to develop novel therapeutic approaches to improve human health. This symposium will explore the multifaceted intersections in which developmental and stem cell biology propel basic biology forward and bring translational efforts closer to clinical applications.

Beyond the scientific breakthroughs showcased in the oral and poster presentations, the exhibit hall will present the latest advancements in technology, reagents, devices, and support services designed to elevate your research endeavors.

We hope you will enjoy the symposium and take advantage of the intimate setting to spend time getting to know each other, to foster new connections, build productive collaborations, and take the ideas and inspiration back to your own laboratories.

On behalf of the organizers and the people who made this event possible, enjoy the meeting and your time in the Queen City.

Sincerely,

Cincinnati Program Organizing Committee:

Shuibing Chen, PhD, *Weill Cornell Medicine, USA*

Makiko Iwafuchi, PhD, *Cincinnati Children's Hospital, USA*

Takanori Takebe, MD, PhD, *Cincinnati Children's Hospital, USA*
Osaka University and Tokyo Medical and Dental University, Japan

James Wells, PhD, *Cincinnati Children's Hospital, USA*

ABOUT THE ISSCR

The mission of the International Society for Stem Cell Research (ISSCR) is to promote excellence in stem cell science and applications to human health. The ISSCR is the largest society in the world dedicated to the advancement of responsible stem cell research—a field that strives to advance scientific understanding, treatments, and cures that better human health. We foster junior scientists, give voice and visibility to scientific advancement, and encourage a positive global environment for future discovery and treatment. Our promise is to help the field of stem cell research reach its potential.

INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

CONTACT US

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ISSCR 2024

THE FUTURE STARTS HERE

HAMBURG GERMANY

10-13 JULY 2024

PLEASE JOIN US FOR OUR 2024 ANNUAL MEETING AT THE CONGRESS CENTER HAMBURG

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MEETING INFORMATION

All times are listed in Eastern Daylight Time (EDT) USA

ONSITE BADGE PICK UP

Pick up your name badge in the designated area below during posted hours. Name badges are required for admission to all sessions, social events, meals/breaks, and the Exhibit & Poster Hall. Badges may be picked up during the following times:

Registration Desk Hours
Memorial Hall Entrance Foyer
1225 Elm Street, Cincinnati, Ohio, 45202

Thursday, 4 April	*8:30 AM – 6:00 PM EDT*
Friday, 5 April	*8:30 AM – 4:00 PM EDT*

ISSCR PROGRAM AGENDA

There will be no printed program book for the 2024 Cincinnati International Symposium. You can access an online version of the program agenda here.

Livestream will not be available for this event.
ISSCR members can access on-demand content after the event. Login to the Member Library with your ISSCR credentials. If you have trouble logging in, first try resetting your password. If the problem persists, please direct questions to isscrdigital@isscr.org. Please allow approximately two weeks post-event for on-demand content to be published in the member library.

SMOKING

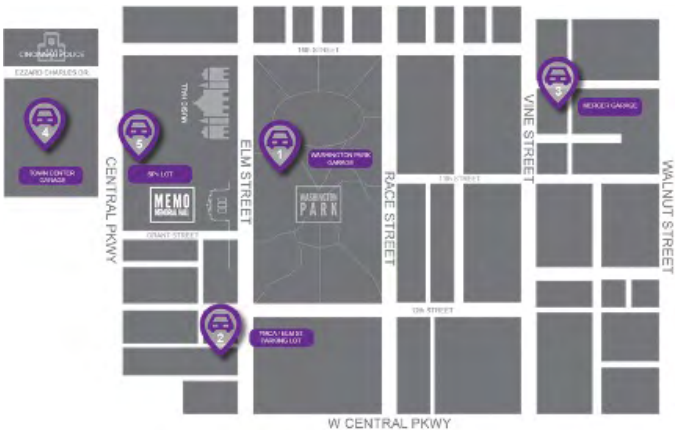
Smoking or the use of e-cigarettes is prohibited at Memorial Hall.

LOST AND FOUND

Please bring found items to the ISSCR Registration Desk during posted hours. If you lost an item, stop by during registration hours for assistance.

PARKING

The most convenient parking option is directly across the street from Memorial Hall at the (1) Washington Park Garage, noted on the parking map. Other options include:



(2) Elm Street Lot on Elm Street

(3) Mercer Commons Garage on Vine Street between 13th and 14th Street

(4) Town Center Garage on Central Parkway opposite Music Hall under WCET

(5) SP+ Surface Lot is located directly between Memorial Hall and Music Hall. Please note that this is the most expensive option and reaches capacity quickly.

MEETING INFORMATION & ISSCR POLICIES

All times are listed in Eastern Daylight Time (EDT) USA

POSTER INFORMATION

Each poster will be presented during a 45-minute session in the Green & Gold Ballrooms on the entrance level of Memorial Hall. **Poster presenters must adhere to the scheduled date and time of their poster display and presentation.**

Thursday, 4 April – Poster Sessions I & II

Poster Session I

Poster Set-up: 3:35 PM – 3:50 PM
Poster Presentation: 5:00 PM – 5:45 PM
Poster Take-down: 5:45 PM

Poster Session II

Poster Set-up: 5:45 PM – 6:00 PM
Poster Presentation: 6:00 PM – 6:45 PM
Poster Take-down: 6:45 PM

Poster presenters are responsible for removing their posters upon completion of their presentation. Any posters that are not removed at the end of the session will be discarded.

CODE OF CONDUCT

The ISSCR is committed to providing a safe and productive meeting environment that fosters open dialogue and discussion and the exchange of scientific ideas, while promoting respect and equal treatment for all participants, free of harassment and discrimination. All participants are expected to treat others with respect and consideration, follow venue rules, and alert staff or security, if at an onsite meeting, of any dangerous situations or anyone in distress. Attendees are expected to uphold standards of scientific integrity and professional ethics.

These policies comprise the Code of Conduct for all ISSCR meetings and events and apply to all attendees, speakers, exhibitors, sponsors, staff, contractors, volunteers, media, and guests.

HARRASSMENT POLICY

The ISSCR prohibits any form of harassment, sexual or otherwise. Incidents should immediately be reported to ISSCR meetings staff at isscr@isscr.org.

RECORDING POLICY

By registering for an ISSCR meeting, you agree to the society's Recording Policy. The ISSCR strictly prohibits the recording (photographic, screen capture, audio and/or video), copying or downloading of scientific results from the sessions, presentations, and/or posters at its meetings.

ABSTRACT CONTENT/ PRESENTATION EMBARGO POLICY

Abstract content may not be announced, publicized, or distributed before the presentation date and time in any way, including in media stories, blogs, and on social media. The ISSCR does permit promotion of general topics, speakers, and presentation days and times. This embargo policy applies to all formats of abstract publication—including abstracts in electronic or print versions of Meeting Program Books/Poster Abstract Books, online via the Program Planner and Poster Abstract PDFs, the Society's website(s), and other publications.

ISSCR POLICIES

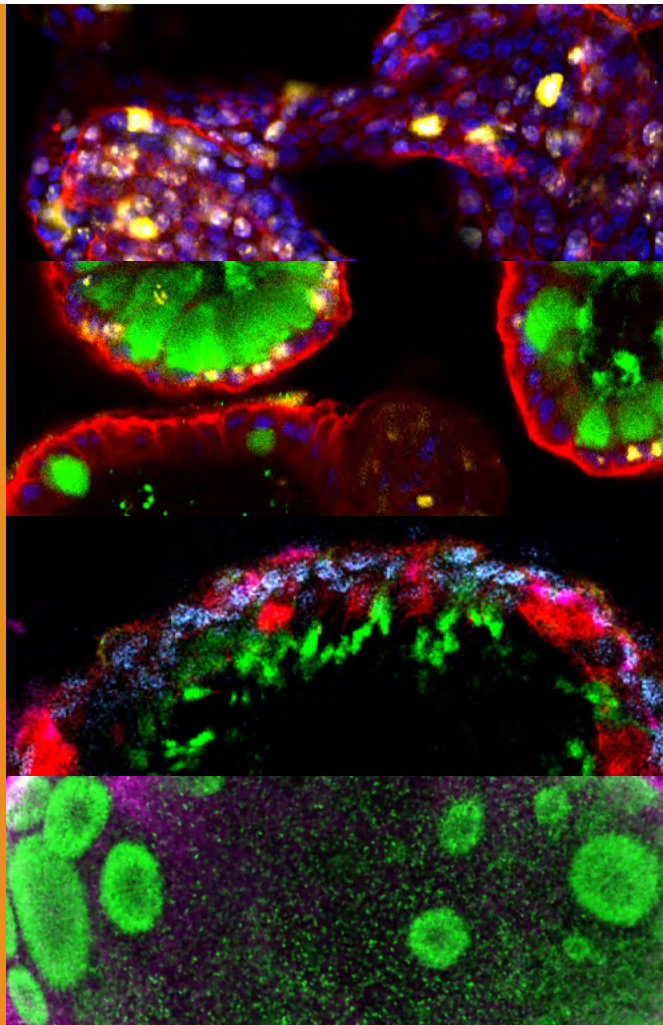
MEDIA POLICY

The ISSCR invites journalists to cover science presented at its meetings and events in adherence with the Society's Media Policy. Still photography, video, and/or audio taping of the sessions, presentations and posters at ISSCR meetings and events are strictly prohibited. Intent to communicate or disseminate results or discussions presented at ISSCR meetings and events is prohibited until the start of each individual presentation. For related questions about the ISSCR Media Policy, please contact Kym Kilbourne at media@isscr.org.

By registering for ISSCR meetings and events, all attendees agree that their image or recording may be used by the ISSCR for promotional purposes in the future.

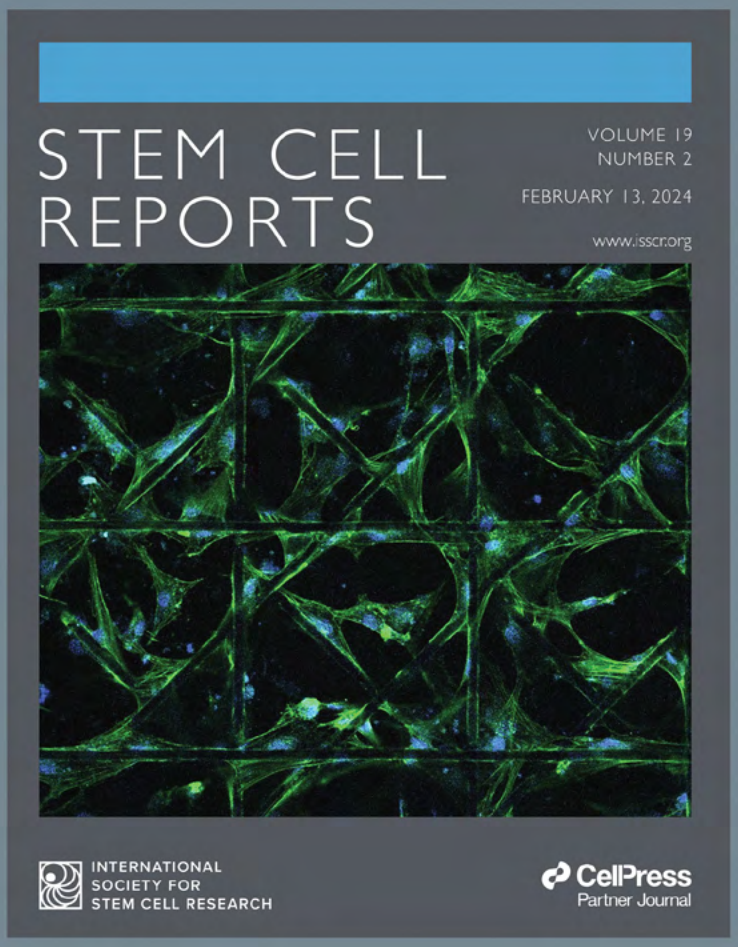
HEALTH & SAFETY

By registering for an ISSCR in-person event, you agree to present proof of COVID-19 vaccination and/or a negative COVID-19 test if requested. You agree to release the ISSCR and its sponsors and exhibitors, and their employees and agents, from and against claims, liabilities and expenses arising from injury, sickness or death from contraction or spread of COVID-19 or other communicable disease due to travel to, or attendance at, an ISSCR event. You agree to take necessary precautions to ensure your own, and others' safety. By registering, you agree not to attend any ISSCR event if you feel sick or have had recent exposure to COVID-19. If you have questions on our health & safety policies, please contact isscr@isscr.org.



FOR
SCIENTISTS.
BY
SCIENTISTS.

ISSCR
INTERNATIONAL SOCIETY
FOR STEM CELL RESEARCH



- Open access, peer reviewed science.
- Articles, Short Reports and Front Matter that challenge science & advance the field.
- Reduced publication fees for ISSCR members.

Your support of ***Stem Cell Reports*** enhances the ISSCR's global mission and outreach to promote, educate and advocate for stem cell research and its application.

STEM CELL REPORTS

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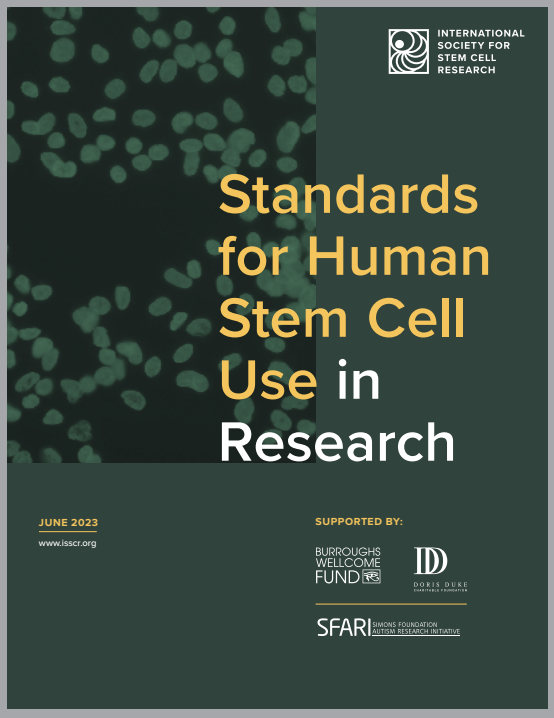
Standards for Human Stem Cell Use in Research



Quality standards and core principles for the laboratory culture and characterization of both adult and pluripotent human stem cells and in vitro model systems using them.

Supported by:

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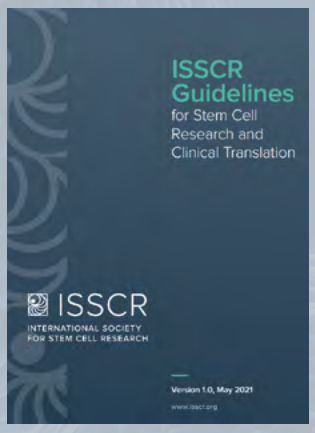
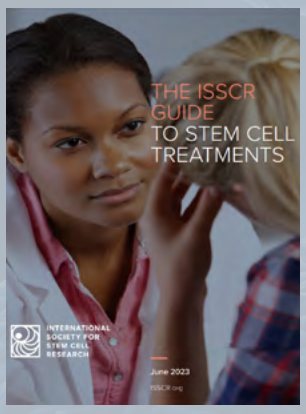
ISSCR RESOURCES

FOR SCIENTISTS
Enhancing rigor and reproducibility in preclinical stem cell research and ultimately strengthening the pipeline of therapies for patients.



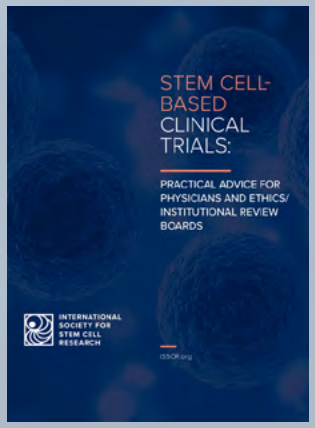
FOR SCIENTISTS
Adherence to the ISSCR Guidelines provides assurance that stem cell research is conducted with scientific and ethical integrity and that new therapies are evidence-based.



FOR THE PUBLIC
This guide provides patients and the public with comprehensive and reliable information to make informed decisions about stem cell treatments, emphasizing safety and efficacy.

FOR CLINICIANS
A professional standard intended to help patients make an informed decision when offered a stem cell "treatment" outside of a clinical trial.

FOR CLINICIANS
A professional standard to help ensure women are well informed prior to donating fetal tissue after an elective termination of pregnancy.



FOR CLINICIANS
Fundamental questions for clinicians and ethics/ institutional review committees to ask when running or reviewing early phase, stem cell-based clinical trials.

Explore all ISSCR resources at **ISSCR.org** and **AboutStemCells.org**

ISSCR INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

PROGRAM SCHEDULE

4 April 2024

Thursday
4 April 2024

All times are listed in Eastern Daylight Time (EDT) USA

10:00 AM – 10:30 AM WELCOME COFFEE

ROOM: Green/Gold Ballrooms

10:30 AM – 12:36 PM FUNDAMENTAL MOLECULAR MECHANISIMS OF EARLY DEVELOPMENT

ROOM: Anderson Theater

Chair: **James M. Wells,** *Center for Stem Cell and Organoid Medicine (CuSTOM), Cincinnati Children's Hospital Medical Center, USA*

10:30 AM – 10:32 AM
Welcome Remarks
Keith Alm, *CEO, International Society for Stem Cell Research (ISSCR)*

10:32 AM – 10:35 AM
Opening Remarks
James M. Wells, *Center for Stem Cell and Organoid Medicine (CuSTOM), Cincinnati Children's Hospital Medical Center, USA*

10:35 AM – 11:15 AM
Opening Keynote
Magdalena Zernicka-Goetz, *Caltech, USA and University of Cambridge, UK*
STEM CELL-BASED EMBRYO MODELS TO UNDERSTAND HOW TISSUES AND ORGANS ARE FORMED AND WORK TOGETHER

11:15 AM – 11:27 AM
Idse Heemskerk, *University of Michigan, USA*
THE TIME INTEGRAL OF BMP SIGNALING CONTROLS DIFFERENTIATION IN 2D HUMAN GASTRULOIDS BY MODULATING SOX2

11:27 AM – 11:39 AM
Kentaro Iwasawa, *Cincinnati Children's Hospital Medical Center, USA*
REDEFINING ENDODERMAL PROGENITOR LANDSCAPE IN EMBRYONIC HEPATO-BILIARY-PANCREATIC DEVELOPMENT

11:39 AM – 11:51 AM
Arend Overeem, *Leiden University Medical Centre, Netherlands*
TGFβ/NODAL SIGNALING CONTROLS HUMAN PRIMORDIAL GERM CELL SPECIFICATION AND MAINTENANCE OF GERM CELL FATE

11:51 AM – 12:16 PM
Aaron M. Zorn, *Center for Stem Cell and Organoid Medicine (CuSTOM), Cincinnati Children's Hospital Medical Center, USA*
FOREGUT ORGANOGENESIS FROM EMBRYOS TO ORGANOID MODELS

12:16 PM – 12:36 PM
Makiko Iwafuchi, *Cincinnati Children's Hospital Medical Center, USA*
PIONEER AND PRDM TRANSCRIPTION FACTORS COORDINATE BIVALENT EPIGENETIC STATES TO SAFEGUARD CELL FATE

PROGRAM SCHEDULE

4 April 2024

12:36 PM – 2:15 PM LUNCH BREAK

ROOM: Green/Gold Ballrooms

12:55 PM – 2:00 PM INNOVATION SHOWCASES

ROOM: Anderson Theater

**Lunches may be brought to the Anderson Theater to enjoy during the Innovation Showcases*

12:55 PM – 1:25 PM

Presented by STEMCELL Technologies Inc.
Jenna Moccia, PhD, *STEMCELL Technologies, Inc., Canada*
ORGANOIDS AND STEM CELL-BASED TISSUE MODELS: POWERFUL TOOLS FOR DRUG DISCOVERY

1:30 PM – 2:00 PM

Presented by Rorze Lifescience Inc.
Tomohiro Takanose, *Rorze Lifescience, Japan*
Taro Kabakino, *Hitachi Global Life Solutions, Japan*
EXIT STRATEGY WITH PIPELINE HOLDERS BY HITACHI GLS-RLS ALLIANCE TOWARD INDUSTRIALIZATION OF REGENERATIVE MEDICINE

2:15 PM – 3:20 PM MORPHOGENETIC MECHANISMS OF EARLY DEVELOPMENT

ROOM: Anderson Theater

Chair: **Silvia Velasco,** *Murdoch Children's Research Institute, Australia*

2:15 PM – 2:40 PM

Olivier Pourquié, *Harvard Stem Cell Institute, Harvard University, USA*
DECONSTRUCTING HUMAN MUSCULO-SKELETAL SYSTEM DEVELOPMENT IN VITRO

2:40 PM – 2:55 PM

Alex Hughes, *University of Pennsylvania, USA*
INTERPRETING THE GEOMETRY AND RHYTHM OF EARLY KIDNEY FORMATION FOR SYNTHETIC MORPHOGENESIS

2:55 PM – 3:20 PM

Minoru Takasato, *RIKEN Center for Biosystems Dynamics Research, Japan*
BLADDER ORGANOIDS GENERATED FROM HUMAN IPS CELLS MIMIC UROTHELIAL STRUCTURE AND FUNCTIONS

3:20 PM – 3:50 PM BREAK

ROOM: Green/Gold Ballrooms

Refreshment Break, sponsored by **Nikon Instruments Inc.**

PROGRAM SCHEDULE

4 April 2024

3:50 PM – 4:55 PM — DEVELOPMENTAL ORIGINS OF DISEASES—PART I: DEGENERATIVE DISEASES

ROOM: Anderson Theater

Chair: **Aaron M. Zorn,** *Center for Stem Cell and Organoid Medicine (CuSTOM), Cincinnati Children's Hospital Medical Center, USA*

3:50 PM – 4:15 PM
Christine L. Mummery, *Leiden University Medical Center, Netherlands*
HUMAN PLURIPOTENT STEM CELLS COME OF AGE IN MODELLING CARDIOVASCULAR DISEASES AND TOXICITY

4:15 PM – 4:30 PM
Orly Reiner, *Weizmann Institute of Science, Israel*
NEUROMORPHOCHIP ORGANOIDS REVEAL A NOVEL MICROCEPHALY MECHANISM

4:30 PM – 4:55 PM
Silvia Velasco, *Murdoch Children's Research Institute, Australia*
HUMAN BRAIN ORGANOIDS BRING NEW INSIGHT INTO THE DEVELOPMENTAL ORIGINS OF BRAIN DISEASES

5:00 PM – 7:00 PM — WELCOME RECEPTION AND POSTER SESSIONS I & II

ROOM: Green/Gold Ballrooms

Supported by **REDI Cincinnati**/**Jobs Ohio**

5:00 PM – 5:45 PM
Poster Session I

6:00 PM – 6:45 PM
Poster Session II

PROGRAM SCHEDULE

Friday
5 April 2024

All times are listed in Eastern Daylight Time (EDT) USA

8:30 AM – 9:00 AM — WELCOME COFFEE

ROOM: Green/Gold Ballrooms

9:00 AM – 10:05 AM — DEVELOPMENTAL ORIGINS OF DISEASES—PART II: CONGENITAL MALFORMATIONS

ROOM: Anderson Theater

Chair: **Magdalena Zernicka-Goetz,** *Caltech, USA and University of Cambridge, UK*

9:00 AM – 9:25 AM

Mingxia Gu, *Center for Stem Cell and Organoid Medicine (CuSTOM), Cincinnati Children's Hospital, USA*
DECIPHERING ENDOTHELIAL AND MESENCHYMAL ORGAN SPECIFICATION IN VASCULARIZED LUNG AND INTESTINAL ORGANOIDS

9:25 AM – 9:40 AM

Vered Shacham Silverberg, *Cincinnati Children's Hospital Medical Center, USA*
MODELLING BARRET'S ESOPHAGUS USING HUMAN IPSC-DERIVED ESOPHAGEAL RAFT CULTURES

9:40 AM – 10:05 AM

Andrew McMahon, *Keck School of Medicine, University of Southern California, USA*
MECHANISTIC AND THERAPEUTIC MODELING OF AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE WITH HUMAN KIDNEY ORGANOIDS

10:05 AM – 10:45 AM — BREAK

ROOM: Green/Gold Ballrooms

PROGRAM SCHEDULE

5 April 2024

10:45 AM – 11:50 AM INFECTIOUS DISEASES

ROOM: Anderson Theater

Chair: **Takanori Takebe,** *Cincinnati Children's Hospital Medical Center, USA and Osaka University and Tokyo Medical and Dental University, Japan*

10:45 AM – 11:10 AM

Shuibing Chen, *Weill Cornell Medicine, USA*
HUMAN PLURIPOTENT STEM CELL-DERIVED ORGANOIDS AND SARS-CoV-2

11:10 AM – 11:25 AM

Robert Schwartz, *Weill Cornell Medicine, USA*
DEVELOPMENT OF A LIBRARY OF NONHUMAN PRIMATE PLURIPOTENT STEM CELLS AND STEM CELL DERIVED LINEAGES TO STUDY SPECIES-SPECIFIC HOST RESPONSES AND RESTRICTIONS OF PATHOGENS

11:25 AM – 11:50 AM

Kyle Loh, *Stanford University School of Medicine, USA*
LINEAGE TRACING BLOOD ORIGINS IN VIVO TO GUIDE EFFICIENT IN VITRO PRODUCTION OF BLOOD PROGENITORS

11:50 AM – 1:45 PM LUNCH BREAK

ROOM: Green/Gold Ballrooms

12:10 PM – 1:15 PM INNOVATION SHOWCASES

ROOM: Anderson Theater

**Lunches may be brought to the Anderson Theater to enjoy during the Innovation Showcases*

12:10 PM – 12:40 PM

Presented by PHC Corporation of North America
Cristie Marko, *PHC Corporation of North America, USA*
LIVE CELL METABOLIC ANALYZER, LICELLMO—PAVING THE WAY FOR METABOLIC RESEARCH & CELL AND GENE THERAPY

12:45 PM – 1:15 PM

Presented by Corning Life Sciences
Tom Bongiorno, PhD, *Corning Life Sciences, USA*
OPTIMIZED SURFACES FOR STEM CELL-DERIVED MODELS OF HUMAN DEVELOPMENT AND DISEASE

PROGRAM SCHEDULE

5 April 2024

1:45 PM – 2:50 PM — CELL AND TISSUE THERAPIES

ROOM: Anderson Theater

Chair: **Mingxia Gu,** *Center for Stem Cell and Organoid Medicine (CuSTOM), Cincinnati Children's Hospital Medical Center, USA*

1:45 PM – 2:10 PM

Miki Ando, *Juntendo University School of Medicine, Japan*
iPSC-DERIVED NEXT-GENERATION T CELL THERAPY FOR REFRACTORY TUMORS

2:10 PM – 2:25 PM

Muhammad Riaz, *Johns Hopkins University School of Medicine, USA*
EXAMINING THE EFFICACY OF HUMAN IPSC-DERIVED CORNEAL ENDOTHELIAL CELLS INJECTION AS AN ALTERNATIVE TO PEDIATRIC ENDOTHELIAL KERATOPLASTY

2:25 PM – 2:50 PM

Charles E. Murry, *University of Washington, USA*
GENOME EDITING STEM CELLS TO PREVENT ENGRAFTMENT ARRHYTHMIAS AFTER CARDIOMYOCYTE TRANSPLANTATION

2:50 PM – 3:30 PM — PANEL DISCUSSION: STEM CELL-BASED MODELS FOR DRUG DISCOVERY

ROOM: Anderson Theater

Sponsored by **Nikon Instruments Inc.**
Chair: **Shuibing Chen,** *Weill Cornell Medicine, USA*

Magdalena Kasendra, *Center for Stem Cell and Organoid Research and Medicine (CuSTOM), USA*
Christine L. Mummery, *Leiden University Medical Center, Netherlands*
Li Pang, *US Food and Drug Administration, USA*
Takanori Takebe, *Cincinnati Children's Hospital Medical Center, USA, Osaka University and Tokyo Medical and Dental University, Japan*

3:30 PM – 4:00 PM — BREAK

ROOM: Green/Gold Ballrooms

PROGRAM SCHEDULE

5 April 2024

4:00 PM – 5:25 PM — MOVING INTO THE FUTURE WITH EMERGING TECHNOLOGIES AND CLOSING REMARKS

ROOM: Anderson Theater

Chair: **Makiko Iwafuchi,** *Cincinnati Children's Hospital Medical Center, USA*

4:00 PM – 4:25 PM	**Samantha Morris,** *Washington University School of Medicine, USA* **NEW GENOMIC TECHNOLOGIES TO DECONSTRUCT AND CONTROL CELL IDENTITY**
4:25 PM – 4:40 PM	**Feng Guo,** *Indiana University Bloomington, USA* **BRAIN ORGANOID COMPUTING FOR ARTIFICIAL INTELLIGENCE**
4:40 PM – 5:20 PM	**Closing Keynote** **Hideyuki Okano,** *Keio University, Japan* **INVESTIGATING THE ROLE OF GLIAL CELLS IN THE PATHOGENESIS AND TREATMENT OF ALZHEIMER'S DISEASE-RELATED DISORDERS USING NEXT-GENERATION BRAIN ORGANOIDS**
5:20 PM – 5:25 PM	**Closing Remarks** **Makiko Iwafuchi,** *Cincinnati Children's Hospital Medical Center, USA*

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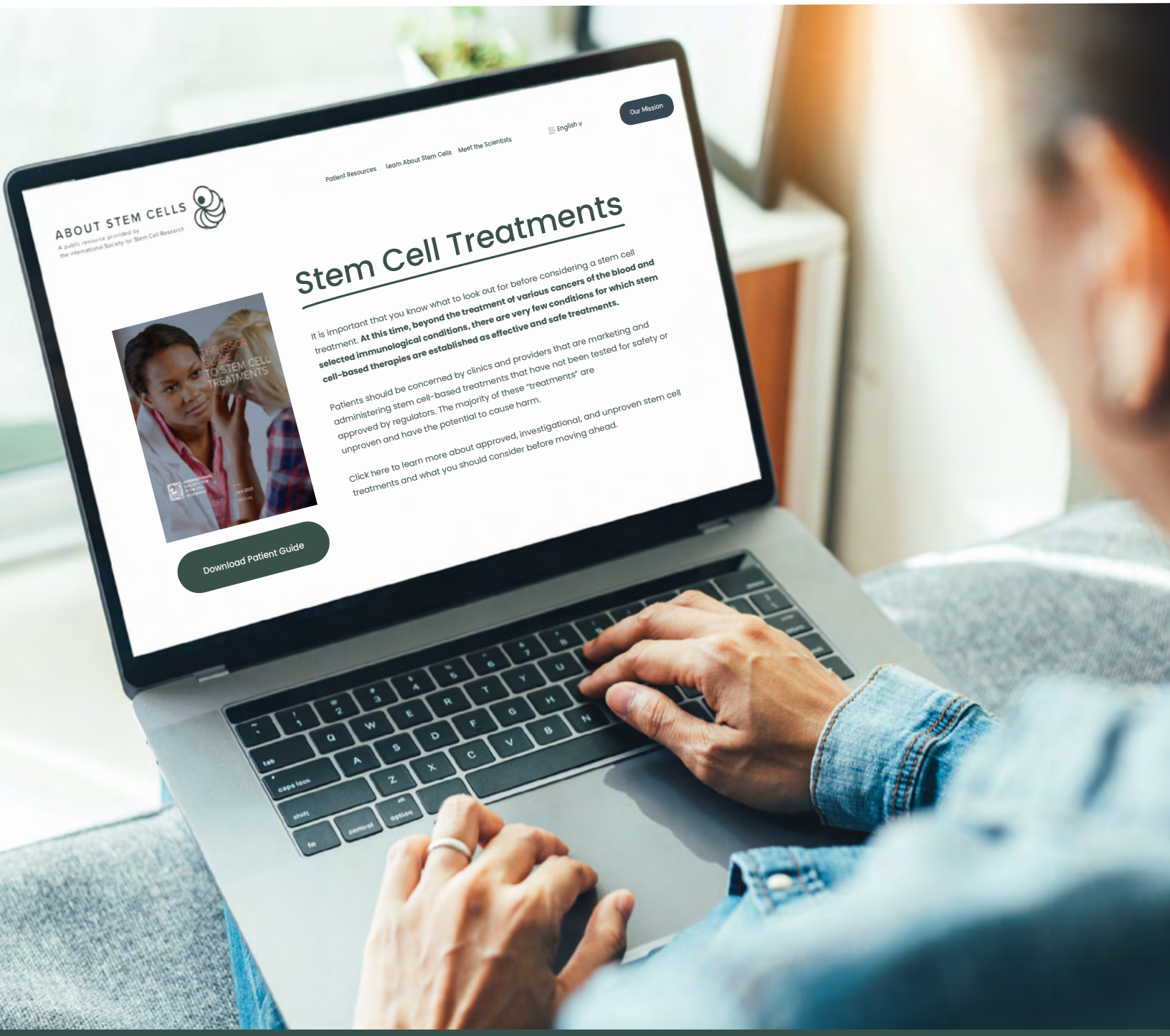

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SEASON 3 EPISODE 3:

THE SELLING OF STEM CELLS

LEIGH TURNER, PHD
UNIVERSITY OF CALIFORNIA, IRVINE, USA

SEASON 3 EPISODE 4:

HUMAN FETAL TISSUE: A LEGACY OF BIOMEDICAL RESEARCH CONTRIBUTIONS

JUSTIN BRUMBAUGH, PHD
UNIVERSITY OF COLORADO, USA

LAWRENCE GOLDSTEIN, PHD
UNIVERSITY OF CALIFORNIA, SAN DIEGO (EMERITUS), USA

TYLER LAMB, JD
INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH, USA

TAMRA LYSAGHT, PHD
UNIVERSITY OF SYDNEY, AUSTRALIA

SPEAKER ABSTRACTS

All times are listed in Eastern Daylight Time (EDT) USA

THURSDAY, 4 APRIL 2024

10:35 AM – 12:36 PM
FUNDAMENTAL MOLECULAR MECHANISMS OF EARLY DEVELOPMENT

10:35 AM – 11:15 AM

STEM CELL-BASED EMBRYO MODELS TO UNDERSTAND HOW TISSUES AND ORGANS ARE FORMED AND WORK TOGETHER

Zernicka-Goetz, Magdalena

Biology and Biological Engineering, Caltech, Pasadena, CA, USA and University of Cambridge, UK

I will talk about our lab's work to establish methods for culturing human and mouse embryos beyond implantation in vitro and how results coming from these studies enabled us to put together multiple stem cell types, programmed to form embryonic and extra-embryonic tissues, to self-organise into complete embryo-like structures. I will detail how we are using these complete mouse and human embryo models to determine the mechanisms behind embryo self-organisation, bringing insight into the cellular and molecular mechanisms that control previously unexplored aspects of early mammalian development until early organogenesis.

Funding Source: NIH, Horizon Ventures, NOMIS Foundation, Open Philanthropy

Keywords: *embryo, models, development*

11:15 AM – 11:27 AM

THE TIME INTEGRAL OF BMP SIGNALING CONTROLS DIFFERENTIATION IN 2D HUMAN GASTRULOIDS BY MODULATING SOX2

Heemskerk, Idse[1], Teague, Seth[2], Primavera Gillian[2], Chen, Bohan[3], Liu, Zong-Yuan[1], Yao, LiAng[1], Freeburne, Emily[1], Khan, Hina[1], Jo, Kyoung[1], Johnson, Craig[1]

[1]Cell and Developmental Biology, University of Michigan, Ann Arbor, MI, USA, [2]Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA, [3]Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI, USA

How paracrine signals are interpreted to yield multiple cell fate decisions in a dynamic context during human development in vivo and in vitro remains poorly understood. We used automated tracking method to follow signaling histories linked to cell fate in large numbers of human pluripotent stem cells (hPSCs). Using an unbiased statistical approach, we discovered that measured BMP signaling history correlates strongly with fate in individual cells. We found that BMP response in hPSCs varies more strongly in the duration of signaling than the level. However, by direct manipulation of signaling we discovered that both the level and duration of signaling activity causally control cell fate choices only by changing the time integral of signaling and that duration and level are therefore interchangeable in this context. In 2D human gastruloids, a stem cell model for patterning of the human embryo, we showed that signaling histories predict the fate pattern and that the integral model correctly predicts changes in cell fate domains when signaling is perturbed. To reveal the underlying mechanism for how cells integrate BMP signaling we performed a screen for transcription factors whose expression reflects integrated BMP signaling, yielding three families of transcription factors. Focusing on the SOX family, we found that direct modulation of SOX2 and genomic data support a key role for SOX2 in integration of BMP signaling.

Funding Source: National Institute of General Medical Sciences (NIGMS R35GM138346) NSF RECODE (2033654)

Keywords: *signaling, gastrulation, quantitative*

SPEAKER ABSTRACTS

11:27 AM – 11:39 AM

REDEFINING ENDODERMAL PROGENITOR LANDSCAPE IN EMBRYONIC HEPATO-BILIARY-PANCREATIC DEVELOPMENT

Iwasawa, Kentaro[1], Koike, Hiroyuki[1], Reza, Hasan Al[1], Kishimoto, Keishi[2], Rankin, Scott[2], Thorner, Konrad[2], Kimura, Masaki[1], Iwafuchi, Makiko[2], Wells, James, M.[2], Zorn, Aaron M.[2], Takebe, Takanori[1]

[1]Division of Gastroenterology, Hepatology and Nutrition, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA, [2]Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

Liver organogenesis commences with invaginating epithelia at the foregut and midgut endoderm border. The prevailing developmental dogma designates the ventral posterior foregut endoderm as the exclusive driver of liver development. Fate mapping on embryonic day (E) 8.5 reveals distinct progenitor populations around the anterior intestinal portal (AIP) lip, contributing broadly to hepato-biliary-pancreatic development. Recent human pluripotent stem cell (hPSC) findings implicate Caudal type homeobox 2 (Cdx2)+ endoderm, a midgut marker, in hepatic precursor specification. However, the expression patterns of CDX2 in the AIP lip remain inadequately characterized, and its developmental relevance is unclear. This study investigates the contribution of Cdx2+ endoderm to early hepatic fate specification in mice and humans, elucidating interactions between regionalized endoderm and mesoderm during hepatic organogenesis. Immunostaining at E8.5 in mice reveals CDX2+ localization in the lateral, but not medial, AIP lip region. Similarly, single-cell RNA sequencing (scRNAseq) of mouse embryos discloses the progressive acquisition of hepatic fate from Foxa2+Cdx2+ posterior gut endoderm. RNA-sequencing of hPSC-derived Foxa2+Cdx2+ posterior gut exhibits upregulation of lateral AIP lip characteristic genes, consistently generating hepatic progenitors in the foregut-midgut boundary model. To decipher the epigenetic landscape of Cdx2+ posterior gut (PG) compared to the Sox2+ anterior gut (AG), we analyze the activating marker H3K4me3 and the repressive marker H3K27me3 on hPSC-derived AG and PG. Significantly distinct Gene Ontology terms reveal upregulation in AG for H3K4me3 and in PG for H3K27me3 in "Wnt-protein binding" and "Wnt-activated receptor activity," indicating a suppressed Wnt pathway response in PG relative to AG. This observation is confirmed with higher peaks in H3K4me3 within AG and H3K27me3 within PG, particularly in Wnt receptors, including Frizzled class receptors 1 (FZD1), FZD2, FZD5, FZD7, and FZD8. Trajectory analysis unveils retinoic acid-dependent expression of secreted Wnt antagonists preceding hepatic transcriptional program activation. Overactivation of Wnt signaling abolishes hepatic specification potential in ex vivo cultures. These experimental insights underscore previously underappreciated endodermal progenitors in vertebrate hepatic organogenesis, emerging through intricate interactions between regionalized endoderm and neighboring mesoderm.

Funding Source: This work was supported by Cincinnati Children's Research Foundation grant, NIH Director's New Innovator Award (DP2 DK128799-01) for TT. KI is a New York Stem Cell Foundation–Druckenmiller Fellow.

Keywords: *Liver, Development, Endoderm*

11:39 AM – 11:51 AM

TGFβ/NODAL SIGNALING CONTROLS HUMAN PRIMORDIAL GERM CELL SPECIFICATION AND MAINTENANCE OF GERM CELL FATE

Overeem, Arend, Chang, Yolanda, Lopes, De Sousa Chuva Susana

Anatomy and Embryology, Leiden University Medical Centre, Amsterdam, Netherlands

The human germline is established around week 2–3 of embryonic development, with the emergence of primordial germ cells (PGCs) induced by bone morphogenic proteins (BMPs). What controls the separation of the germline from other BMP-induced somatic lineages is not fully resolved. Moreover, even after specification, PGCs may not be fully committed to the germ cell lineage, as exemplified by the possibility to generate pluripotent embryonic germ cells (EGCs) from mouse and human PGCs. Such lineage plasticity is also observed in PGC-like cells (PGCLCs) derived from pluripotent stem cells (PSCs). As a consequence,

SPEAKER ABSTRACTS

PGCLCs in prolonged culture tend to lose germ cell identity, hampering research on germ cell maturation. Recently we showed that applying BMP4 with extracellular matrix (ECM) components induces formation of human PGCLCs from PSCs with 50% efficiency. Building on this work, we report that TGFβ/Nodal signaling controls the specification of PGCLCs, and subsequently, the maintenance of germ cell fate. Whereas, blocking or strongly inducing TGFβ/Nodal signaling disrupts PGCLC specification, establishing low levels of TGFβ/Nodal signaling enables PGCLC induction at 75%-80%. After specification, inhibiting TGFβ/Nodal signaling proves crucial for maintaining the germ cell fate. Continued TGFβ/Nodal inhibition allows the feeder-free maintenance and proliferation of isolated PGCLCs. During this extended culture, PGCLCs sustain expression of key PGC markers, namely SOX17, TFAP2C, and POU5F1, while also upregulating CD38 and SUSD2, perhaps indicating of a transition to migratory-stage germ cells. Notably, hPGCLCs cultured without TGFβ/Nodal inhibition assume an embryonic germ cell (EGC)-like state, expressing pluripotency markers POU5F1 and SOX2, but lacking SOX17 expression. Our study identifies the differential role of TGFβ/Nodal signaling during and after specification of PGCLCs. These findings allow for the efficient generation and culture of PGCLCs, providing a new human in vitro model that can be used for investigating the elusive processes of germ cell migration and maturation.

Funding Source: This work was supported by Novo Nordisk Foundation (reNEW NNF21CC0073729) and the Dutch Research Council the “Nederlandse organisatie voor gezondheidsonderzoek en zorginnovatie” (ZonMw).

Keywords: *Germline, PGC, Gametogenesis*

11:51 AM – 12:16 PM

FOREGUT ORGANOGENESIS FROM EMBRYOS TO ORGANOID MODELS

Zorn, Aaron[1], Kishimoto, Keishi[2], Iwasawa, Kentaro[3], Han, Lu[1], Eicher, Alexandra[1], Helmrath, Michael[4], Morimoto, Mitsuru[5], Wells, James M.[1]

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Visceral organs, such as the lungs, esophagus, stomach and liver, are derived from the fetal foregut through a series of inductive interactions between the definitive endoderm and the surrounding splanchnic mesoderm (SM). While endoderm patterning is fairly well studied, the paracrine signaling controlling SM regionalization and how this is coordinated with epithelial identity during organogenesis is obscure. This knowledge gap has hindered efforts to generate complex multi-lineage PSC-derived organoid models. With single cell transcriptomics we generated a high-resolution cell state map of the embryonic mouse foregut identifying a diversity of SM lineages that develop in close register with the organ-specific epithelium. We infer a spatiotemporal signaling network of endoderm-mesoderm interactions that orchestrate foregut organogenesis. Using this signaling roadmap, we developed protocols to direct the differentiation of different SM subtypes from human pluripotent stem cells (hPSCs), which previously have been elusive. Addition of this hPSC-derived mesenchyme to foregut endoderm organoids results in enhanced tissue complexity and maturation. This opens the door to next-generation organoids for modeling human development and disease.

Keywords: *foregut, organoigenesis, mesenchyme*

SPEAKER ABSTRACTS

12:16 PM – 12:36 PM

PIONEER AND PRDM TRANSCRIPTION FACTORS COORDINATE BIVALENT EPIGENETIC STATES TO SAFEGUARD CELL FATE

Iwafuchi, Makiko[1], Matsui, Satoshi[1], Granitto, Marissa[1], Buckley, Morgan[1], Ludwig, Katie[1], Koigi, Sandra[1], Shiley, Joseph[1], Zacharias, William[2], Mayhew, Christopher[1], Lim, Hee-Woong[3]

[1]Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA, [2]Division of Pulmonary Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA, [3]Division of Biomedical Informatics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

Pioneer transcription factors (TFs) possess a unique ability to open target sites on chromatin, facilitating access for multiple epigenetic and transcriptional regulators for initiating new gene regulatory programs. Current dogma is that pioneer TFs play activating roles in epigenetic and gene regulation, by opening local chromatin to activate enhancers and target genes. However, cell fate control requires the coordination of both lineage-specific gene activation and repression of alternative lineage programs, a process that is poorly understood. Our recent studies revealed unexpected roles of FOXA pioneer TF in repressing alternative-lineage gene expression programs, while simultaneously activating endodermal genes during human endoderm differentiation. In our new CRISPR interference model, which targeted the three redundant FOXA genes, we observed not only the anticipated loss of endoderm gene expression but also the de-repression and ectopic expression of genes typically expressed in alternative lineages or during later stages of endoderm differentiation. Through proteomics and cistrome analyses, we gained insight into the repressive mechanisms by which FOXA cooperates with PRDM1 TF to recruit Nucleosome Remodeling and Deacetylation (NuRD) and Polycomb Repressive Complexes (PRC) to establish bivalent enhancers. Similarly, OCT4 coordinates with PRDM14 to form bivalent enhancers and repress cell differentiation programs in human pluripotent stem cells, suggesting that this may be a common mechanism. Our findings reveal a critical mechanism whereby pioneer TFs, in cooperation with PRDM TFs, prevent precocious and alternative-lineage gene expression by employing epigenetic repression to safeguard cell fate.

Funding Source: The National Institutes of Health (P30 DK078392 and 1R01GM143161) and the Cincinnati Children's Research Foundation (Trustee Awards and Center for Pediatric Genomics Pilot Awards).

Keywords: *Pioneer-transcription-factors, Cell-fate-control, Bivalent-epigenetic-state*

12:55 PM – 2:00 PM
SPONSORED INNOVATION SHOWCASES

12:55 PM – 1:25 PM

ORGANOIDS AND STEM CELL-BASED TISSUE MODELS: POWERFUL TOOLS FOR DRUG DISCOVERY

Presented by STEMCELL Technologies Inc.

Jenna Moccia, PhD

STEMCELL Technologies, Inc., Canada

As a research community, we understand better than ever the mechanisms and signals that lead stem cells and progenitor cells to form functional tissues. This understanding has been harnessed to create stem cell-derived in vitro tissue models with cells that exhibit the structural features, as well as transport and metabolic capabilities that are characteristic of in vivo tissues. Using stem cell-based tissue models, including organoids, scientists working to discover new therapies can now scalably gain more predictive insights into how prospective new drugs are likely to impact disease states and healthy tissues. This talk showcases how epithelial organoids can be used as in vitro models to predict potency and tissue-specific toxicities. We further describe how epithelial progenitor cells can be cultured as monolayers at the air-liquid interface or apical-out structures to generate cell models that have the physical and practical characteristics necessary to apply them to drug discovery applications.

SPEAKER ABSTRACTS

1:30 PM – 2:00 PM

EXIT STRATEGY WITH PIPELINE HOLDERS BY HITACHI GLS-RLS ALLIANCE TOWARD INDUSTRIALIZATION OF REGENERATIVE MEDICINE

Presented by Rorze Lifescience Inc.

Tomohiro Takanose

Rorze Lifescience, Japan

Taro, Kabakino

Hitachi Global Life Solutions, Japan

New treatments are being developed worldwide through the pursuit of many great challenges and technological developments related to regenerative medicine. In the process of transitioning from basic research to seeds and Leeds, it is evident that almost all cell culture processes are currently performed manually. The final drug must meet the requirements of not only product safety but also stability, productivity, price, as well as being able to be industrialized and delivered to patients as soon as possible. Having a strategy is essential for achieving this. Since 2019, Hitachi Global Life Solutions and Rorze Lifescience have been working as strategic partners on industrialization, working together with several pipeline holders in Japan and Asia. In this showcase, we will present our endeavors and some case studies.

2:15 PM – 3:20 PM MORPHOGENETIC MECHANISMS OF EARLY DEVELOPMENT

2:15 PM – 2:40 PM

DECONSTRUCTING HUMAN MUSCULO-SKELETAL SYSTEM DEVELOPMENT IN VITRO

Pourquié, Olivier

Harvard Stem Cell Institute, Harvard University, Cambridge, MA, USA

Skeletal muscles and vertebrae derive from precursors located in the embryonic segments called somites. These structures form periodically from a posterior tissue called Presomitic Mesoderm (PSM). The rhythmic formation of somites involves a molecular oscillator called segmentation clock which drives pulses of Notch, Wnt and FGF signaling in the PSM. Virtually nothing is known on human somitogenesis as it proceeds between 3- and 5-weeks post conception when embryos are extremely difficult to access. We have developed protocols to differentiate human pluripotent stem cells (ES/iPS) in vitro into PSM. Single cell RNA-sequencing comparison of these human cells differentiating in vitro with mouse embryo PSM reveals that they faithfully recapitulate the PSM differentiation sequence in vitro. Using our in vitro system as a proxy for human somitogenesis, we were able to demonstrate that human iPS reporter cells harboring a HES7 fluorescent reporter differentiated to PSM exhibit 5-hour oscillations, thus identifying the human segmentation clock. We have also succeeded in generating PSM organoids that can sequentially form somites exhibiting a normal antero-posterior pattern in vitro. By mimicking key signaling events leading to muscle formation in the embryo, we developed directed differentiation protocols which recapitulate the developmental sequence of myogenesis. Our work provides a framework to study early stages of human myogenesis which are poorly accessible in the embryo.

Funding Source: NIH, French Muscular Dystrophy Association (AFM)

Keywords: *somite, skeletal muscle, embryo*

2:40 PM – 2:55 PM

INTERPRETING THE GEOMETRY AND RHYTHM OF EARLY KIDNEY FORMATION FOR SYNTHETIC MORPHOGENESIS

Hughes, Alex[1], Viola, John[1], Liu, Jiageng[1], Grindel, Samuel[1], Davis, Sachin[1], Prahl, Louis[1], Huang, Aria[1], Chan, Trevor[1], Hayward-Lara, Ella[2], Porter, Catherine[1], Zhang, Jitao[3]

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In this work we discover rhythmic mechanical and differentiation ‘pace-making’ in nephron-forming kidney niches using live imaging, packing theory, organoids, and spatial sequencing. The kidney develops through branching of ureteric bud epithelial tubules (the future urinary collecting ducts), stroma, and nephron

SPEAKER ABSTRACTS

progenitors in the cap mesenchyme that surrounds each tubule tip. Dynamic interactions between these tissues set nephron numbers for life, impacting adult disease. How then are the rates of nephron formation and ureteric tubule branching balanced? Here we study the consequences of tubule tip packing at the embryonic kidney surface for tip organization and nephron formation. Over developmental time, kidney curvature reduces and 'tip domains' pack more closely, creating a semi-crystalline tip geometry at the kidney surface. This causes a rigidity transition to more solid-like tissue properties at later developmental stages, confirmed by micromechanical measurements. We then define a tip 'life-cycle' between branching events, and find that nephrogenesis rate varies over this life-cycle. Applying force inference techniques adapted from a cell vertex model and validating with laser ablation shows that tip domains experience a cyclical mechanical transient over each life-cycle. We then hypothesized that tip duplication periodically creates a mechanical microenvironment permissive to nephrogenesis. Indeed, mimicking a mechanical transient in human iPSC-derived nephron progenitor organoids increased Wnt-driven commitment to early nephron cell aggregates. The data suggest that temporal waves of mechanical stress within nephron progenitor populations could constitute a clock that synchronizes nephron formation and ureteric tubule duplication. In unpublished spatial sequencing data, we find that the avalanche-like commitment of nephron progenitors to early nephrons reflects rhythmic transcriptional priming associated with the ureteric bud branch life-cycle. This may act to peg nephron formation rate to the ureteric bud branching rate. Ongoing work will clarify variation in nephron endowment between kidneys and advance engineered replacement kidney tissues for regenerative medicine.

Funding Source: University of Pennsylvania MRSEC DMR-2309043, NIH T32HD083185 (JMV), NIH NIGMS MIRA R35GM133380 (AJH), NIH NIDDK R01DK132296 (AJH), NSF CAREER award 2047271 (AJH), Penn CPE4H pilot grant (AJH).

Keywords: *organogenesis, rhythmic biology, organoids*

2:55 PM – 3:20 PM

BLADDER ORGANOIDS GENERATED FROM HUMAN IPS CELLS MIMIC UROTHELIAL STRUCTURE AND FUNCTIONS

Takasato, Minoru, Ofuji, Kazuhiro, Wymeersch, Filip Jos

RIKEN Center for Biosystems Dynamics Research, Kobe, Japan

The bladder is a sac-like organ that stores and excretes urine produced by the kidneys and protects the body from uremic toxins. The developmental process of the bladder is unique to mammals, with the ventral side of the cloaca separating from the hindgut and developing into the urothelium. Recent advances in biotechnology have made it possible to directly differentiate human pluripotent stem cells into 3-dimensional tissue structures called organoids. Here, we have differentiated human iPS cells (hiPSCs) into bladder organoids with structural and functional characteristics specific for the bladder. We show that hiPSCs-derived hindgut/cloaca spheroids have bipotency to the intestine and the bladder and that BMP4-mediated ventralization of them is required for their fate determination to the bladder. We also demonstrate that ATRA, FGF10 and BMP4 induce bladder urothelial progenitors from the ventral hindgut/cloaca spheroids, and that subsequent regulation of ATRA concentration and long-term culture promote their maturation. Our induced mature bladder organoids had a sac-like structure, with a stratified epithelial layer consisting of all types of bladder urothelium, and revealed a barrier function. Bladder organoids provide a powerful model for understanding human bladder development as well as disease models and therapeutic cells in future.

Funding Source: JSPS KAKENHI Grant Number JP21H03801, AMED under Grant Number JP21bm0704062 and Otsuka Pharmaceutical Co., Ltd.

Keywords: *Bladder, Organoid, hPSCs*

SPEAKER ABSTRACTS

4 April 2024

3:50 PM – 4:55 PM
DEVELOPMENTAL ORIGINS OF DISEASES: DEGENERATIVE DISEASES

3:50 PM – 4:15 PM

HUMAN PLURIPOTENT STEM CELLS COME OF AGE IN MODELLING CARDIOVASCULAR DISEASES AND TOXICITY

Mummery, Christine L.

Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands

Our lab creates models for cardiovascular disease based on pluripotent stem cells (hPSCs). We use these for understanding disease mechanisms and cardiotoxic effects of drugs. We can predict the toxic effects of test drugs with almost 80% accuracy in (immature) cardiomyocyte monolayer cultures. When we require mature cells, we combine hPSC-cardiomyocytes with cardiac fibroblasts and endothelial cells in "microtissues". The cardiomyocytes develop electrical, metabolic and functional features allowing us to model postnatal onset diseases or dissect which cell types in the heart are actually responsible for the disease phenotype. We showed for example fibroblasts in the heart can contribute to abnormal heart contraction in patients with arrhythmogenic cardiomyopathy. These complex cell systems are paving the way towards better understanding of disease mechanisms and drug discovery.

Funding Source: Novo Nordisk Foundation grant (NNF21CC0073729; reNEW)

Keywords: *cardiovascular, pluripotent, disease*

4:15 PM – 4:30 PM

NEUROMORPHOCHIP ORGANOIDS REVEAL A NOVEL MICROCEPHALY MECHANISM

Reiner, Orly[1], Fu, Jianping[2], Tshuva, Rami Yair[1], Bok, Jeyoon[2], Xu, Xufeng[2], Bhattacharya, Bidisha[1], Sapir, Tamar[1]

[1]Molecular Genetics and Molecular Neuroscience, Weizmann Institute of Science, Rehovot, Israel, [2]Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA

We are introducing a groundbreaking advancement in neural organoid technology. Our innovative approach involves the development of a patterned central nervous system (CNS) neural tube organoid, from head to tail, derived from human pluripotent stem cells. This novel organoid exhibits remarkable organization along both the rostral-caudal and dorsal-ventral axes, showcasing gene expressions from key regions such as the head, forebrain, midbrain, and hindbrain. The orchestrated expression of HOX genes along the neural tube further emphasizes the precision of our methodology, culminating in the development of the pallium and subpallium through continued culture. Single-cell RNA sequencing validates the authenticity of our system, with striking parallels to developing human embryos, notably aligning with Carnegie Stage 12 at Day 9. Leveraging this advanced model, we are actively investigating neurodevelopmental diseases, yielding promising results. We delved into the genetic intricacies underlying microlissencephaly by genome-engineering NDE1 knockout human pluripotent stem cells. The subsequent differentiation into various brain organoid types revealed a profound impact on brain patterning. Our findings indicate a unique mechanism for microcephaly, marked by changes in early brain patterning.

Funding Source: Weizmann Institute, ISF grant 545/21, BSF; Grant No. 2017006, National Institute of Neurological Disorders and Stroke of the National Institutes of Health under Award Number R21NS127983.

Keywords: *Brain, Malformations, Patterning*

SPEAKER ABSTRACTS

4:30 PM – 4:55 PM

HUMAN BRAIN ORGANOIDS BRING NEW INSIGHT INTO THE DEVELOPMENTAL ORIGINS OF BRAIN DISEASES

Velasco, Silvia

Stem Cell Biology, Murdoch Children's Research Institute, Parkville, Australia

Pluripotent stem cell-derived organoids represent a significant advance in modeling human brain development in vitro and provide an invaluable opportunity to investigate cellular and molecular processes underlying brain diseases. Mutations in genes encoding epigenetic modifiers have emerged as a leading cause of developmental conditions presenting megalencephaly/macrocephaly, autism, and intellectual disability, suggesting that the establishment and maintenance of precise epigenetic states is fundamental for normal brain growth, development, and function. However, the biological role of most epigenetic regulators during brain development remains unknown. By leveraging highly reproducible forebrain organoid models and single cell multiomics approaches, gene regulatory network reconstruction analysis, and high-throughput morphometric screenings, we investigated the molecular mechanisms through which haploinsufficiency of the SUV420H1/KMT5B and NSD1/KMT3B genes, which encode histone lysine methyltransferases, are involved in the pathogenesis of neurodevelopmental disorders. By uncovering cell-type specific abnormalities associated with epigenetic dysregulation during neurodevelopment, our work contributes to shed light on the link between abnormal brain growth and neurocognitive dysfunction and provides an experimental paradigm for investigating the molecular mechanisms and ultimately identify effective therapies for neurodevelopmental disorders.

Funding Source: Silvia Velasco is Principal Investigator at the Novo Nordisk Foundation Center for Stem Cell Medicine, reNEW, which is supported by a Novo Nordisk Foundation grant number NNF21CC0073729.

Keywords: *Brain, Organoids, Neurodevelopment*

FRIDAY, 5 APRIL 2024

9:00 AM – 10:05 AM
DEVELOPMENTAL ORIGINS OF DISEASES: CONGENITAL MALFORMATIONS

9:00 AM – 9:25 AM

DECIPHERING ENDOTHELIAL AND MESENCHYMAL ORGAN SPECIFICATION IN VASCULARIZED LUNG AND INTESTINAL ORGANOIDS

Gu, Mingxia

CuSTOM, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

During organogenesis, the orchestrated co-development of vasculature, mesenchyme, and neighboring epithelial cells is crucial for organ formation, maturation, and the acquisition of organ-specific characteristics. Here, utilizing a mesoderm-endoderm co-differentiation method regulated by BMP signaling, we constructed a human pluripotent stem cell-derived organoid system comprising lung or intestinal epithelium surrounded by mesenchyme and vasculature. Notably, the microenvironment of the organoid facilitated the development of organotypic features in the endothelium and mesenchyme, capturing inter-lineage communications found in human and mouse fetal organ atlas through single-cell RNA-sequencing. Utilizing this model, we identified novel marker genes in human anterior vs. posterior gut tube mesenchyme, and elucidated critical signaling pathways directing cell fate determination in organotypic endothelium and mesenchyme during early organ formation. After transplantation under the mouse kidney capsule, the vascularized organoid integrated with the host circulation, developing perfusable human capillary structures adjacent to the epithelial layer and maturing into highly specialized lung endothelium resembling the gas exchange barrier observed in human alveoli. Furthermore, we generated vascularized lung and intestinal organoids using iPSCs derived from patients with Alveolar Capillary Dysplasia with Misalignment of Pulmonary Veins, a complex congenital disorder caused by FOXF1 mutation. Our model recapitulated the cell autonomous and non-autonomous abnormalities in the alveolar endothelium and epithelium. The establishment

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of the vascularized organoids provides a unique platform for elucidating the mechanisms driving endothelium and mesenchyme specification during early organogenesis, and investigating complex cell-cell communications in human gut tube development and disease.

Keywords: *organoid, co-differentiation, vascularization*

9:25 AM – 9:40 AM

MODELLING BARRET'S ESOPHAGUS USING HUMAN IPSC-DERIVED ESOPHAGEAL RAFT CULTURES

Silverberg, Vered Shacham, Wells, James

Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

Barrett's Esophagus (BE) is a chronic condition caused by acid reflux from the stomach to the esophagus. BE causes gastric and intestinal metaplasia of the esophagus epithelium, affects approximately 1.3 million people in the USA alone and is also the leading risk factor in developing esophagus adenocarcinoma (EAC). Our understanding of BE and related deadly conditions has been very limited to date because of the lack of good esophageal models. We recently developed human iPSC-derived esophageal raft cultures (ERC) that show high resemblance to human esophagus, and we aim to study whether this new culture system is suitable to model BE by chronically exposing ERC to acidic or control media. Chronic exposure of normal esophageal cultures to acidic environment resulted in ectopic expression of gastric and intestinal markers in the ERC epithelium, as well as the loss of expression of normal esophageal markers and transition from squamous to columnar epithelium, which are all known phenotypes of BE. Our results demonstrate that our new human iPSC-derived ERC are suitable to model and study BE. Chronic exposure of these cultures to acidic environment recapitulates the phenotype of BE patients' biopsies- gastric and intestinal metaplasia. Once we established our cultures as a good model system, we can now proceed into using these cultures to study the development of the disease, to identify possible risk factors for developing EAC and to study possible treatments for BE.

Keywords: *Esophagus, Barrett's, metaplasia*

9:40 AM – 10:05 AM

MECHANISTIC AND THERAPEUTIC MODELING OF AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE WITH HUMAN KIDNEY ORGANOIDS

McMahon, Andrew

Stem Cell Biology and Regenerative Medicine, Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research/Keck School of Medicine of USC, Los Angeles, CA, USA

Autosomal dominant polycystic kidney disease (ADPKD), an inherited disorder leading to a loss of kidney function in approximately 8 million world-wide, occurs in patients inheriting a single inactive allele of either PKD1 or PKD2, on de novo mutation or epigenetic silencing of the remaining active allele. Though human cystic disease initiates in the adult, with a mean onset in the late 40's and mid 50's, mouse studies show germline removal of Pkd1 or Pkd2 activity results in dramatic cyst growth in the developing fetal kidney. Thus, ADPKD is a disease target potentially suited to disease modeling in functionally immature, pluripotent stem cell-derived human kidney organoids. Using a scalable manufacturing system and automated multi-day tracking of cyst development, we generated PKD1 and PKD2 mutant kidney organoid models and performed in vitro screening of annotated compound libraries, identifying a small molecule inhibitor of epithelial cyst formation. Analysis of compound action links cyst initiation and later cyst growth to the mitochondrial respiratory chain. At late cyst stages, several lines of evidence point to a role for nuclear YAP in proliferative expansion of cysts. Applying chemical genetic approaches to human ADPKD models identified a link between atypical protein kinase C, an apical membrane localized suppressor of Hippo signaling, and nuclear localization of YAP, in renal cyst growth. These studies provide new insight for therapeutic evaluation in ADPKD.

Keywords: *Kidney, Organoid, Disease-modeling*

SPEAKER ABSTRACTS

10:45 AM – 11:50 PM
INFECTIOUS DISEASES

10:45 AM – 11:10 AM

HUMAN PLURIPOTENT STEM CELL-DERIVED ORGANOIDS AND SARS-COV-2

Chen, Shuibing

Department of Surgery, Weill Cornell Medicine, New York, NY, USA

Human pluripotent stem cell (hPSC)-derived cells and organoids provide a powerful platform for studying infectious diseases. We have established an hPSC-based platform that encompasses over 15 types of hPSC-derived cells and organoids. This platform allows us to systematically examine viral tropism, host responses, immune cell-mediated host damage, and conduct antiviral drug screening. Using hPSC-derived forebrain organoids, we identified hippeastrine as a compound that inhibits ZIKV infection. In response to the COVID-19 pandemic, we assembled a large consortium team to leverage hPSC-derived cells and organoids for the study of SARS-CoV-2. We conducted the first chemical screen using hPSC-derived lung and colonic organoids, which led to the identification of several anti-SARS-CoV-2 drugs. Furthermore, we reported a host-specific response to SARS-CoV-2 infection. This response included cell apoptosis in lung organoids, ferroptosis in cardiac pacemaker cells, and pancreatic beta cell transdifferentiation. Additionally, we developed an immune-cardiac platform to model the impact of immune cell-mediated heart damage and identified a JAK inhibitor that protects host cells from immune cell-mediated damage. Concurrently, the FDA approved the Emergency Use Authorization of a JAK inhibitor for COVID-19 patients.

Keywords: *Organoids, SARS-CoV-2, drug screening*

11:10 AM – 11:25 AM

DEVELOPMENT OF A LIBRARY OF NONHUMAN PRIMATE PLURIPOTENT STEM CELLS AND STEM CELL DERIVED LINEAGES TO STUDY SPECIES-SPECIFIC HOST RESPONSES AND RESTRICTIONS OF PATHOGENS

Schwartz, Robert[1], Bram, Yaron[1], Chandar, Vasuretha[1], Frankel, Angela[1], Gaska, Jenna[2], Ploss, Alexander[2]

[1]Department of Medicine, Weill Cornell Medicine, New York, NY, USA, [2]Department of Molecular Biology, Princeton University, Princeton, NJ, USA

Infectious diseases contribute significantly to human morbidity and mortality. 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 and the NHP iPSC library. Human, chimpanzee, and bonobo HLCs had robust HBV viral entry and infection while orangutans HLCs were permissive had less robust HBV infection. Rhesus macaque HLCs were not permissive to HBV viral entry or infection but transduction with the human HBV (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

SPEAKER ABSTRACTS

these species-specific differences to identify HBV host dependency factors that can then be used for targeted HBV therapy.

Keywords: *Virology, HBV, PSC*

11:25 AM – 11:50 AM

LINEAGE TRACING BLOOD ORIGINS IN VIVO TO GUIDE EFFICIENT IN VITRO PRODUCTION OF BLOOD PROGENITORS

Loh, Kyle, Fowler, Jonas L., Zheng, Sherry Li

Department of Developmental Biology, Institute for Stem Cell Biology & Regenerative Medicine, Stanford University School of Medicine, Stanford, CA, USA

The developmental origin of blood-forming hematopoietic stem cells (HSCs) is a longstanding question. Here our non-invasive genetic lineage-tracing in mouse embryos pinpoints that artery endothelial cells generate HSCs. Arteries are transiently competent to generate HSCs for 2.5 days (~E8.5-E11), but subsequently cease, delimiting a narrow timeframe for HSC formation in vivo. Guided by the arterial origins of blood, we efficiently and rapidly differentiate human pluripotent stem cells (hPSCs) into posterior primitive streak, lateral mesoderm, artery endothelium, hemogenic endothelium, and >90% pure hematopoietic progenitors within 10 days. hPSC-derived hematopoietic progenitors generate T, B, NK, erythroid, and myeloid cells in vitro and, critically, express hallmark HSC transcription factors HLF and HOXA5-HOXA10, which were previously challenging to upregulate. We differentiated hPSCs into highly-enriched HLF+ HOXA+ hematopoietic progenitors with near-stoichiometric efficiency by blocking formation of unwanted lineages at each differentiation step. hPSC-derived HLF+ HOXA+ hematopoietic progenitors could avail both basic research and cellular therapies.

Keywords: *Blood, Vasculature, HSC*

12:10 PM – 1:15 PM
SPONSORED INNOVATION SHOWCASES

12:10 PM – 12:40 PM

LIVE CELL METABOLIC ANALYZER, LICELLMO— PAVING THE WAY FOR METABOLIC RESEARCH & CELL AND GENE THERAPY

Presented by PHC Corporation of North America

Cristie Marko

PHC Corporation of North America, USA

Among the various biological functions that cells carry out to maintain life, metabolism is the key activity by which individual cells process nutrient molecules and is closely associated with cell proliferation and differentiation. The investigation and understanding of cellular metabolic mechanisms has become increasingly crucial. These analyses include basic stem cell research involving ES and iPS cells, cancer immunotherapy like CAR-T and TCR-T therapy, and development stages of manufacturing processes for the commercialization of cell and gene therapy (CGT) products. To meet the need in the field, PHC Corporation of North America is developing a continuous metabolic analyzer, LiCellMo™, which is being designed to achieve real-time visualization of the metabolic condition of living cells. This instrument is being designed to drive the opportunity to make new discoveries that have not been seen in previous studies. The PHCbi brand LiCellMo paves the way not only for metabolic research, but also the manufacturing of significant CGT products.

12:45 PM – 1:15 PM

OPTIMIZED SURFACES FOR STEM CELL-DERIVED MODELS OF HUMAN DEVELOPMENT AND DISEASE

Presented by Corning Life Sciences

Tom Bongiorno, PhD

Corning Life Sciences, USA

Spheroids and organoids hold tremendous promise for models of human development and disease. Using cell culture vessels with optimized geometry and surface chemistry, together with appropriate biochemical factors, small numbers of stem cells can be induced to representative models of developmental states and various diseases. In this series of studies, embryoid

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bodies, neurospheres, and intestinal organoids were generated from induced pluripotent stem cells. Within each study, the size and biomarker expression of the multicellular models were consistent, demonstrating the scalability of this approach to generate large numbers of models for further interrogation, such as detailed characterization or drug screening.

1:45 PM – 2:50 PM
CELL AND TISSUE THERAPIES

1:45 PM – 2:10 PM

IPSC-DERIVED NEXT-GENERATION T CELL THERAPY FOR REFRACTORY TUMORS

Ando, Miki

Hematology, Juntendo University School of Medicine, Hongo, Japan

Functionally rejuvenated virus-specific CTLs (rejTs) generated from iPSCs robustly suppress virus-associated tumors, such as EBV-associated lymphomas and cervical cancer. However, autologous rejT generation is time-consuming, leading to difficulties in treating patients with advanced cancer. Although the use of allogeneic rejTs can obviate this, rejection by patient immune system is a major obstacle. To overcome this problem, we have generated HLA class I-edited virus-specific rejTs using CRISPR/Cas9 technology. These rejTs not only suppress recipient immune rejection, but also retain more robust cytotoxicity compared to the original CTLs. Single-cell RNA sequencing analysis revealed that these rejTs were highly enriched in tissue-resident memory T cells and showed high expression of genes associated with cytotoxicity. These features may have promoted stronger TCR activation and increased TCR-mediated target cell death. We are currently generating clinical grade rejTs from a master cell bank of HLA-edited iPSCs derived from virus-specific CTLs at our cell processing center. We believe that these next-generation T cells, generated using iPSC and CRISPR/Cas9 technology, offer a sustainable and promising approach to “off-the-shelf” T cell therapy.

Funding Source: These studies were supported by a grant from the Japan Agency for Medical Research and Development (JP21bk0104117).

Keywords: *CTL, CRISPR-Cas9, Next-generation*

2:10 PM – 2:25 PM

EXAMINING THE EFFICACY OF HUMAN IPSC-DERIVED CORNEAL ENDOTHELIAL CELLS INJECTION AS AN ALTERNATIVE TO PEDIATRIC ENDOTHELIAL KERATOPLASTY

Riaz, Muhammad[1], Izzi, Jessica M.[2], Hutchinson, Eric K.[2], Riazuddin, S. Amer[1]

[1]The Wilmer Eye Institute, Johns Hopkins University School of Medicine, Baltimore, MD, USA, [2]Department of Molecular and Comparative Pathobiology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

The cornea is the outermost, transparent tissue of the eye composed of five layers with corneal endothelium (CE) as the innermost layer. The genetic and environmental insults lead to corneal endothelial cell (CEC) loss and if left untreated result in blindness. Corneal endothelial dystrophies are the leading cause of corneal transplantation performed in the United States each year. Congenital hereditary endothelial dystrophy (CHED) is a recessive blinding disease characterized by corneal opacification, degenerating CE, and thickened Descemet’s membrane (DM). Loss of function of a membrane protein, SLC4A11, results in CHED. This progressive disease develops during infancy and donor tissue-dependent transplantation surgery is the only treatment option. Although keratoplasty has been successful, high graft rejection in pediatric patients due to a more active immune system, challenging surgical procedure, and the global shortage of donor tissue advocate for alternate therapies. We previously documented that cryopreserved human embryonic stem cell (hESC)-derived CECs form a functional CE on a central 8-mm denuded DM in rabbits and monkeys. Further, we recently have confirmed that hESC-derived CECs form a functional CE and DM on central 4-mm denuded stroma in adult rabbits and monkeys. Here, we evaluated the efficacy of cryopreserved human induced pluripotent stem cell (iPSC)-derived CECs to form a functional CE and DM in juvenile rabbits and monkeys. Here, we first generated corneal edema rabbit and monkey models after stripping the central 5-mm CE and DM by Descemetorhexis. A few days later, following the development of corneal edema, we injected cryopreserved human iPSC-derived CECs into the

SPEAKER ABSTRACTS

anterior chamber of juvenile rabbits and monkeys and allowed the injected CECs to settle on posterior stroma by placing the animals in an eye-down position for four hours. The CEC-injected eyes developed transparent corneas a few weeks after the injection of CECs. Importantly, the pachymetry of the CEC-injected eyes using a handheld pachymeter illustrated similar corneal thickness to the untreated eyes. We are the first to report examining the efficacy of cryopreserved iPSC-derived CECs in juvenile rabbit and monkey corneal edematous models as an alternative to donor tissue in pediatric endothelial dystrophy patients.

Funding Source: This work is supported, in part, by the Knights Templar Eye Foundation (grant number 2023-22).

Keywords: *cornea, keratoplasty, rabbit*

2:25 PM – 2:50 PM

GENOME EDITING STEM CELLS TO PREVENT ENGRAFTMENT ARRHYTHMIAS AFTER CARDIOMYOCYTE TRANSPLANTATION

Murry, Charles E., Marchiano Silvia, Bertero Alessandro, Reinecke Hans, Klaiman Jordan, Yang Xiulan, Pabon Lil, Sniadecki Nathan, Nakamura Kenta, Neidig Lauren, Thies Scott, MacLellan Robb.

Institute for Stem Cell and Regenerative Medicine, University of Washington, Seattle, WA, USA,

Stem cell-based heart regeneration has immense promise but has proven difficult to achieve. My lecture will describe our efforts to eliminate arrhythmias accompanying cardiomyocyte transplantation. We used genome editing to identify the culprit ion channels that carry the arrhythmic currents post engraftment (spoiler alert: pacemaking channels), creating non-arrhythmic grafts that remain excitable. When transplanted into porcine hearts, these edited cells reduce arrhythmias by ~95%. We are currently testing whether these edited cells retain their ability to restore contractile function in the infarcted heart.

Funding Source: NIH, Sana Biotechnology, UW Medicine Heart Regeneration Program

Keywords: *Cardiomyocyte transplantation, arrhythmia, gene editing*

2:50 PM – 3:30 PM
PANEL DISCUSSION: STEM CELL-BASED MODELS FOR DRUG DISCOVERY

Sponsored by Nikon Instruments Inc.

Kasendra, Magdalena

(CuSTOM) Center for Stem Cell and Organoid Research and Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

Mummery, Christine L.

Anatomy and Embryology, University of Leiden Medical Center, Leiden, Netherlands

Pang, Li

Food and Drug Administration, USA

Takebe, Takanori

Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA and Osaka University and Tokyo Medical and Dental University, Japan

4:00 PM – 5:25 PM
MOVING INTO THE FUTURE WITH EMERGING TECHNOLOGIES AND CLOSING REMARKS

4:00 PM – 4:25 PM

NEW GENOMIC TECHNOLOGIES TO DECONSTRUCT AND CONTROL CELL IDENTITY

Morris, Samantha

Developmental Biology, Washington University School of Medicine, St. Louis, MO, USA

Understanding how cell identity is regulated represents the fundamental biological focus of my research program, providing a foundation for the precision engineering of clinically relevant cell types. The ectopic expression of transcription factors (TFs) is a common approach to directly reprogram fully differentiated cells to alternate fates. Despite intense investment in this strategy, most protocols generate low yields of incompletely specified cells, rendering them unsuitable for therapeutic application and disease modeling. Why does direct reprogramming fail to efficiently recapitulate target cell identity, and how can cells be faithfully guided toward defined functional states to support new regenerative therapies? How cell identity

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is defined in molecular and functional terms is central to these questions, representing a critical gap in knowledge that my research program seeks to address. To deconstruct cell identity quantitatively, my team develops innovative experimental and computational genomic technologies that we apply to a broad range of development, differentiation, and reprogramming paradigms. To date, our single-cell multiomic lineage tracing has revealed mechanistic insight into reprogramming failure, suggesting that cells transit through rare malleable states that are primed for fate conversion. Our novel computational tools to interrogate gene regulatory networks (GRNs) have revealed drivers of cell fate specification and conversion, delivering a systems-level understanding of cell identity. We continue to build on this work to characterize the narrow gene regulatory states within which ectopic TFs can effectively reconfigure cell identity, where exploiting these malleable states promises to unlock fate potential. Further, we are currently developing a framework to empirically deconstruct gene regulation in cell fusion models, offering unique insight into efficient and faithful reprogramming of cell identity. Finally, with these foundational principles, we are developing new in vitro and in silico models of intestinal failure, applying our digital blueprint for cell engineering to correct cell dysfunction in human disease.

Keywords: *Reprogramming, single-cell lineage tracing, gene regulatory networks, intestinal failure*

4:25 PM – 4:40 PM

BRAIN ORGANOID COMPUTING FOR ARTIFICIAL INTELLIGENCE

Guo, Feng

Intelligent Systems Engineering, Indiana University Bloomington, IN, USA

In recent years, the demand for computing power has surged with the rapid evolution of artificial intelligence (AI), including machine learning and artificial neural network models. However, the current state of computing hardware faces challenges related to energy efficiency and processing power, particularly when tasked with running complex models. In response to these challenges, neuromorphic computing systems, drawing inspiration from the structure and function of the human brain, are undergoing development. One avenue of exploration involves leveraging human brain organoids—three-dimensional brain-like tissues derived from human pluripotent stem cells. These organoids can replicate certain aspects of the human brain's structure and function. In this context, we present the development of a hybrid neuromorphic computing system by integrating conventional computing hardware with a human brain organoid. Our approach involves implementing the organoid within a reservoir computing framework, a type of artificial neural network. The organoid serves as a dynamic physical reservoir, adept at capturing and processing information based on a sequence of inputs. Silicon computer hardware is employed for the input and output layers, with the output layer trained to interpret the reservoir layer's output, enabling predictions or classifications from the original input data. To showcase the versatility of this hybrid system, we demonstrated its application in speech recognition and the prediction of nonlinear equations. This innovative integration of traditional computing and organic neural elements holds promise for advancing biocomputing, brain-machine interfaces, and translational medicine. The hybrid neuromorphic computing system presented herein opens new avenues for exploration, offering potential insights into the synergy between artificial and biological systems.

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Funding Source: This work was funded with support from the National Institute of Health Awards (DP2AI160242, R01DK133864, and U01DA056242).

Keywords: *organoids, AI, Biocomputing*

4:40 PM – 5:20 PM

CLOSING KEYNOTE—INVESTIGATING THE ROLE OF GLIAL CELLS IN THE PATHOGENESIS AND TREATMENT OF ALZHEIMER'S DISEASE-RELATED DISORDERS USING NEXT-GENERATION BRAIN ORGANOIDS

Okano, Hideyuki

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Advancements in iPSC technology have been pivotal in regenerative medicine, disease modeling, and drug discovery, particularly in neuroscience. Our study presents novel differentiation protocols for deriving diverse neuronal and glial cells from iPSCs, establishing over 40 neurodegenerative disease models for pathophysiological analysis. Despite the progress, a significant challenge remains in bridging gaps between iPSC-based drug research and clinical applications. Our research highlights the potential of glial cells, which are crucial in neurodegenerative diseases progression. Current iPSC-derived organoids inadequately represent glial populations, lacking microglia and exhibiting immature neurons. We successfully directed iPSCs to differentiate into astrocytes, oligodendrocytes, and microglia, creating co-culture systems and brain organoids inclusive of microglia to investigate glial roles in conditions like dementia. Astrocytes expressing the APOE4 gene, linked to Alzheimer's disease (AD) risk, were studied against APOE3 astrocytes. APOE4 astrocytes inhibited dendritic spine formation, with an overexpression of extracellular matrix factor EDIL3, which was also found co-localized with amyloid plaques in AD patient brains, suggesting its implication in AD pathology. We also examined the APOE Christchurch mutation, associated with familial AD resistance, finding it reduces tau propagation and the transformation of native astrocytes into reactive states. Additionally, our analysis of iPSC-derived astrocytes from Kii ALS/PDC patients showed a drastic reduction in CHCHD2, a mitochondrial gene, hinting at its pivotal role in disease etiology. Our goal is to target glial cell anomalies to innovate treatments for neurodegenerative diseases. This research marks a step towards closing the gap between iPSC research and tangible clinical outcomes, providing insights into the underlying mechanisms of neurodegeneration and paving the way for targeted therapeutic strategies.

Funding Source: The present study is supported by the Japan Agency for Medical Research and Development (AMED) grant (JP17pc0101006).

Keywords: *Alzheimer's, glia, ApoE4*

5:20 PM – 5:25 PM

CLOSING REMARKS

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POSTER SESSION I

TOPIC: CELL AND TISSUE THERAPIES

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ENGINEER CAR-NEUTROPHILS FROM HUMAN PLURIPOTENT STEM CELLS FOR TARGETED CHEMOIMMUNOTHERAPY AGAINST GLIOMA

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Glioblastoma (GBM), the most common type of primary brain tumor, is characterized by high mortality rate, short lifespan, and poor prognosis with a high tendency of recurrence. Functional therapeutics, including PRMT5 inhibitors, radiosensitizers, and emerging chimeric antigen receptor (CAR)-T immunotherapy, have been developed to treat GBM. However, the existence of physiological blood-brain barrier (BBB) or blood-brain-tumor barrier has impeded the efficient delivery of such promising therapeutics into the brain and limited their therapeutic efficacy. Given the native ability of neutrophils to cross BBB and penetrate the brain parenchyma, here we tested the therapeutic concept that neutrophils could be engineered with synthetic CARs to specifically target GBM and effectively deliver chemo-drugs to brain tumor as a novel dual chemoimmunotherapy for the first time. Primary neutrophils are short-lived and resistant to genetic modification. Therefore, we genetically engineered human pluripotent stem cells with different chlorotoxin (CLTX) CARs and differentiated them into functional CAR-neutrophils. As compared to CAR-natural killer (NK) cells, systemically administered hPSC-derived CLTX CAR-neutrophils significantly reduced tumor burden in xenograft mouse models and extended their lifespan, suggesting superior abilities of neutrophils in crossing BBB and penetrating GBM xenograft in mice. We also loaded hypoxia-activated prodrug tirapazamine (TPZ) into CAR-neutrophils using silica nanoparticles with rough surfaces (R-SiO2-TPZ) and demonstrated their enhanced antitumor activities in xenograft mouse models, serving as a novel dual chemoimmunotherapy against GBM. Our results established that CAR neutrophil-mediated drug delivery may provide an effective and universal strategy for specific targeting of solid tumors.

Funding Source: NCI R37CA265926

Keywords: *Immunotherapy, CAR-neutrophils, hPSCs*

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SYNTHETIC HYDROGELS AS A DEFINED ALTERNATIVE TO MATRIGEL FOR HUMAN EPITHELIAL ALVEOLAR ORGANOID CULTURE

Eiken, Madeline K.[1], Childs, Charlie[2], Plaster, Eleanor[1], Spence, Jason[2], Loebel, Claudia[3]

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Human pluripotent stem cell (hPSC)-derived organoids, three-dimensional in vitro organ-like structures, recapitulate some of the cell types and functions of in vivo organs. Due to their experimental tractability, renewable cell source and relative complexity, organoids are promising models for drug screening and discovery. Organoids are typically grown in Matrigel, a mouse sarcoma-derived basement membrane matrix that is complex, poorly defined, and does not offer control over mechanical properties. Organoid culture in Matrigel complicates drug screening due to poor control of both the matrix and the organoids themselves. Synthetic hydrogels have emerged as an alternative to Matrigel for organoid culture. In addition to providing a chemically defined matrix, synthetic hydrogels also provide tunable mechanical properties to model changes in the extracellular matrix (ECM) such as matrix stiffening that occurs in fibrotic diseases. Here, we utilize a hyaluronic acid-based hydrogel for epithelial alveolar organoid culture. First, alveolar organoids are pre-aggregated in hydrogel-based microwells, enabling precise control over organoid size,

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density, and growth. Next, aggregates are embedded in hydrogels of multiple stiffnesses, reflecting a range of pathological and physiological stiffnesses in the in vivo alveolar region. Within 14 days, cells within aggregates proliferate and grow into viable alveolar organoids that maintain their alveolar epithelial identity. During culture in both microwells and upon embedding into hydrogels, cells secrete their own nascent matrix as visualized using a biorthogonal labeling approach. This capacity to assess changes in matrix deposition may be a useful screening parameter for anti-fibrotic drugs. These results highlight the potential of synthetic hydrogels as a platform for organoid-based drug screening that provides improved control of both the properties of the matrix and organoids.

Funding Source: This work was supported by funding from the NIH (R01 to JRS, R00 to CL, F31 to CJC), American Lung Association, Cystic Fibrosis Foundation, Chan Zuckerberg Initiative, and the NSF GRFP (MKE).

Keywords: *organoids, hydrogels, ECM*

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ENGINEERING GLOBAL ALIGNMENT OF HUMAN IPSC-DERIVED SKELETAL MUSCLE TISSUE TO SYNCHRONIZE CONTRACTION AND IMPROVE MATURATION

Eicher, Alexandra K.[1], Rodríguez de la Rosa, Alejandra[1], Uzel, Sebastien[2], Lewis, Jennifer[2], Pourquié, Olivier[1]

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Research on muscle degeneration is limited by the lack of cellular models and sources of muscle progenitor cells. However, the Pourquie lab has developed a directed differentiation protocol using human induced pluripotent stem cells (hiPSCs) to generate millimeter-long striated myofibers and their progenitors, satellite cells. The protocol recapitulates embryonic and fetal stages of muscle development, and the resulting hiPSC-derived myofibers demonstrate functionality through spontaneous contraction and local alignment into myofiber bundles. However, their maturation is incomplete, comparable to late fetal myofiber bundles, with nuclei surrounded by proliferative progenitor cells rather than non-proliferative progenitor cells characteristic of postnatal or adult muscle tissue. Two challenges are hindering the maturation of these myofibers: long-term continued growth in vitro and global alignment of the myofiber bundles. To overcome these challenges, we leveraged a protocol pioneered by the Lewis lab to engineer three-dimensional (3D) hiPSC-derived anisotropic organ building blocks (aOBBs) comprised of skeletal muscle. Mononucleated muscle progenitor cells were harvested from hiPSC-derived myofibers grown for approximately 21 days in culture, suspended in a collagen gel, and seeded onto micropillar arrays for overnight curing into centimeter-long aOBBs. The production of these skeletal muscle aOBBs must be scaled up to generate enough material for compaction into a dense cellular bioink, which can be shear-aligned during bioprinting. Synchronized muscle contractions and peripherally localized nuclei will mark enhanced maturation of aligned bio-printed human skeletal muscle tissues. Generating functional skeletal muscles with enhanced alignment, maturation, and function will open new avenues for studying disease etiologies, modeling muscle damage and repair, and potentially providing transplantable material for clinical therapies.

Funding Source: Funded by NIH T32 5T32EB016652-10—Organ Design and Engineering Training (ODET) Program

Keywords: *muscle, bioink, bioengineering*

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QUIESCENT ADIPOSE-DERIVED MESENCHYMAL STROMAL CELLS (ADSCS) REJUVENATE THEMSELVES AND PROVIDE FACTORS THAT REMOTELY RESTORE CELLULAR FUNCTIONS OF AGED ADSCS

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Although a number of studies have shown therapeutic effects of mesenchymal stem cells (MSCs) on severe autoimmune diseases and inflammatory diseases, clinical applications of MSCs for these diseases are

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still limited. One of the reasons is aging of MSCs due to older donor age and ex vivo culture of MSCs before usage, which results in attenuation of their anti-inflammatory/immunomodulatory functions, as well as their proliferation/differentiation potencies. We previously reported that adipose tissue-derived stromal cells (ADSCs), one type of MSCs used in multiple clinical trials, exhibit anti-inflammatory/immunomodulatory functions when they become quiescent in gels with a stiffness mimicking that of adipose tissue. We also showed that transplants containing quiescent ADSCs enhanced diabetic wound healing more than did those containing non-quiescent ADSCs. Here, we investigated the effect of inducing quiescence in aged ADSCs on themselves and their neighboring ADCSs. ADSCs at high passage exhibited a flattened shape and impaired DNA damage repair response, accompanied by attenuated proliferation and increased secretion of monocyte chemoattractant protein-1 (MCP-1). However, introducing quiescence into these aged ADSCs by embedding them in soft gels eliminated these phenotypes associated with aging. Furthermore, administration of conditioned medium derived from ADSCs rendered quiescent at high passage significantly improved age-associated impaired proliferation and biased differentiation (impaired osteogenic differentiation) of ADSCs at high passage. These results suggest that quiescence rejuvenates ADSCs, which in turn provide factors that contribute to rejuvenation of other ADSCs. Thus, introducing quiescence in ADSCs may contribute to overcoming challenges in ADSC-based cell therapies due to their aging.

Keywords: *MSCs, aging, rejuvenation*

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POLY (LACTIC-CO-GLYCOLIC ACID) AND GRAPHENE OXIDE NERVE GUIDANCE CONDUIT PROMOTES SAFE AND FUNCTIONAL AXON REGENERATION WITH A POTENTIAL TO TREAT PERIPHERAL NERVE INJURIES

Harley-Troxell, Meaghan E.[1], Pedersen, Alisha[2], Newby, Steven[2], Stephenson, Stacy[3], Masi, Tom[4], Crouch, Dustin[5], Anderson, David[2], Dhar, Madhu[2]

[1]Large Animal Clinical Sciences, University of Tennessee, Knoxville, TN, USA, [2]Large Animal Clinical Sciences, University of Tennessee College of Veterinary Medicine, Knoxville, TN, USA, [3]Plastic and Reconstructive Surgery, University of Tennessee Medical Center, Knoxville, TN, USA, [4]Graduate School of Medicine, University of Tennessee, Knoxville, TN, USA, [5]Mechanical, Aerospace, and Biomedical Engineering, University of Tennessee, Knoxville, TN, USA

Peripheral nerve injuries (PNI) caused by trauma and disease impact the quality of life of millions of people. The current gold standard of treatment, the autograft, fails to restore nerve function in approximately one-third of patients and is often associated with untoward effects. The overall goal of this study is to use tissue engineering and regenerative medicine to develop a nerve guidance conduit (NGC) that will stimulate axonal elongation and restoration of nerve function to effectively cure PNIs. Towards this goal we fabricated a novel 3D printed NGC composed of 50:50 poly (lactic-co-glycolic acid) (PLGA) and 0.25% graphene oxide (GO), seeded with one million human mesenchymal stem cells (MSCs). Cell attachment was confirmed using DiI labelling, followed by confocal microscopy. Three neuro-specific markers were validated using immunofluorescence. Cytocompatibility and neural differentiation of cells in response to the NGC were confirmed in vitro. A rat sciatic nerve defect model was used to evaluate systemic toxicity, using an immune panel run through flow cytometry. Results showed no evidence of toxicity associated with the NGC, including MSCs, throughout the 6-month study. Evaluation of localized toxicity in the kidney, lung, spleen, and liver was performed using transmission electron microscopy. The in vivo model also was used to evaluate functional regeneration using gross muscle

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and gait analyses, as well as expression of neural proteins via immunohistochemistry. Results show that the PLGA/GO NGC and MSCs were biocompatible, and functional regeneration with the NGC was comparable to that of the autograft, suggesting that the specific combination of PLGA and GO alone may provide an effective biomimetic therapy to repair the PNI. While the addition of MSCs did not further improve functional repair, current studies are investigating the mechanisms of neurogenesis to better understand the response of MSCs to the NGC, and thus support nerve repair in vivo.

Funding Source: University of Tennessee: Office of Research, Innovation, and Economic Development Seed Award.

Keywords: *MSC, Graphene, Nerve*

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IN VITRO CHARACTERIZATION AND EVALUATION OF STABILITY OF CANINE MESENCHYMAL STEM CELLS FOR OFF-THE-SHELF CLINICAL USE

Rivera Orsini, Michael A.[1], Ozmen, Emine[2], Miles, Alyssa[3], Newby, Steven[2], Springer, Nora[3], Dhar, Madhu[4]

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Patients are brought to the clinic every day in need of a treatment that will modulate the immune system and induce repair in damaged tissues. Cell-based therapies are increasingly used in both human and veterinary medicine to treat patients with diseases and injuries. Mesenchymal stem cells (MSCs) are pluripotent, non-hematopoietic cells with self-renewal capability to differentiate into specific tissue lineages. The availability of methods for isolation, characterization, and ex vivo expansion of MSCs, presents them as the most commonly used cells in regenerative medicine and tissue regeneration. MSCs can be readily isolated from any somatic tissue including bone marrow, fat, umbilical cord, and skin. The use of autologous MSCs has been the golden standard due to the donor and the recipient being the same, hence there is a reduced risk of life-threatening complications. The performance of the MSCs depends on various factors including lack of uniform isolation and cell culture methods, donor source, donor age, and donor health. Due to these weaknesses, a fully characterized and cryobanked allogenic source of MSCs with consistent performance, such as an off-the-shelf bank of MSCs for immediate application is needed. Based on our data, we hypothesize that CD90-positive and MHC II-negative MSCs can be used safely as an allogenic source treatment. As a first step to prove our hypothesis, in this study, we isolated and characterized adipose-derived MSCs from a 13-month-old female Pitbull mix. We evaluated the viability of the cells post-cryopreservation under extreme temperatures and time. Each gram of adipose tissue yielded 2.5x106 cells after four days in culture. Starting at 1,143 cells/cm2 proliferation in 10 days resulted in 7.5x104 cells/cm2. Greater than 70% of cells were positive for CD90, CD44, and CD29 and <2% were negative for MHC II expression. Cells were removed from the cryofreezer and their viability was recorded to be >87% for 48 hours when kept on ice with ambient temperatures reaching upwards of 52.5°C. Further investigation is underway to ensure in vivo allogeneic MSC safety. In conclusion, we have a cryobank of CD90+ and MHCII- canine adipose tissue-derived MSCs which can be used as an off-the-shelf therapy either in the clinic or be shipped to other practices.

Keywords: *Off-The-Shelf, Canine, MSC*

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SCALABLE PRODUCTION OF PLURIPOTENT STEM CELL-DERIVED LIVER ORGANOIDS IN ROTATING CULTURE

Elzobair, Tahlil, Kimura, Masaki, Iwasawa, Kentaro, Santangelo, Constance, Takebe, Takanori

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Liver transplantation is the standard care for patients with end-stage liver disease, but the lack of organ donors results in over 1,000 pre-transplant deaths in the

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US alone. Finding alternative therapies to replace organ transplantation is becoming an urgent call. Human pluripotent stem cell-derived liver organoids (HLOs) have recently been a powerful tool to explore liver replacement therapies, demonstrating an initial promise in alleviating fatal hepatic diseases in multiple rodent models. However, the available culture techniques for organoids are still challenging to generate large enough numbers for human-relevant applications. Here we report a dynamic rotating culture method for scalable production of functional HLOs in spinner flask with 50% less use of the mouse sarcoma derived basement membrane extracellular matrix (Matrigel) and unexpectedly by using the spinner flask we were able to generate functional HLOs in Matrigel-free culture. We observed high proliferation and expansion ability of HLOs generated in the flask displayed in exponential growth curve. Gene expression analysis of hepatocytic markers such as ALB, MRP3 and HNF4a, were highly upregulated and more than 4-fold higher albumin secretion in comparison to static culture were detected. Immunohistochemical staining also displayed markers such as PROX1, E-cadherin and CDX2 suggesting the presence of hepatic lineage. Mice received intraperitoneal Injection of spinner flask generated HLOs started expressing 2-fold more serum albumin after 24 hours compared to the static cultured HLOs. These findings qualify the rotating culture method (spinner flask) to be an effective technique for generating large number of highly proliferating mature HLOs without using any animal-derived material. This rotating culture method we established will move the organoid medicine field one step forward by offering a massive number of functional organoids for tissue replacement therapies.

Keywords: *Organoids, Rotation, Matrigel-free*

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CHARACTERIZATION OF MUSCLE STEM CELL GYLCOSYLATION PROFILED IN SKELETAL MUSCLE

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Glycosylation, a post-translational modification, is essential for proper protein function and playsa key role for many biological processes. Mutations in glycosyltransferases, enzymes that catalyze the addition of glycans to proteins, have been linked to many diseases, collectively referred to as Congenital Disorders of Glycosylation (CDGs), some of which can cause mild to severe skeletal muscle phenotypes. Therefore, the effects of defective glycosylation need to be studied and understood in the context of muscle regeneration and atrophy. Current experimental techniques used to study glycosylation require tissue dissociation, leading to loss of spatial and environmental context. We have developed a set of reagents to map glycosylation patterns in situ using CODEX (co-detection by indexing), a multiparametric tissue staining technique. By detecting 12 different glycosylation moieties with resident cell population markers, we uncovered that muscle cell types display diverse glycosylation patterns. In particular, changes in the glycosylation of muscle stem cells, a key cell population that bears skeletal muscle regenerative capacity, is necessary for their repair function. Our aim is to characterize muscle stem cell glycosylation patterns via CODEX analysis on young and aged mice muscle tissue during homeostasis and regeneration. Results will be validated by isolating muscle stem cells via FACS, and subsequently culturing and differentiating muscle stem cells into myoblasts, myocytes, and myotubes. Glycan content of each cell type will be assayed using lectin binding and mass spectrometry to highlight different glycosylation patterns. Our analyses provide further understanding of the impact of glycosylation on muscle regeneration and may reveal potential implications for therapeutic interventions in CDGs that affect muscle function.

Keywords: *Muscle, Glycosylation, Congenital*

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ADDRESSING BIOREACTOR HIPSC AGGREGATE STABILITY, MAINTENANCE AND SCALEUP CHALLENGES USING A DESIGN OF EXPERIMENT APPROACH

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Stem cells derived cell therapies hold the potential for treatment of regenerative clinical indications. Conventional static culture flasks are impractical for large scale production needs. Static culture has a limited ability to scale up. Suspension culturing can be used to produce target cells in large quantities. In this study, we uncover insights about the 3D environmental factors that are process parameters for controlling pluripotency, growth and aggregate stability using a bioreactor culture system. Utilizing a design of experiments approach we evaluated media additives that have versatile properties such as reducing shear stress by decreasing surface tension, enhancing extracellular matrix, increasing aggregate stability, and preventing aggregate fusion. Multiple response parameters were chosen to assess cell growth, pluripotency maintenance and aggregate stability in response to five additive inputs, and mathematical models were generated and tuned for maximal predictive power. For each model the relationship between additive/response parameter could be analyzed and optimized towards desirability criteria. In all desirability contexts, process capability as a measurement of process robustness was favorable. Optimal conditions for maximal cell growth required combinatorial additive use. Similarly, optimization of the maintenance of pluripotency was also dependent on combinatorial signaling. Based on measurements of aggregate size and size variance optimization could be performed to increase aggregate homogeneity. In all cases, underlying models included multiple interaction terms, revealing the criticality of simultaneous testing using combinatorial designs. We conclude that DoE-based interaction testing performed within a manufacturing-relevant environment allows for process understanding of the biomanufacturing process. The method identifies critical process parameters; their interacting criticality, while returning a deep process understanding.

Keywords: *iPSC, Bioreactors, suspension*

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INVESTIGATING CELLULAR NECROSIS, STRESS, AND MATURATION IN ASTROCYTE-ENRICHED ORGANOIDS

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Brain organoids provide a physiologically relevant model for studying human brain development and disease. However, due in part to a lack of vasculature, brain organoids develop necrotic cores upon reaching approximately 1.5 mm in diameter. This necrosis is caused by a variety of factors: hypoxia, build-up of waste products, and lack of nutrient penetration to the organoid's core. This increases cellular stress and influences cellular maturation, distorting in vitro organoids' likeness to associated in vivo counterparts. Using protocols developed in our lab, we incorporated MatriGel drops, approximately 100–500 um in diameter, into the center of astrocyte-enriched organoids (AEOs) creating a "hollow" core free of cells, thus displacing cells that might become necrotic. We have found that intact PSC colonies regularly engulf these MatriGel drops when plated in ultra-low attachment tissue culture plates. The resulting MatriGel-enriched AEOs continue to develop complex neuroepithelium observed via brightfield imaging. Immunohistochemical analysis (IHC) of brain organoids lacking MatriGel-enriched cores have necrotic cores by approximately day 70, although multi-electrode array recordings demonstrate both increasing electrophysiological activity as organoids develop and robust electrophysiological activity by approximately nine months. We will now compare electrophysiological activity of AEOs with and without MatriGel cores in addition to cell death and apoptosis measured via viability and tunnel assays. Cellular stress markers, including HIF1a, PGK1, ARCN1, and GORASP2, in addition to cellular maturation will be analyzed by

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single-cell sequencing. We predict that MatriGel AEOs may show less hypoxic, pre-apoptotic, and cellular stress markers. These findings will establish a scalable method, increasing cellular viability and maturation, to develop a closer approximation of brain organoid models to human brain functionality.

Keywords: *Organoids, Necrosis, Brain*

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PROMOTION OF THE MATURATION OF ENGINEERED CARDIAC TISSUE DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS THROUGH SYNCHRONIZED ELECTRO-DYNAMIC STIMULATION

Maihemuti, Wusiman, Murata, Kozue, Masumoto, Hidetoshi

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Electrical and dynamic stimulation have been widely used to enhance the maturation of engineered cardiac tissue (ECT) generated from induced pluripotent stem cells (iPSCs), given to their presence in the physiological environment of the heart. However, the existing literature lacks a direct comparison between electrical and dynamic stimulation, primarily due to the diversity in systems used for creating ECT and variations in outcome measures assessing functionality. This study aimed to achieve two main objectives: first, to directly assess the effects of dynamic and electrical stimulation on ECTs, and second, to determine the importance of synchronizing these two stimuli. Our hypothesis suggested that synchronized electro-dynamic stimulation would enhance the maturation and function of the constructs. To test this hypothesis, ECTs were created by co-culturing a mixture of human iPSC-derived cardiovascular cells with Collagen I and Matrigel for a duration of 2 weeks. Subsequently, comparative cultures with no stimulation, dynamic stimulation, electrical stimulation (3volts), and synchronized electro-dynamic stimulation using a synchronization sensor capable of generating electrical stimulation at the timing minimized by dynamic stimulation was conducted for 2 weeks. In our optimized protocol, we observed that the 30rpm dynamic culture significantly enhanced the cardiomyocyte (CM) component and collagen alignment compared to the 60rpm group. Synchronized stimulation notably enhanced the CM component and collagen alignment of the ECTs associated with increased TNNT2 expression levels. Additionally, synchronically stimulated ECTs exhibited a positive force-frequency relationship in terms of contractility, indicating tissue maturation. In summary, electro-dynamic stimulation demonstrated the capability to create ECT with superior functional and structural properties, accompanied by tissue maturation, compared to either electrical or dynamic stimulation alone.

Keywords: *iPSC, Maturation, Organoid*

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A FIRST-IN-HUMAN CLINICAL STUDY OF LAPAROSCOPIC AUTOLOGOUS MYOBLAST SHEET TRANSPLANTATION TO PREVENT DELAYED PERFORATION AFTER DUODENAL ENDOSCOPIC MUCOSAL DISSECTION

Kanetaka, Kengo[1], Higashi, Miki[1], Kobayashi, Shinichiro[2], Hashiguchi, Keiichi[3], Nakao, Kazuhiko[3], Eguchi, Susumu[2]

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The detection rate of superficial non-ampullary duodenal epithelial tumors (SNADET) has recently been increasing. Large tumors may contain malignant lesions and early therapeutic intervention is recommended. Endoscopic mucosal dissection (ESD) is considered a feasible treatment modality, however, the anatomical and physiological characteristics of the duodenum create a risk of postoperative perforation after ESD. We previously showed that myoblast sheet transplantation on the serosal side after duodenal endoscopic submucosal dissection (ESD) prevented delayed perforation in a porcine model. We herein present the result of a first-in-human (FIH) clinical

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trial of laparoscopic autologous myoblast sheets transplantation after duodenal ESD to prevent delayed perforation. Three patients with SNADET >20 mm in diameter underwent autologous cell sheet transplantation on thin duodenal serosa after ESD. In case 1, autologous myoblast sheets which were fabricated from muscle tissue obtained seven weeks before ESD were transplanted laparoscopically onto the serous side of the ESD. The patient's postoperative course was uneventful. Endoscopy and abdominal computed tomography revealed no signs of delayed perforation. Despite incomplete mucosal closure in case 2, and multiple micro perforations during ESD in case 3, cell sheet transplantation could prevent the postoperative massive perforation after ESD, and endoscopy on day 49 after transplantation revealed no stenosis. In conclusion, this FIH clinical trial showed the safety, efficacy, and procedural operability of this novel regenerative medicine approach involving transplanting an autologous myoblast sheet laparoscopically onto the serosa after ESD in cases with a high risk of delayed perforation. This result indicates the potential application of cell sheet medicine in treating various abdominal organs and conditions with minimal invasiveness in the future.

Funding Source: This research was supported by AMED under Grant Number JP20bk0104112h0001.

Keywords: *cell, duodenum, laparoscopy*

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COORDINATED DIFFERENTIATION OF NEURAL STEM CELLS FOR NEUROREGENERATION IN MOUSE SCIATIC NERVE INJURY

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Nerve injury repair requires delicate interplays between neurotrophic factors and neural stem cells (NSCs). Deciphering the roles of neurotrophic proteins and their mechanisms of action on neuro-regeneration could benefit patients with neural damage and neurodegeneration. We showed FGF1 could improve sciatic nerve injury repair in rats. We also showed that NSCs could improve nerve repair but there appeared a lag when compared to FGF1. We hypothesized that NSCs might secrete a second protein to optimize nerve repair. Using proteomic analysis, we identified IL12 p40-homodimer (IL12p80). We further showed human IL12p80 (hIL12p80, made in insect cells) improved sciatic nerve regeneration in mice. As such, the group of PLA conduit with NSCs and hIL12p80 (CNI) showed better recovery than the other groups in the sciatic functional index (SFI), Rotarod performance, and compound muscle action potential (CMAP) analyses. Moreover, the CNI group recovered faster, outperforming the other groups in SFI and Rotarod performance tests beginning in the fourth-week post-surgery. Histology staining showed that the CNI group increased the diameter of the newly regenerated nerve two-fold ($P < 0.01$). In vitro studies showed that hIL12p80 stimulated the differentiation of mouse NSCs to oligodendrocytes through phosphorylation of Stat3. Further, implantation using PLGA conduits (C2.0 and C2.1) showed better recovery in the Rotarod test and CMAP than using PLA conduits. In B6 mice, the group with C2.1+NSCs+hIL12p80 (C2.1NI) not only promoted sciatic functional recovery but also reduced experimental autotomy. These results suggested that hIL12p80, combined with NSCs, enhanced the functional recovery and accelerated the regeneration of damaged nerves in mice. We will also present the regeneration results using hIL12p80 that our lab purified from CHO-S cells. The synergistic effects of FGF1-induced neurogenesis and IL12-induced myelinogenesis could likely optimize the effect of nerve repair.

Funding Source: This research is supported by the National Science and Technology Council, Taiwan, NSTC 112-2314-B-039-050 and NSTC 112-2314-B-039-071; China Medical University, Taiwan, CMU110-YT-01 and CMU112-MF-15.

Keywords: *NSCs, Neurodegeneration, Oligodendrocytes*

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HUMAN IPSC-DERIVED INTESTINAL ORGANOIDS TO STUDY HUMAN CONGENITAL DISEASES

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The gastrointestinal (GI) tract consists of highly specialized organs from the proximal esophagus to the distal colon, each with unique functions. Rare congenital malformations of the GI tract, including organ atresia, agenesis or mis-patterning are linked to gene mutations although the molecular basis of these malformations has been poorly studied due to lack of model systems to study human development. We identified a patient with compound heterozygous mutations in the transcription factor RFX6 with pancreatic agenesis as previously described. In addition, the patient had duodenal mal-rotation and atresia suggesting that establishment of the proximal small intestine was impaired in these patients. To identify the molecular basis of the intestinal malformation we generated induced pluripotent stem cell lines from this patient, and derived human intestinal organoid (HIOs) to identify how mutations in RFX6 impact intestinal patterning and function. We identified that the duodenal identity of HIOs and patient tissues had adopted a more distal small intestinal signature, including expression of SATB2, normally expressed in the ileum and colon. CRISPR-mediated correction of RFX6 restored duodenal identity, including expression of PDX1, which is required for duodenal development. Using transcriptomic approaches in HIOs and Xenopus embryos we identified that PDX1 is a downstream transcriptional target of RFX6 and that PDX1 expression in a RFX6 mutant background was sufficient to rescue duodenal identity. However, RFX6 had a PDX1-independent role in regulating expression of components of WNT, HH, and BMP signaling pathways that are critical for establishing early regional identity in the GI tract. In summary, we have identified that RFX6 is one of the most upstream regulators early intestinal patterning in vertebrates and that it acts by regulating key transcriptional and signaling pathways.

Keywords: *Endoderm-Patterning, Intestinal-Organoid, Mitchel-Riley*

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ADVANCING TYPE II DIABETES MODELING: FROM IMPROVED STEM CELL PROTOCOLS TO NOVEL USER-FRIENDLY MICROPHYSIOLOGICAL PLATFORM

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There is a critical need for improved in vitro systems to model type II diabetes to advance therapeutic drug candidates to preclinical studies. Significant advances have been made in this direction, with human pluripotent stem cells (PSCs) holding the highest potential as a limitless source of beta-cells for disease modeling and cellular therapy for diabetes. While many protocols have been published describing the differentiation of insulin-expressing cells from PSCs, it is unclear which yield the most functional cells with reproducible glucose-stimulated insulin secretion (GSIS). In this study, we performed a side-by-side comparison between conventional and state-of-the-art protocols to determine which yields the most functionally mature cells. Our results unequivocally demonstrate that the newer protocols yield clusters with enhanced maturity, characterized by mono-hormonal insulin expression and robust, reproducible GSIS. Besides being glucose-responsive, these pancreatic aggregates exhibit responsiveness to incretins hormones, further underscoring their physiological relevance. Additionally,

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we demonstrated that cryopreserved pancreatic progenitors yield aggregates that are capable of GSIS. Lastly, our team has developed an easy-to-use, low-cost pillar/perfusion plate that does not require pumps and specialized equipment and allows for studies of inter-organ endocrine crosstalk. Overall, this platform has the potential to facilitate drug development by providing more predictive outcomes and human models for preclinical studies.

Funding Source: This research was supported by: NIH, U19 AI116491, P01 HD093363, UH3 DK119982, Digestive Disease Research Center (P30 DK078392), the Shipley Foundation and the Allen Foundation (JMW).

Keywords: *organoids, diabetes, MPS*

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TUNING TRANSCRIPTION FACTOR LEVELS TO RECONCILE DOSAGE SENSITIVITY AND ROBUSTNESS IN DEVELOPMENT

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Transcription factors (TFs) are key drivers of cellular and developmental identity; as a result, their function must be robust to perturbation. At the same time, evidence from human genetics indicates that TF dosage reductions of ~50% or less frequently underlie both severe birth defects and normal-range phenotypic variation; reconciling this dosage sensitivity with transcriptional robustness requires quantitative studies of TF dosage, which are lacking to date. We developed an approach to precisely modulate TF levels in stem cell-derived human facial progenitors (cranial neural crest cells) and applied it to SOX9, a TF associated with craniofacial variation and defects (Pierre Robin Sequence, PRS). Most SOX9-dependent regulatory elements (REs) are buffered against small decreases in SOX9 dosage, but REs directly and primarily regulated by SOX9 show heightened sensitivity to SOX9 dosage. These RE responses can be partially predicted from DNA sequence alone and themselves predict expression responses of nearby genes. Sensitive REs and genes underlie the vulnerability of both in vitro chondrogenesis and variation along the PRS phenotypic axis to SOX9 dosage perturbation. We propose a model in which REs and genes sensitive to SOX9 dosage transmit quantitative TF dosage changes to specific cellular and morphological effects underlying craniofacial defects, while other phenotypically important REs and genes are regulated by SOX9 but highly buffered. This model reconciles transcriptional robustness with dosage sensitivity, can explain the phenotypic impact of quantitative perturbations to SOX9 dosage, and is further supported by application of our approach to another dosage-sensitive TF, TWIST1.

Funding Source: This work was funded by the Helen Hay Whitney Foundation, Howard Hughes Medical Institute, and NIH grant K99DE03272901.

Keywords: *Dosage-sensitivity, Neural-crest, Transcription*

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MAPPING THE CELLULAR LANDSCAPE OF CORTICAL DEVELOPMENT IN TUBEROUS SCLEROSIS COMPLEX

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Tuberous Sclerosis Complex (TSC) is a genetic neurodevelopmental disorder caused by mutations in the TSC1/TSC2 genes, resulting in upregulation of mammalian target of rapamycin (mTOR) signaling and unregulated cell growth. Within the brain, dysplastic cortical lesions called tubers emerge during fetal development. Although tuber growth appears to stop postnatally, repercussions do not. TSC patients suffer debilitating neurological sequelae from these tubers,

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including epilepsy, autism, and neuropsychiatric disorders. The exact molecular mechanisms underlying tuber development remain unclear, although recent work has implicated epidermal growth factor receptor (EGFR) signaling. In this study, we sought to understand TSC-specific changes in cellular identity during cortical development of TSC2 mutant cells and their association with tuber formation. Using CRISPR-Cas9 technology, we generated isogenic lines of TSC patient-derived induced pluripotent stem cells (iPSCs) with pathogenic mutations in the TSC2 gene. iPSCs were differentiated into neural lineages and collected at several timepoints for analysis. Immunoblot analysis revealed an inverse relationship between TSC2 (tuberin) and EGFR expression throughout neural differentiation in TSC2 mutant neurons with differential regulation of downstream targets. Mass cytometry analysis on the single-cell level in both early TSC2 mutant neural precursor cells and mature excitatory neurons revealed unique populations with upregulated mTOR-specific and mTOR-independent markers, including p-S6 240/244, p-STAT3 S727, and YAP1. Mass cytometry analysis of resected tubers from TSC patients with epilepsy also showed distinct cellular identities with unique populations exhibiting differential expression of mTOR downstream targets and stem cell/astrocytic markers. Taken together, these results indicate that an interplay of EGFR and mTOR signaling with cell-specific changes during cortical development may play a role in tuber formation and these changes can be effectively modeled using human iPSCs.

Funding Source: This research is supported by R01NS118580 (RAI, KCE).

Keywords: *neurodevelopment, epilepsy, autism*

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DEFICITS OF SOX10 IN ENS PROGENITORS ALTER EARLY ENTERIC NEURON LINEAGE ALLOCATION

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Multipotent neural crest progenitors form the Enteric Nervous System (ENS) giving rise to enteric neurons and glia that mediate gut peristalsis. Sox10 is an essential transcription factor necessary for initial migration of progenitors that colonize the fetal intestine. Mice carrying mutant alleles of Sox10 exhibit aganglionosis modeling human Hirschsprung disease. Interestingly, Sox10 mutations also produce an imbalance of enteric neuron subtypes in regions of postnatal ganglionated bowel. Because Sox10 is initially expressed in enteric vagal progenitors and is not present in enteric neuronal lineages, we hypothesized that deficits of Sox10 dysregulate gene networks essential for enteric neurogenesis that feed forward and alter lineage allocation of distinct enteric neuron subtypes. A dual transgenic strategy that labels ENS progenitors (Tg. Sox10-H2BVenus) and developing neurons (Tg.Phox2b-H2BCerulean) was utilized to capture the full profile of enteric cell types by flow sorting from fetal mouse intestine at 15.5 days post coitus (dpc). Single cell RNA-sequencing of captured populations for wildtype and Sox10 mutant (Sox10Dom) littermates was performed to detect differences between progenitor populations and emerging neurons. When abundance of progenitor and neuronal clusters were compared between wildtype and Sox10 mutants, a unique progenitor cluster was detected in Sox10Dom populations accompanied by reduction in one discrete neuronal lineage branch and a compensatory increase in an alternate neuronal lineage. Transit-amplifying populations also were decreased in Sox10Dom mutants. While Tricycle analysis found cell cycle progression is comparable between wildtype and mutants, transition rates between

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states are notably reduced for Sox10Dom mutants. Differential gene expression analysis between wildtype and Sox10 mutants identified alterations in multiple positive transcriptional regulators that are known to play key roles in enteric neurogenesis including Phox2b and multiple Hox genes. Hybridization chain reaction validated differential gene expression between genotypes coupled with immunohistochemistry to pinpoint early differentiating neurons. Our analysis elaborates the gene regulatory networks that are essential for building diversity of enteric neurons from neural crest progenitors.

Funding Source: National Institutes of Health R01 DK127178

Keywords: *neural crest, Enteric Nervous System, Sox10*

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HUMAN IPSC-DERIVED MODELS OF SPECIAL POPULATION: DOWN SYNDROME

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Therapeutic development for special populations of patients, including individuals with genetic conditions such as Down Syndrome (DS), is challenging. The extra copy of chromosome 21 in DS patients leads to a wide range of health problems including intellectual disability, digestive tract malformations, missing nerves in the large intestine, and chronic constipation. The genetic and physiological differences between mouse models and humans have hindered accurate models to recapitulate DS pathology. While studies done in discordant monozygotic twins help to understand the impact of chromosomal abnormalities on transcriptomics; these studies are limited because this population is quite rare. The lack of knowledge of the mechanisms causing the pathological features of DS is a roadblock to developing effective therapeutic strategies to improve the quality of life for these individuals. Induced pluripotent stem cells (iPSCs) have revolutionized the landscape of disease modeling by offering unprecedented opportunities to study complex disorders. Patients' iPSCs can be differentiated into specific cell lines or used to generate organoids to model disease-related cellular and molecular pathologies. Here, we leverage human iPSCs derived from an individual mosaic for DS (+Ts21, C1-DS1, and isogenic control C1-DS2U) to generate DS- intestinal organoids and neural progenitor cells representative of monozygotic twins discordant for Ts21. Gene expression analysis indicated upregulation of drug-metabolizing enzymes CYP3A4 and UGT2B7 in DS1-IO compared to the isogenic control. These hiPSC-derived cells and organoids representative of monozygotic twins discordant for Ts21 could be further incorporated into microfluidic devices to guide drug dosing. Such tools can facilitate answering mechanistic questions related to the gut-brain axis in DS and related diseases. Altogether, this study paves the way for personalized and effective therapeutic interventions for individuals with DS.

Keywords: *DownSyndrome, iPSC, Organoids*

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INVESTIGATING THE ROLE OF SMAD6 IN THE DEVELOPMENT OF TRACHEOESOPHAGEAL BIRTH DEFECTS

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Esophageal atresia (EA) with or without tracheoesophageal fistula (TEF) affects 1 in 3000 live births in the USA. EA occurs when the esophagus fails to form a continuous tube from the oral cavity to the stomach, preventing the passing of ingested foods and liquids to the stomach. TEF occurs when there is an abnormal connection between the trachea and esophagus, resulting in swallowed food or liquids being moved into the lungs. The etiology of EA/TEF occurs during fetal development, at the stage when the common foregut tube separates into the esophagus and respiratory tract. This process is essential for proper

respiratory and gastrointestinal functioning and involves an array of complex molecular and morphological processes. While a small number of risk genes have been associated with EA/TEF in humans, the vast majority remain unknown. As part of a program project to identify the genes causing tracheoesophageal defects, we identified a patient with EA/TEF who presented with a mutation in SMAD6. SMAD6 functions as an inhibitor of BMP signaling and modulates transcription, suggesting that altered BMP signaling may underlie EA/TEF in this patient. SMAD6 mutations have been correlated with defects in cardiovascular, endochondral bone, and blood vessel formation. However, the role of SMAD6 in EA/TEA formation, as well as esophageal/tracheal development, remains unknown. We hypothesize that SMAD6 is essential for normal tracheoesophageal development, and we will dissect the role of SMAD6 in human esophageal/tracheal development using pluripotent stem cell-derived organoids. Furthermore, work in progress aims to identify the molecular pathways impacted by SMAD6 deficiency.

Funding Source: Funding sources are provided by the L.B. Research and Education Foundation (University of Cincinnati Medical Scientist Training Program), NIH T32GM149200-01, and NICHD P01HD093363.

Keywords: *BMP, SMAD6, morphogenesis*

TOPIC: DEVELOPMENTAL ORIGINS OF DISEASES: DEGENERATIVE DISEASES

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EXPLORING LIPID DROPLET LOAD AND IMPROVING SYNAPTIC DENSITY IN APOE4 INDUCED NEURONS

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The risk of developing sporadic Alzheimer's disease (AD), a neurodegenerative disorder resulting in cognitive decline, is influenced by polymorphisms in the Apolipoprotein E (APOE) gene. Of this gene, APOE4 is high-risk, APOE3 is neutral, and APOE2 is protective against AD. The APOE4 gene is linked to alterations in microglia metabolism, which is associated with neuronal dysregulation. We are assessing whether APOE-driven phenotypes in microglia alter metabolic or synaptic function in neurons. In particular, we are interested in extracellular vesicles from iPSC-derived human microglial-like cells (iMGLs) as a candidate for cellular communication that conveys APOE-driven phenotypes in glial cells to NGN2 induced human neurons (iNs). Preliminary data suggests that iNs of APOE4 genotypes have a greater lipid droplet (LD) load compared to APOE3 genotypes. To investigate this, we assessed the expressions of genes involved in lipolysis and lipophagy by RT-qPCR using RNA extracted from isogenic APOE3 and APOE4 iNs. No significant difference in transcription was found between these lines. Future plans include assessing protein levels via western blots and exploring whether treating iNs with iMGL exosomes affects their expression of LD regulators. This study will contribute to our understanding of the observed APOE-dependent LD load. Furthermore, our iNs form sparse synapses with our current induction protocol. To enhance synapse density to better examine the effects of exosomes on synapses, we co-cultured iNs with primary mouse astrocytes at various densities. Co-cultures were fixed and analyzed for pre- and post-synaptic marker co-localization (Synaptophysin I and PSD95) through immunocytochemistry and confocal microscopy. Preliminary data suggests that the presence of astrocytes improves synaptic density in iNs at Day 24 and 38 when cultured at a 1:1 ratio or higher. This improvement will allow us to further explore the effects of iMGL exosomes on iN synapses.

Keywords: *APOE, Alzheimers, Neurons*

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MODELING METABOLIC DYSFUNCTION-ASSOCIATED STEATOTIC LIVER DISEASE AND CHRONIC LIVER INJURY IN HUMAN LIVER ORGANOIDS

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Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common chronic liver disease, affecting more than 25% of the global population. It is a progressive disease that starts with steatosis, can develop into steatohepatitis, progress to fibrosis, and eventually lead to cirrhosis and hepatocellular carcinoma. Human liver organoids (HLOs), derived from pluripotent stem cells, have emerged as a model to study liver disease in human cells. With their multicellular organization, HLOs can mimic the liver's cellular heterogeneity, cell-cell communications, and cellular functions. To recapitulate major features of MASLD and chronic liver injury, we treated HLOs in conditions that included free fatty acids and TGF-b (Transforming growth factor-beta). We developed a toolbox of assays to evaluate MASLD progression and chronic injury in HLOs. Bodipy staining and triglyceride quantification assays were applied to quantify steatosis, immunofluorescence was used to evaluate collagen production, fluorometric assays were performed to detect oxidative stress, and single-cell RNA sequencing (scRNA-seq) was applied to evaluate gene expression in individual cell types. We also found that locked nucleic acids (LNAs) could deplete RNAs in whole organoids and inhibit progression of fibrosis. Taken together, free fatty acids and TGF-b treated HLOs exhibited major features of MASLD and chronic liver injury, including lipid accumulation, inflammation, oxidative stress, and fibrosis, which make HLOs an excellent model to study MASLD development and chronic liver injury and investigate antifibrotic therapies.

Keywords: *Liver, MASLD, Organoids*

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THE ROLE OF ORPHAN NUCLEAR RECEPTOR ESTROGEN-RELATED RECEPTOR GAMMA (ERR GAMMA) IN THE DIFFERENTIATION OF ACID-SECRETING PARIETAL CELLS FROM MOUSE GASTRIC STEM CELLS

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Numerous diseases, from heartburn to gastric cancer, feature dysregulation of acid-pumping parietal cells (PCs) which are generated from gastric epithelial stem cells throughout life. Surprisingly, we know little about the mechanisms governing how gastric epithelial cell types differentiate and how that contributes to disease progression. Here we introduce a new mouse model with a single allele (Atp4bDtr) that causes rapid diphtheria toxin-mediated PC-ablation followed by synchronous differentiation of PCs from progenitors. We molecularly and morphologically characterized regenerating PCs at multiple "Phoenix Stage" timepoints (the time frame of minimal residual mature PCs and maximal differentiating PC progenitors). Stepwise analysis of single-cell RNA sequencing and histological studies of regenerating PCs showed that the orphan nuclear receptor Estrogen-related receptor gamma (ERRÎ[3], encoded by Esrrg) marked early PC progenitors reemerging from stem cells after genetic ablation in mice. Immunostaining of human tissue revealed that ERRÎ[3] also identifies immature PCs and PC progenitors in human autoimmune gastritis. To examine the fate of Esrrg+ progenitors, we generated EsrrgP2A-CreERT2 mice for lineage tracing. Indeed, we observed that Esrrg progenitors predominantly became PCs during homeostasis and regeneration. Early PC progenitors, a previously unrecognized cell population, preferentially expressed genes that were SMAD4 and SP1 targets and that encoded the cytoskeletal and GTPase proteins that help establish PC-specific architecture and acid signaling. Energetic, metabolic, and acid transporter genes were induced later during PC maturation. Epithelium- or PC lineage-specific deletion of Esrrg completely depleted the PC

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census. Thus, Esrrg, which regulates cellular metabolism and maturation in other tissues, is uniquely required in the stomach for both cell specification and subsequent metabolic maturation of PCs from gastric epithelial stem cells. The results suggest ERRÎ³ dynamically controls gastric cell census by coordinating metabolism with differentiation, making it a promising therapeutic target for PC-related disorders.

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Keywords: *Differentiation, Stomach, Regeneration*

TOPIC: FUNDAMENTAL MOLECULAR MECHANISMS OF EARLY DEVELOPMENT

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NUCLEAR FACTOR I GENES ORCHESTRATE THE TIMING OF CORTICAL DEVELOPMENT

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Cortical development is a remarkably regulated process involving the generation of several cell types at precise developmental stages. Radial glial cells (RGCs) proliferate early in embryogenesis and sequentially differentiate to establish the final architecture of the cerebral cortex. This stereotyped temporal progression of mammalian RGCs competency follows intrinsic transcriptional programs and requires extracellular signals; however, the fundamental mechanisms involved are still elusive. To address this, we study the role of Nuclear Factor I genes (NFIs) in corticogenesis, a family of transcription factors crucial for brain development and whose dysfunction results in distinct neurodevelopmental disorders. Exploiting conditional mouse lines, we explored the independent and combinatorial roles of NFIs by partially or totally deleting those factors in RGCs. We found that when Nfia, Nfib and Nfix are concomitantly perturbed, cortical phenotype is strikingly impaired, with a remarkable increase in progenitors retaining a proliferative state and do not undergoing proper cell fate choice. Although NFIs share similar DNA binding motifs, we identified independent and distinct roles during corticogenesis. Notably, we observed a medial to lateral gradient in cell-specific alterations that also involve upper layer (UL) neurogenesis, suggesting that a joint expression of NFIs orchestrate pallial patterning. Using human pluripotent stem cell models of cortical development, we confirmed the importance of NFIs for human corticogenesis as we identified a specific role of NFIX in potentially regulating the progression of UL neurogenesis. Since current protocols lack these critical neuron subtypes, we may have uncovered the molecular controllers for the temporal progression of cortical development.

Keywords: *NFI, Neocortex, Development*

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THE ROLE OF EIF2ALPHA PHOSPHORYLATION IN REGULATION OF PLURIPOTENCY AND LINEAGE COMMITMENT

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Phosphorylation of eIF2α (p-eIF2α) integrates a diverse set of environmental cues and stress, such as nutrient status, viral infection and ER stress, to direct eukaryotic mRNA translation. We demonstrate eIF2α phosphorylation is transiently increased in the epiblast layer of pre-implantation embryo. To establish the role of eIF2α phosphorylation in self-renewal and differentiation of pluripotent cells, we have derived mouse embryonic stem cells (ESCs) carrying wild-type eIF2α (eIF2α+/+) or phosphorylation-resistant mutant eIF2α (eIF2αA/A), where the phosphorylation site of eIF2α at serine 51 has been mutated to alanine.

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We identified that phosphorylation of eIF2α plays an important role in exit from naïve pluripotency. We employed a genome wide translatome analysis approach (ribosome footprinting) to identify the subset of genes regulated by phosphorylation of eIF2α. In addition to known p-eIF2α targets such as ATF4, we uncovered novel p-eIF2α sensitive gene networks that orchestrate fundamental cellular processes such as glutathione metabolism and chromosome organization. Strikingly, we discovered extensive p-eIF2α-dependent transcriptional reprogramming that promotes vascular development but inhibits neuron differentiation. The p-eIF2α regulatory map of pluripotent cells described in this study will provide a foundation for future interrogation of the relationships between translational control, pluripotency, glutathione metabolism, chromosome organization and lineage commitment.

Funding Source: NIH-R01HL163806-01 awarded to S.T. Canadian Institutes of Health Research (CIHR) (FND-148423) awarded to N.S.

Keywords: *eIF2α, translation, pluripotency*

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QUANTIFYING POSITIONAL INFORMATION FROM COMBINATORIAL SIGNALING IN 2D HUMAN GASTRULOIDS WITH ITERATIVE IMMUNOFLUORESCENCE

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The idea that developmental patterning relies on positional information encoded by morphogen gradients is a cornerstone of developmental biology. This concept has been mathematically formalized in the framework of Shannon's information theory. Information theoretic analysis has resulted in surprising findings that suggest optimal decoding of positional information from morphogen gradients in the fly embryo and vertebrate neural tube. Here we apply this framework to quantify how accurately instantaneous cell signaling reflects position and in turn predicts cell fate in 2D micropatterned human gastruloids. In this system, exogenous BMP4 initiates self-organized patterning of human pluripotent stem cells into concentric rings of different cell types associated with gastrulation. 2D human gastruloids are patterned into seven fates by at least four signaling pathways. To relate all of these in individual cells, we adapted an iterative immunofluorescence protocol to measure eight signaling readouts and eighteen cell-fate markers in the same sample. We found that combining information from multiple signaling readouts substantially reduces error in readout of position. Strikingly, while the information in individual signals is localized, the combined precision of all signals is approximately uniform in space; around 10% of the colony radius (3–4 cell diameters). Although this precision is preserved at different doses of BMP4, different signals carry this positional information, suggesting redistribution of that information between pathways. This is likely inconsistent with a model where instantaneous signaling thresholds predict fate.

Funding Source: This work was supported by the National Institute of General Medical Sciences (NIGMS685 R35GM138346) and NSF RECODE (2033654)

Keywords: *information, morphogens, gastruloids*

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DECIPHERING LIGAND-MEDIATED AEROCYTE SPECIFICATION IN VITRO USING A VASCULAR ORGANOID MODEL SYSTEM

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Recent studies revealed that alveolar capillaries consist of two specialized endothelial subtypes -aerocytes (aCap) and general capillary (gCap) cells. However, the developmental origins of aCap in mammalian lungs remain ambiguous. Early studies have already shown that cell-cell communication between the primordial epithelial and mesenchymal compartments is essential for lung development. We hypothesize that the microenvironment of developing lungs contains key signals that drive aCap specification. Here, we describe

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efforts to interrogate ligand-receptor interactions found to be enriched in the developing mammalian alveolar niché in vitro using induced pluripotent stem cell (iPSC)-derived blood vessel organoids (VOs) with the goal of deciphering signals governing aCap specification. We found that Endothelin-1 (EDN1) and VEGFA-189 were able to effectively induce the expression of aCap markers such as EDNRB and HPGD in the iPSC-VOs. The ligand-treated VOs displayed significant expansion in vessel area, akin to the complex morphological changes undergone by aCap during development. The expansion in vessel area was not due to cell proliferation but bona fide morphogenetic changes. Accompany vessel expansion was evidence of cytoskeletal remodeling and activation of an aCap transcriptional program—an increase in F-actin condensates was noted in 'large' vessels of ligand-treated VOs and elevated levels of transcription factor GATA2 were observed. Thereafter, we determined that mechano-transduction Rho/ROCK pathway could potentially be involved in ligand-mediated aCap specification. We report that inhibiting Rho/ROCK signaling in both ligand-treated VOs and human pulmonary microvascular endothelial cells resulted in the loss of EDNRB-expressing CD31+ aCap-like cells. Restoring Rho/ROCK signaling alleviated the loss of aCap-like cells. In summary, our results suggest that ligands such as EDN1 and VEGFA-189 are critical aCap specifiers; both ligands induce drastic morphogenetic changes to the endothelium in VOs perhaps by inducing cytoskeletal remodeling and a core transcription factor network in a Rho/ROCK-dependent manner. Our study, to our knowledge, is the first to identify aCap specifying factors and to determine the relationship between cell shape and fate during aCap development.

Funding Source: NIH LungMAP2 Pilot Award American Heart Association Pre-doctoral Fellowship (1013861), University of Cincinnati Graduate Student Government Research Fellowship

Keywords: *lung, endothelial, development*

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IN VITRO STEM CELL-BASED MODEL HELPS IDENTIFY TFAP2A AS REGULATOR OF HUMAN AMNION DIFFERENTIATION

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Prior to implantation, the human embryo proper consist of a cyst of pluripotent epiblast cells. Implantation triggers amniogenesis within a subset of the epiblast cells surrounding the pro-amniotic cavity. We previously established a culture system that supports amniogenesis from human pluripotent stem cells (hPSC) and identified that BMP signaling is a critical regulator of human amniogenesis. However, the cell population is not restricted to amnion, hindering reproducible molecular investigations. In this study, we developed a new hPSC-based 3D amnion culture system where exogenous BMP4 was introduced to trigger amniogenesis in all cells, allowing for highly robust mechanistic analyses. RNAseq was performed to examine the transcriptional characteristics of this amnion tissue. First, we merged the RNAseq data with a published scRNAseq dataset of a CS7 human embryo and found that the in vitro amnion clustered with developing in vivo human amnion cells, validating the amniotic nature of this new model. To investigate the temporal cascades post BMP4 treatment, we analyzed the expression of established amnion markers. The earliest detected transcription factor is GATA3, followed by TFAP2A. To identify their role, we generated amnion tissue carrying loss-of-function mutations in GATA3 or TFAP2A. While GATA3-KO appeared largely normal, TFAP2A-KO cysts were asymmetrically patterned, in which squamous cells with amniotic characteristics formed on one side, and columnar pluripotent epiblast-like cells on the other. We also identified WNT3A as a potential downstream target of TFAP2A. To verify the role of WNT signaling during amniogenesis, we utilized small molecule inhibitors for WNT-signaling, and found that inhibition of WNT signaling leads to asymmetric tissue similar to TFAP2A-KO. Here, we identified the role of TFAP2A, as well as WNT signaling during human amniogenesis.

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Funding Source: R01-HD098231 (K.T.) Lalor Foundation Fellowship (N.S.) AHW Project #5520766 (N.S.)

Keywords: *Amnion, BMP, TFAP2A*

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FULL-LENGTH LAMININS ARE CRUCIAL FOR RECREATING THE CELLULAR NICHE IN TISSUE IN VIVO

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Laminins are an extracellular matrix protein family of 16 different tissue-specific-isoforms and serve an important role in the formation and maintenance of the basement membrane architecture, thus they are vital for tissue homeostasis.

Intact laminins are essential, as mutations in genes encoding laminins can cause a wide spectrum of diseases. Mutations in different domains of the laminin protein result in BM weakness and various muscle, kidney, nerve, and eye disorders. Several human congenital diseases are caused by laminin chain mutations, such as Pierson syndrome and epidermolysis bullosa.

The biological effects of laminins are vastly mediated by cell surface receptors which link laminin matrices to intracellular signaling pathways. Hence, laminins are not only relevant for cell adhesion but also for various physiological functions (i.e., cell survival, migration, differentiation, cell maturation, polarization, and organization of specialized cell types in different tissues and organs, such as brain, pancreas, vasculature, lung, liver, kidney, muscle, and skin). In vivo, most laminin receptors are integrins and non-integrins like dystroglycan, syndecan and Lutheran blood group glycoprotein. Laminins also have a high binding affinity to growth factors. Such interactions are essential for controlling GF release kinetics in vivo, thus modulating tissue morphogenesis. Mimicking the cell microenvironment in vitro with full-length laminins is crucial for development of successful differentiation protocols, predictable disease models and effective gene editing. Our internal studies confirm the important role of full-length laminins. We show higher efficiency on ES cell cultivation and faster proliferation rate with standard morphological appearance, compared to truncated laminin.

Laminins play a key role in development and tissue morphogenesis. Full-length laminins are highly suitable matrices for recreating the natural cell niche in vitro for a variety of tissues.

Keywords: *Laminin, Microenvironment, Development*

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DISSECTING LYMPHOID PROGENITOR ONTOGENY IN ZEBRAFISH HEMATOPOIESIS

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Hematopoietic stem cells (HSCs) are multipotent, self-renewing cells that maintain the entire blood system by their ability to differentiate into mature blood cell types of the erythroid, myeloid, and lymphoid lineages. During embryogenesis, HSC-independent lymphoid progenitor cells (LyPs) emerge simultaneous with HSCs and are the major contributor of early thymic T-cells. The ontogeny of the lymphoid lineage in fetal hematopoiesis is convoluted, with specific lymphocytes arising from distinct hematopoietic stem and progenitor cells. One of the major limitations in the field is the inability to distinguish these LyP cells from HSCs at the time of formation, which makes it difficult to define the factors distinctly critical for LyP vs HSC generation. Through single-cell RNA-sequencing of nascent hematopoietic stem and progenitor cells from developing zebrafish, we identified genes that discriminate LyPs from HSCs. We are using whole mount in-situ hybridization (ISH) to delineate the spatiotemporal expression of these newly identified LyP markers during embryogenesis. So far, we performed ISH of two top LyP markers smarcd1 and meis1b. Smarcd1 is required for early lymphopoiesis via an interaction with the bHLH transcription factor E2A, and Meis1b is a homeobox domain protein that is required for definitive hematopoiesis. In wildtype zebrafish at 24hpf, and to a lesser extent at 30 and 52 hpf, both smarcd1 and meis1b are expressed in the

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posterior blood island (PBI), a region known to give rise to the first thymic-seeding lymphoid cells. Runx1 is a hematopoietic transcription factor whose loss severely impairs definitive hematopoiesis. In zebrafish, some runx1 loss-of-function mutants survive and recover most of their blood system, suggesting that a subset of HSC/progenitors are Runx1-independent. Through ISH experiments at 24, 30, and 52 hpf, we observed no difference in smarcd1 or meis1b expression based on runx1 genotype. Taken together, our data suggest that LyPs may originate before definitive HSCs within the PBI in a Runx1-independent manner. Completion of this project will advance our understanding of the factors that drive multipotency and self-renewal properties of HSCs and LyPs which is critical for harnessing stem cells for regenerative medicine.

Funding Source: Funded by T32GM145438 and R01DK131445

Keywords: *Hematopoietic Stem Cells, Lymphoid Progenitors, Zebrafish*

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THE ROLE OF SONIC HEDGEHOG SIGNALING ON CRANIAL NEURAL CREST POTENCY

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Most cells within the embryo are restricted to give rise to derivatives of their respective germ layers, but cranial neural crest cells (CNCCs), often called "the fourth germ layer", represent an interesting exception. Although CNCCs are derived from ectoderm, they can differentiate into both traditionally ectodermal (e.g., neurons/glia) and mesodermal derivatives (e.g., bone, cartilage). Despite a robust interest in CNCCs, the molecular signaling mechanisms that convey this pleistopotency are not well understood. Previous in vitro studies suggested that exposure to Sonic hedgehog (Shh) signaling conveyed pleistopotency upon CNCCs, as it increased the proportion of CNCCs capable of differentiating into both ectodermal and mesodermal derivatives; however, the mechanism behind this observed phenomenon remain unknown. To investigate the role of Shh signaling in CNCC potency, we performed RNAscope and showed that early CNCCs co-express Oct4 and Shh, suggesting a possible link between Shh signaling and potency. In vivo data supported these findings by revealing that Gli transcription factors (Gli TFs) were expressed during CNCC induction and specification, and computational analysis revealed that a significant number of Gli motifs were present at enhancers of pluripotency genes (e.g., Oct4 and Nanog). Furthermore, CNCCs lacking Gli TFs (Gli2f/f; Gli3f/f; Wnt1-Cre) failed to properly initiate differentiation into mesodermal derivatives. To determine if Gli-mediated Shh signaling is essential for conveying potency or rather for initiating differentiation, we generated conditional knockout lines that delete Gli TFs at different stages of CNCC development (induction, specification, and migration). Together, these results not only provide insight into the molecular mechanisms necessary for establishing CNCCs pleistopotency but also establish a novel role for Shh signaling during CNCC development.

Funding Source: NIH R35DE027557 (SAB) NIH F31DE033565 (SJYH)

Keywords: *Sonic-hedgehog, Cranial-neural-crest-cells, Pleistopotency*

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THE ROLE OF TRANSCRIPTION FACTOR REX1/ ZFP42 IN TRANSLATIONAL CONTROL OF MOUSE EMBRYONIC STEM CELLS

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Rex1 (reduced expression 1, also known as Zfp42) is a highly specific pluripotency marker that plays an important role in epigenetic and transcriptional regulation of mouse embryonic stem cells (mESCs). Rex1 is a DNA-binding Gliâ-Kruppel type zinc finger transcription factor (TF) that is evolutionarily related to Yin Yang 1 (YY1) and Yin Yang 2 (YY2) TFs. Previous studies have focused on the role of Rex1, YY1 and YY2 in the nucleus, however some evidence suggest that these TFs might be present in the cytoplasm or interact with RNA. To identify novel interacting partners of Rex1, we employed HEK293 Flp-In T-REx cell line system to induce GFP tagged YY1, YY2, and Rex1, followed by GFP affinity purification (in duplicate) and mass spectrometry (AP-MS). Surprisingly, AP-MS data demonstrated that Rex1 specifically binds to several ribosomal proteins in both small and large ribosomal subunits. Immunostaining and subcellular fractions demonstrated that a large portion of Rex1 is enriched in the cytoplasm. Importantly, polysome profiling showed Rex1 co-migrates with translating ribosome in mouse embryonic stem cells (mESCs). These results suggest an unexpected role of Rex1 in the translational control of mESCs. To validate this hypothesis, we will test the impact of Rex1 on global and mRNA-specific translation in WT and Rex1 KO mESCs using a puromycin incorporation assay, polysome profiling, and ribosome footprinting. Additionally, we will use an enhanced Crosslinking and immunoprecipitation (eCLIP) assay to identify RNAs that directly bind to Rex1. This study will advance our understanding of stem cell translational regulation as well as uncovering an unexpected and novel role for Rex1 in regulating mRNA translation in mESCs.

Keywords: *Rex1, Transcription, Translation*

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SYSTEMATIC EVALUATION OF RETROVIRAL LTRS AS CIS-REGULATORY ELEMENTS IN MOUSE EMBRYOS

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In mammals, many retrotransposons are de-repressed during zygotic genome activation (ZGA). However, their functions in early development remain elusive largely due to the challenge to simultaneously manipulate thousands of retrotransposon insertions in embryos. Here, we applied CRISPR interference (CRISPRi) to perturb the long terminal repeats (LTR) MT2_Mm, a well-known ZGA and totipotency marker that exists in ~2667 insertions throughout the mouse genome. CRISPRi robustly perturbed 2485 (~93%) MT2_Mm insertions and 1090 (~55%) insertions of the closely related MT2C_Mm in 2-cell embryos. Remarkably, such perturbation caused down-regulation of hundreds of ZGA genes and embryonic arrest mostly at the morula stage. Mechanistically, MT2 are globally enriched for open chromatin and H3K27ac and function as promoters/enhancers downstream of OBOX/DUX proteins. Thus, we not only provide direct evidence to support the functional importance of MT2 activation in development, but also systematically define cis-function of MT2 in embryos by integrating functional perturbation and multi-omic analyses.

Funding Source: NIH: R00HD104902 and the Trustee award from CCHMC

Keywords: *ERVs, ZGA, CRISPRi*

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PROTEOMIC ANALYSIS OF DIFFERENTIALLY RECRUITED CHROMATIN-BOUND TCF/SOX-β-CATENIN INTERACTING PROTEINS

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The Wnt/β-catenin signaling pathway regulates many aspects of embryonic development, stem cell maintenance and regeneration, and when disrupted can lead to diseases, including cancer. These processes are governed by differential Wnt target gene transcription which is initiated upon the binding of Wnt ligands to Frizzled and LRP5/6 co-receptors, facilitating the inhibition of the β-catenin destruction complex and stabilization and translocation of β-catenin to the nucleus. Here, β-catenin forms the Wnt enhanceosome, transcriptional complexes composed of chromatin-bound TCF and SOX transcription factors and co-regulatory proteins whose interactions with additional protein constituents and the surrounding chromatin contribute greatly to transcriptional specificity. ChIP-qPCR data collected in the lab revealed consistencies between the core TCF and SOX enhanceosome, including BCL9, PYGO, MED12, BRG1, and CBP. However, the composition of each respective enhanceosome beyond these components and the dependence of SOX17 on the composition of the Wnt enhanceosome is unknown. To address this, we performed RIME against β-catenin, TCF7L2, and SOX17 in wild type and SOX17-/- definitive endoderm differentiated human embryonic stem cells in a Wnt:On or Wnt:Off state. β-catenin exclusively interacted with 75 proteins in the Wnt:On condition, 29 of which were dependent on the presence of SOX17. Zero of these proteins interacted with TCF7L2 in a Wnt dependent manner indicating that β-catenin recruits a unique subset of proteins to the SOX17 Wnt enhanceosome. Additional whole-interactome analyses revealed that 31 proteins exclusively interacted with TCF7L2 and 353 proteins with SOX17 (n=415) in the Wnt:On condition, suggesting that each transcription factor recruits a nearly independent subset of co-regulatory proteins in response to Wnt signaling though not dependent on the presence of β-catenin. Together, these data provide novel insight into differential co-factor recruitment and specificity of gene transcription in response to Wnt signaling.

Funding Source: CuSTOM

Keywords: *Wnt, SOX17, Enhanceosome*

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INTEGRATING URETERIC-BUD DERIVED COLLECTING SYSTEMS INTO HUMAN KIDNEY ORGANOIDS TO PROMOTE DISTAL NEPHRON ANASTAMOSIS AND DRAINAGE

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Human kidneys comprise approximately 1 million individual nephrons, each of which must connect at its distal end to a collecting duct (CD) that transports urine out of the kidney for eventual excretion. The anastomosis between the nephron and CD, which derive from distinct metanephric and ureteric bud (UB) progenitor populations, respectively, is therefore among the most critical developmental determinants of kidney function. Since conventional hPSC-derived kidney organoids do not contain UB precursors, their nephrons do not fuse to CDs and thus remain non-functional, blind-ended tubules. We sought to address this limitation and promote these critical tissue interactions by assembling UB spheroids, differentiated in parallel, into metanephric-like kidney organoids. We elucidated efficient methods for incorporating the UBs and promoting growth of both tissue compartments in the integrated organoids. Fluorescent labeling showed that UB progenitors underwent rapid growth and extension into a tubular network resembling the CD system of the developing kidney. Remarkably, the resulting organoids contained nephron epithelia that were connected to UB-derived CDs. Consistent with normal kidney development and anatomy, only distal ends of the nephrons fused to CDs, recapitulating a typical proximal-distal polarity. Morphologic and transcriptional analyses revealed normal differentiation trajectories of UB and metanephric lineages, and the fusion process occurred during an early period of

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nephrogenesis analogous to the S-shaped body stage at which the anastomosis occurs in vivo. In conclusion, we established methods for integrating CD networks into kidney organoids, and this is the first described system that recapitulates anastomoses between distal nephron and CD epithelia. This innovative system is an important advance in kidney tissue engineering since generating a continuous drainage pathway from nephrons is an obligatory step toward production of functional renal tissue.

Keywords: *Kidney, Nephrogenesis, Organoid*

TOPIC: MORPHOGENETIC MECHANISMS OF EARLY DEVELOPMENT

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BREAKING THE BARRIER OF RPE NEUROCOMPETENCE

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The retinal pigment epithelium (RPE) is a monolayer of cells that is essential for retina health and physiology, with several functions such as providing support and nutrients to the retina. During eye morphogenesis, the RPE and retina originate from the proximal and distal portions of the optic cup, respectively. RPE is a plastic tissue that can regenerate neural retina in embryonic amniotes via cell reprogramming. However, RPE neurocompetence is only present in the early embryonic stages. In chicken embryos, RPE can reprogram into neural retina after retinectomy and FGF2 stimulation at embryonic day 4 (E4), but not at embryonic day 5 (E5) or later. Based on recent genomic analyses, including bulk and snRNA/ATAC-seq, we hypothesized that signaling pathways and intrinsic cell fate control during eye morphogenesis are coupled mechanisms that restrict RPE neurocompetence. In order to identify transcription factors and signaling pathway candidates for functional perturbation to promote RPE reprogramming in the late embryonic state, we used single-nucleus multimodal profiling to differentiate chicken RPE from E3-E7. Multimodal data analysis pointed to 9 up-regulated and 9 down-regulated transcription factor-encoding genes and accompanying changes in motif accessibility that coincided with RPE neurocompetence restriction. Our data suggested that enhanced activity of the Hippo-YAP pathway, and manipulation of transcription factors, such as NFIA and NFIB, could restrict RPE neurocompetence. Inhibition of the Hippo-YAP pathway significantly increased cell proliferation in E4 and E5 RPE explants in the absence of FGF2. This increase in cell proliferation did not coincide with the induction of retina. Hippo-YAP signaling inhibition also up-regulated eye field transcription factors, EMT regulators, and cell cycle-related genes, while suppressing RPE identity. In contrast, inhibition of NFIA transiently caused an increase in the size of RPE explants, but only at E4. Altogether, our data suggest that the neurocompetence of embryonic RPE is jointly regulated by intrinsic cues and extrinsic signals, with differing effects on RPE cell behavior. Further, these findings indicate that cell proliferation and gene regulatory networks may be responsible for controlling RPE reprogramming and restriction of neurocompetence.

Funding Source: R01 EY026816 and R01 EY034980 to KDRT; F99 NS129167 to JAT

Keywords: *RPE, reprogramming, neurocompetece*

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SIGNALING REQUIREMENTS FOR HUMAN PRIMORDIAL GERM CELL-LIKE DIFFERENTIATION ARE TIME-DEPENDENT

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Human primordial germ cells (hPGCs) are the precursors of eggs and sperm, which perpetuate genetic information to the next generation. hPGCs are specified around the onset of gastrulation, after which they migrate into the gonads and further develop into gametes. Dysregulated development leads to germ cell tumors or infertility. Investigating germ cell development is critical for the fundamental understanding of early human development and advancing in vitro

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gametogenesis (IVG), which holds great potential for improving reproductive health. However, many aspects of hPGC specification and further development remain poorly understood, due to limited access to the early post-implantation human embryo and a lack of suitable experimental systems. We previously showed highly reproducible hPGC-like cells (hPGCLC) differentiation in a micropatterned human pluripotent stem cells (hPSCs) model for gastrulation. This model allowed us to understand how the relative timing of the BMP and Nodal signaling pathways controls hPGCLC specification. Here, we further characterize the subsequence maintenance and maturation of our hPGCLC over 7 days post the 2-day induction from hPSCs. We found hPGCLCs stably maintain early markers including TFAP2C, PRDM1, NANOG, and SOX17. We further observed progressive maturation, reflected in the increase of markers including CD38, ITGA6, and KIT based on which the cells could be effectively sorted. We investigated the signaling requirements of hPGCLC maintenance and observed a clear shift in signaling requirements for hPGCLCs after induction. Specifically, ERK signaling emerges as crucial for both hPGCLC fate maintenance and further maturation, whereas BMP, Wnt, and Nodal signaling are no longer necessary. This underscores distinct phases in hPGC development. In summary, our data suggest distinct phases of hPGC differentiation with time-dependent signaling requirements, and we leveraged these insights to greatly improve the efficiency of hPGCLC differentiation, which is a crucial first step for IVG.

Keywords: *Germline, gastrulation, Signaling*

TOPIC: MOVING INTO THE FUTURE WITH EMERGING TECHNOLOGIES

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DECIPHERING ENDOTHELIAL AND MESENCHYMAL ORGAN SPECIFICATION IN VASCULARIZED LUNG AND INTESTINAL ORGANOIDS

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During organogenesis, the orchestrated co-development of vasculature, mesenchyme, and neighboring epithelial cells is crucial for organ formation, maturation, and the acquisition of organ-specific characteristics. Here, utilizing a precisely controlled mesoderm-endoderm co-differentiation method regulated by BMP signaling,

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we constructed a human pluripotent stem cell-derived organoid system comprising lung or intestinal epithelium surrounded by mesenchyme and vasculature. Notably, the microenvironment of the organoid facilitated the development of organotypic features in the endothelium and mesenchyme, capturing inter-lineage communications found in human and mouse fetal organ atlas through single-cell RNA-sequencing. Utilizing this model, we identified novel marker genes in human anterior vs. posterior gut tube mesenchyme, and elucidated critical signaling pathways directing cell fate determination in organotypic endothelium and mesenchyme during early organ formation. Notably, Semaphorin proteins were crucial for lung specification, and GDF15 for intestinal specification. After in vivo transplantation , the vascularized organoid integrated with the host circulation, developing perfusable human sub-epithelial capillary structures and maturing into highly specialized lung endothelium resembling the gas exchange barrier observed in human alveoli. Furthermore, we generated vascularized lung and intestinal organoids using iPSCs derived from patients with Alveolar Capillary Dysplasia with Misalignment of Pulmonary Veins, a complex congenital disorder caused by FOXF1 mutation. Our model recapitulated the cell autonomous and non-autonomous abnormalities in the alveolar endothelium and epithelium. The establishment of the vascularized organoids provides a unique platform for elucidating the mechanisms driving endothelium and mesenchyme specification during early organogenesis, and investigating complex cell-cell communications in human gut tube development and disease.

Funding Source: Heart Association Pre-Doctoral Fellowship 1013861 and 906513, NHLBI/NIH grants: DP2 grant DK128799-01, N01-75N92020C00005, R01HL095993, S10OD026717-01; Human Disease Model Award of the Erasmus MC

Keywords: *Vascularization, Organoid, Gut*

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DESIGN AND CONTROL OF IN VITRO MICROENVIRONMENTS TO REPLICATED COMPLEX IN-VIVO ENVIRONMENT FOR ORGANOID ARCHITECTURE

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In vitro 3D and organoid culture methods that mimic the rich complexity of cell population and extracellular matrix (ECM) components of in vivo tissues contribute greatly to furthering our understanding of various biological phenomena. However, achieving precise control over the complex shapes, architectures, and interactions between different tissues within cultured organoids remains a challenge. Current organoid development heavily relies on cellular self-organization, yet the uniform culture conditions in vitro fail to provide accurate spatial cues to cells. Conversely, leveraging engineering principles offers a promising avenue to tailor the design, composition, and construction of organoids based on specific research objectives. We have developed an in vitro experimental platform for the organoid culture to design and control microenvironment. The simple cube device, which comprises a polycarbonate frame with rigid agarose walls and an inner ECM hydrogel, can be used as a carrier of organoid to (i) control the spatial distribution of cells by employing 3D-printed carbohydrate moulds to create cell seeding pockets in the ECM hydrogel, (ii) design tissues with localized ECM by isolating ECM hydrogels of varying the composition or stiffness in separate compartments, (iii) facilitate integration with microfluidics to generate the concentration gradient of morphogens to direct cell growth and differentiation, (iv) assemble multi-CUBE with organoids or tissues to express tissue-tissue interactions. By employing above technologies, we were able to replicate the notochord signal during the development of the neural tube. The Shh gradient, facilitated through a 100 Î¼m slit on a CUBE device, was applied to the neural plate, resulting in the generation of a localized expression pattern on the neural tube organoid.

Funding Source: JSPS KAKENHI (23H04723, 23KK0205)

Keywords: *Organoid, Chip, Gradient*

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CO-CULTURE SYSTEM OF HUMAN IPS CELL AND ADIPOSE-DERIVED STEM CELLS TO PRODUCE BRAIN ORGANOIDS

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Human cerebral organoids generated from induced pluripotent stem cell (iPS cells) provide useful models for various neuronal experiments. Although mature cerebral organoids have cortical structures, their physiological functions are limited due to the lack of vascular structures, and insufficient oxygen and nutrients in the central region of the organoid. To induce angiogenesis within a brain organoid, we induced vascularization by co-culturing iPS cells and adipose-derived stem cells (ASCs) during neuronal differentiation. We produced embryoid bodies (EBs) from iPS cells, then fused them with spheroids of ASCs which were produced by the original biodevice. We usually produce EBs from 8000~9000 iPS cells. In this study, however, we produced EBs from 2000 cells and co-cultured them with 2000 cells of ASCs labeled with a 605 nm quantum dot (QD) (iPS: ASC=1:1). In addition, EBs were mixed with ASC spheroids at ratios of 2:1, 1:1 and 1:2 to induce neural differentiation. The QD-labeled ASCs migrated to the central region of the brain organoids after neural maturation. The morphological characteristics of brain organoids higher ratios of ASCs, formed into vessel-like structures in the central region, expressing blood-vessel marker CD31. The results of electrophysiological analysis by multi-electrode array (MEA) in co-cultured organoids showed different current patterns compared to normal organoids. These results indicated that iPS cell-derived brain organoids, co-cultured with ASCs showed signs of angiogenesis, but it was not clear whether this vascularization provided a supporting function for neurons. Further confirmation is required before determining more appropriate methods of supporting neurons within this three-dimensional structure.

Funding Source: With thanks to the Foundation of Global Health Care

Keywords: *Organoid, iPS, QD*

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UTILIZING ADIPOSE-DERIVED STEM CELLS ON DECELLULARIZED BLADDER SCAFFOLDS FOR FUNCTIONAL BLADDER MUCOSA REGENERATION

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Treatment for Bladder Cancer (BCa) often involves radical cystectomies, requiring bladder reconstruction. Current methods, like enterocystoplasty, have limitations, highlighting the need for better alternatives. Tissue engineering offers potential solutions, including using cellular or acellular scaffolds. Acellular scaffolds, like the bladder acellular matrix, show promise of providing a foundation for native cell growth. However, obtaining normal bladder cells from BCa patients is challenging, prompting the use of alternative sources like patient-derived stem cells. Adipocyte-derived stem cells (ASCs) have shown potential for differentiating into urothelial cells, leveraging this ability this project focuses on transdifferentiating ASCs into functional urothelial cells for bladder reconstruction. The methodology involves isolating ASCs from adipose tissue obtained during liposuction, transdifferentiating them into urothelial-like cells using a co-culture technique and then evaluating their characteristics and functionality. Results indicate successful isolation and characterization of ASCs, displaying positive markers for stem cells as guidelines mandate. The co-culture of ASCs with SV-HUC cells resulted in changes resembling epithelial cells, indicating a potential transdifferentiation process, and is corroborated by the mRNA and protein levels. For the functional assay, urothelial-like cells were seeded onto decellularized bladder tissues. ASCs and SV-HUC cells were used as controls. After 10 days in culture, the urothelium barrier function will be assessed by analyzing uroplakin and tight junction proteins. The outcomes of this project hold promise for advancing bladder reconstruction methods, offering a potential alternative to current approaches. The successful

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transdifferentiation of ASCs into functional urothelial cells could pave the way for innovative, more effective strategies in BCa treatment and reconstruction.

Funding Source: We gratefully acknowledge the Canadian Institute of Health Research for their financial support CIHR-401512 that facilitated this research.

Keywords: *ADSC, Bladder-regeneration, Tissue-engineering*

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BMP SIGNALING PROMOTOES IMMUNE CELL DIFFERENTIATION IN HUMAN INTESTINAL ORGANOIDS

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Pluripotent stem cell-derived (PSC) human intestinal organoids (HIOs) are a complex in vitro model system that recapitulates some aspects of the intestinal architecture and function and can be used to model intestinal physiology and pathophysiology. Recently our lab has shown that culture conditions and growth factors influence the cellular composition of HIOs which can be induced to include epithelial, mesenchymal, endothelial, neuronal, serosal and smooth muscle cells. Despite these incredible advances, HIOs have still lacked persisting immune cell populations until recently, when the Wells lab showed that BMP is important for patterning the human intestine into the colonic region and can also induce immune cells within human colonic organoids (HCOs). Here, my goal was to leverage this prior knowledge to develop a small intestinal HIO model that possesses all cell lineages, including immune cells. A major challenge to overcome are the opposing roles of BMP required for small intestinal patterning and immune cell induction. That is, inhibition of BMP via NOGGIN is required for patterning into a small intestinal fate, and activation of BMP leads to both immune cell induction and colonic patterning. To overcome this, I have developed a multi-step induction protocol, where HIOs are first patterned into small intestine, and subsequently treated with BMP4 to induce immune-like cells not present during normal HIO differentiation. This preliminary set of experiments took place over several days, suggesting that early HIO mesoderm/mesenchyme is maintained in a state of high plasticity. I hypothesize that undifferentiated mesoderm progenitors are maintained for several days during the early spheroid phase of the HIO, giving rise to many different tissue lineages, including immune cells. This work begins to outline a method for creating a more complete, immunologically competent HIOs, while also testing the plasticity of mesoderm progenitors in the HIOs.

Funding Source: NIH T32 GM145470

Keywords: *BMP4, HIOs, Immunity*

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CELLOTYPE-PHENOTYPE ASSOCIATIONS ANALYSIS OF MASLD USING EN MASSE ORGANOID PANELS

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Organoid technology thrives due to its ability to mimic human development for investigating disease causes. The "in-a-dish" cellotype-phenotype association is a potent strategy to study donor-specific genetic traits altering phenotype in a cell-type specific regulatory context. En masse phenotyping, combined with donor pooling techniques, offers rich insights into cellotype-phenotype associations under controlled exposures. This approach aids in discovering biomarkers and therapeutic targets for precision medicine against heterogeneous metabolic diseases. We have applied donor pooling and liver organoid technology to develop a multiple-donor Population Organoid Panel (PoP) platform. Utilizing this model, a simultaneous analysis

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of cellotype-phenotype association in MASLD was conducted to examine individual differences. To induce PoP, we utilized genotyped iPSCs and first induced into the foregut endoderm by manipulating Activin, BMP, FGF and Wnt signaling. Subsequently, highly proliferative foregut progenitor cells were seeded under a clonal density along with substrates rich in extracellular matrix proteins, following treatment with FGF2, EGF, VEGF, GSK-3 inhibitor, and TGF-beta/SMAD inhibitor. This allows for the concomitant formation of multi-donor mosaic organoid formation, while each organoid harbors single donor-derived cells. Furthermore, exposing the organoids to high concentrations of fatty acids and insulin induced a state resembling metabolic dysfunction-associated steatohepatitis (MASH), closely mimicking the symptoms observed in patients with MASH. Genetic correlation analysis of the multi-donor MASLD -like PoP revealed associations between known MASLD risk genotype, such as PNPLA3 and GCKR, and lipid accumulation phenotype in organoids. Despite a limited donor pool, the PoP approach demonstrated its ability to establish correlations between genetic factors and phenotype while effectively controlling external factors. Additionally, the single-cell genomics approach combined with PoP enables highly accurate downstream analysis of each donor's characteristics. These analyses facilitate the investigation of the effects of environmental changes on different cell types and pathways, all while considering the genetic background of each donor.

Keywords: *Organoid, Liver, MASH*

THURSDAY, 4 APRIL 2024

6:00 PM – 6:45 PM
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TOPIC: CELL AND TISSUE THERAPIES

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CO-BINDING OF PIONEER TRANSCRIPTION FACTORS AND PRDM1 ENHANCES EFFICIENT POLYCOMB REPRESSIVE DOMAINS FORMATION DURING ENDODERM SPECIFICATION

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Pioneer Transcription Factors (TFs) play a primary role in cell reprogramming and transdifferentiation by their ability to change the chromatin state of target sites and establish the competence for cell fate determination. A recent study in our lab has revealed unexpected novel roles of the pioneer TF FOXA in preventing the activation of alternative-lineage genes by interacting with the transcriptional repressor PRDM1. To better understand the function of PRDM1 in early development, we took advantage of a doxycycline inducible CRISPR interference (CRISPRi) system to knockdown PRDM1 in human pluripotent stem cells (hPSCs) differentiated to the definitive endoderm (DE) stage, where PRDM1 is mostly expressed. We performed RNA-seq and Gene Ontology analysis and found that several processes associated with mesodermal and ectodermal lineages are derepressed after PRDM1 knockdown, supporting its role as a repressor involved in cell fate specification. Furthermore, we did Histone ChIP-seq assays for several repressive (H3K9me3, H3K9me2, H3K27me3 and H2AK119Ub1) and active (H3K27ac and H3K4me1) histone marks and showed that PRDM1 is directly involved in the deposition of Polycomb Repressive Complexes 1 and 2 (PRC1/2) associated marks. To better understand the pleiotropic effects of PRDM1 in cell fate determination, we performed Motif Analysis on PRDM1 ChIP-seq peaks to look for potential co-regulators. Interestingly,

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we found that motifs for several pioneer TFs expressed at the DE stage (i.e. SOX17, GATA6, EOMES) are overrepresented in PRDM1 peaks and their co-binding sites are significantly enriched in repressive marks compared to PRDM1-only or pioneer TF-only peaks. We also demonstrated that PRDM1 physically interacts with these pioneer TFs and that the loss of PRDM1 significantly reduces their DNA binding. Altogether, these results suggest that PRDM1 may control cell fate by cooperating with different pioneer TFs to repress lineage-specific regulatory elements.

Funding Source: This project is supported by the National Health Institute, NIH R01GM143161.

Keywords: *Polycomb, CellFate, PioneerTFs*

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THE HUMAN GLUCOCORTICOID RECEPTOR VARIANT, RS6190, PROMOTES HYPERCHOLESTEROLEMIA AND ATHEROSCLEROSIS THROUGH TRANSACTIVATION OF PCSK9 AND BHLHE40

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Elevated cholesterol poses a significant cardiovascular risk, yet the epigenetic factors influencing the risk and management of hypercholesterolemia remain poorly understood. The glucocorticoid receptor (GR) is a nuclear transcription factor that regulates the metabolism of virtually all major nutrients. However, its role in cholesterol regulation remains unknown. Rs6190, a coding single nucleotide polymorphism (SNP) in the GR protein (R23K), has been associated with metabolic health, but the mechanisms remain unelucidated. We probed the large cohort of the UK Biobank (N=485,895), where this low-frequency coding variant associated with increased cholesterol levels in women (beta=0.055, P=0.0087) independently from known cholesterol-regulating variants. The effect was additive according to the number of SNP alleles (homo > hetero > reference). SNP homozygosity in women associated with increased odds ratio for hypercholesterolemia and death by cardiovascular diseases. To understand the underlying mechanisms, we generated mice and human induced pluripotent stem cells (hiPSCs) genocopying the SNP in the GR gene locus using CRISPR editing. In SNP-bearing littermate mice, the SNP was sufficient to increase total, LDL-, and HDL-cholesterol levels on regular, high-fat, and high-cholesterol diets according to SNP zygosity. Liver RNA-seq and ChIP-seq data revealed that the SNP increased GR transactivation of Pcsk9 and Bhlhe40, negative regulators of LDL receptor and HDL receptor in liver, thereby elevating cholesterol in circulation and risk and severity of atherosclerotic lesions. Remarkably, we also found that this mechanism was conserved in CRISPR-engineered hiPSC-derived hepatocyte-like cells (HLCs). Taken together, our study leverages a non-rare human variant to uncover a novel GR-dependent mechanism contributing to atherogenic risk, particularly in women.

Funding Source: CuSTOM Research Funding 2022 Award

Keywords: *Glucocorticoidreceptor, hypercholesterolemia, atherosclerosis*

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TENDON TISSUE ENGINEERING: EVALUATION OF RABBIT TENDON-DERIVED MESENCHYMAL STEM CELLS AS A SOURCE FOR AN OPTIMAL SCAFFOLD CONSTRUCT

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In the USA, 50% of musculoskeletal injuries involve tendons and ligaments. Tendon injuries often are challenging to manage and can cause severe permanent disabilities. We hypothesize that a tissue engineering

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strategy of combining biomaterials with mesenchymal stem cells (MSCs) will provide an optimal treatment for tendon injuries. This approach requires the identification of suitable biomaterials and the characterization of suitable MSCs. In this project, MSCs were isolated from rabbit tendons using collagenase I and dispase II solutions. These MSCs were characterized by immunofluorescence to demonstrate the expression of MSC-specific markers. While CD44 and CD29 showed some expression, CD90 showed robust expression, suggesting that these cells met the criteria for MSCs. The differential potential of the MSCs into tenocytes is characterized when exposed to tenocyte induction media including TGFÎ²-3. Tenocyte differentiation was confirmed by the positive expression of tenocyte-specific markers, tenomodulin, tenascin-C, and collagen I on Day 3 and Day 6 post-differentiation. In addition to the increased expression of tenocyte markers, we observed changes in cell morphologies and a discrete pattern of the key extracellular matrix protein, fibronectin. Subjectively, the morphologies of the cells were distinctly different compared to both the time points and the undifferentiated controls. Cellular morphometric analyses using a combination of ImageJ and CellPose were used to better understand the cell response to TGFÎ²-3. These cell analyses and characterizations are crucial to forecast how these cells will respond to the tissue microenvironment and when they are exposed to novel biomaterials for tendon healing and repair. In conclusion, these analyses will help understand cell response to novel biomaterials and identify candidate strategies for the treatment of tendon injuries.

Funding Source: Research grant for Biomedical Innovations in SMART tendon technologies using novel additive manufacturing funded by the University of Tennessee, Human Health and Wellness Initiative

Keywords: *MSCs, Tenocytes, Morphometrics*

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HUMAN MESENCHYMAL STEM CELLS DISPLAY DISTINCT PATTERN OF EXTRACELLULAR MATRIX PROTEIN IN RESPONSE TO LIGNIN DERIVED CARBON QUANTUM DOTS

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Carbon quantum dots (CQDs) have been investigated for biomedical applications recently in medical imaging due to their fluorescent properties, overall long-term stability, and excellent biocompatibility. Furthermore, CQDs are at the frontier of regenerative medicine through the investigation of the ability of CQDs to promote stem cell proliferation and differentiation. Carbon quantum dots have been derived from a variety of precursors, such as citric acid, aloe vera, and carbon nanotubes. Recently, novel CQDs have been derived from lignin, a polymer found in the tissue of woody plants. In this study, two colloidal suspensions of lignin derived CQDs in water were synthesized. Lignin is an organic polymer in the tissues of woody plants. Lignin is also inexpensive and considered a byproduct of the wood and pulp industries, making it an abundantly available renewable resource. Material characterization was carried out on the lignin derived CQDs, including Fourier transform infrared spectroscopy (FTIR), emission, excitation and absorbance spectra, zeta potential, transmission electron microscopy (TEM), and scanning electron microscopy (SEM). Thin films of lignin derived CQDs were formed on glass and silicon substrates to assess the in vitro response of human mesenchymal stem cells (hMSCs) to the CQDs. Observations suggest that the two forms of CQDs promote cell attachment and proliferation within 24 hours. Additionally, discrete differences in the cytoskeletal organization and spatial expression of fibronectin were observed after 7 days, suggesting distinct cell responses between the two forms of CQDs. This confirms that lignin-derived CQDs

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trigger a response in hMSCs, and provides evidence that lignin derived CQD have the potential to guide lineage-specific responses, making these a promising biomaterial for future tissue engineering applications.

Funding Source: Sun Grant Initiative

Keywords: *lignin, CQD, MSC*

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A MULTIDISCIPLINARY APPROACH REVEALS DISTINCT BIOLOGICAL AND MATERIAL PROPERTIES OF GRAPHENE OXIDE AND REDUCED GRAPHENE OXIDE NANOPARTICLES OPTIMAL FOR BONE TISSUE ENGINEERING

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Tissue Engineering-Regenerative Medicine (TERM) relies on the contribution of biological, material and manufacturing, to achieve success. Thus, a team science approach to establish technologies, which can be translated from benchtop to bedside, would significantly advance this field. Mesenchymal stromal cells (MSCs) are the most extensively studied and used source of cells in TERM. MSCs replicate, self-renew, and maintain anti-inflammatory, and regenerative properties in vitro and in vivo. Data from our laboratory suggests the use of graphene-based nanoparticles (GNPs) in fabrication of biomimetic scaffolds in combination with MSCs for TERM. Hence, our goal in this study was to leverage the expertise of material scientists and cell biologists and use a multidisciplinary team approach to develop novel technologies for TERM. We hypothesize that GNPs serve as multifunctional platforms, and their size and surface topography can influence cell fate. GNPs with specific topographical features will result in cells creating ECM-mediated signaling. These platform designs will result in controlled cell responses which can be evaluated in vitro prior to in vivo application. In this study, we compared the material properties of graphene oxide (GO) with reduced graphene oxide (rGO) using laser, optical and scanning electron microscopy. Furthermore, the material properties were correlated with the response of human mesenchymal stem cells to each of the nanoparticles. The rGO films demonstrated ten times higher surface roughness and two-fold lower modulus compared to the GO films. Additionally, rGO films showed significant upregulation in the expression of osteocyte-specific genes, suggesting that higher porosity which manifests as higher surface roughness and lower indentation modulus presents an osteogenic environment for MSCs. In conclusion, our data shows that rGO containing nanocomposites have a higher potential to be effective bone substitutes.

Funding Source: University of Tennessee, One Health Initiative

Keywords: *graphene, nanoparticles, ECM*

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REGULATION OF FIBROADIPOGENIC PROGENITORS BY CD163+ MACROPHAGES AFTER SKELETAL MUSCLE DENERVATION

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Patients suffering nerve damage from acute trauma, chronic diseases, neuromuscular disorders, sciatica and aging experience a painful, debilitating quality of life. The persistent loss of innervation to skeletal muscle leads to the replacement of muscle with fibrous connective tissue which further prevents the restoration of the muscle-nerve connection. The signals that control this fibrotic process are unknown and the source of secreted factors that influence fibroadipogenic precursors (FAPs) have not been thoroughly investigated. We found that CD163+ macrophages (M2 macrophages), an immune cell, increase in number in chronically denervated skeletal muscle. Moreover, using highly multiplexed CODEX immunofluorescence assay, we found that M2 macrophage localization in denervated muscles are dispersed throughout the muscle, whereas M1 macrophages were localized at the site of motor nerve damage. We hypothesize that secreted signaling molecules from M2 macrophages will

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promote FAP differentiation into fibroblasts which leads to fibrosis. CD163+ are anti-inflammatory macrophages found around the motor nerve and skeletal muscle; we aim to determine the molecular signal that causes them to expand after denervation. Moreover, we will isolate and characterize how M2 macrophages influence fibrosis. CD163+ macrophages will be prospectively isolated by fluorescence activated cell sorting (FACS) and characterized through RNA-sequencing. Transcriptome differences between CD163+ macrophages from innervated and denervated muscles will be analyzed for pathways that could lead to their expansion. We will also identify secreted factors that could signal to FAPs to promote fibrosis. These secreted molecules that regulate FAP fate hold opportunity for development for therapeutics to prevent fibrosis during muscle regeneration.

Keywords: *Macrophages, Muscle, Fibrosis*

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NOVEL SYNTHETIC PEPTIDE-BASED GROWTH FACTORS AND THEIR APPLICATIONS

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Conventional growth factors (GFs) used in the manufacturing of regenerative medicine and cell therapy products face various challenges, such as lot-to-lot quality variations, potential contamination with biological impurities, low stability, and high manufacturing costs. Leveraging the technology called PDPS (Peptide Discovery Platform Systems), PeptiGrowth Inc. has been working on developing a series of synthetic peptides that possess the same activities and functions as conventional GFs. These peptides can address and overcome all these challenges. They are entirely chemically synthesized and free from animal components, making them suitable for all cell culturing applications. Currently, eight peptides have been developed, including HGF alternative peptide, TGF-Î²1 inhibitor, BDNF alternative peptide, Noggin-like peptide, BMP7 selective inhibitor, BMP4 selective inhibitor, VEGF alternative peptide, and Wnt3a alternative peptide. In this document, we present some application data for the GF-alternative peptides in cell culturing.

Keywords: *growth factors, cell manufacturing, regenerative medicine*

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ANALYZING THE DNK-EVT INTERACTIONS USING MATCHED MATERNAL AND PLACENTAL-DERIVED IPSCS FROM TERM PREGANCIES

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Successful placentation is essential for pregnancy. The key cell types involved in this process are maternal decidual natural killer (dNK) cells and placental epithelial cells called extravillous trophoblast (EVT). These maternal and placental cell interactions allow the development of a semiallogeneic placenta and protect the placenta and fetus from being rejected by the mother. Abnormal interaction of dNK and EVT have been linked to pregnancy complications. However, there is limited information about dNK-EVT crosstalk due to the lack of an accessible model system. Therefore, we aim to develop a coculture system using induced pluripotent stem cells (iPSC) derived from maternal and fetal cells from the same pregnancy. Maternal- and placenta-derived iPSC from healthy term pregnancy were used. EVT are differentiated from placental iPSC, and dNK from maternal iPSC, using protocols established in the lab. On day 5 of EVT differentiation, media was changed to NK media containing dNK at 1:1 ratio, and cocultured for 24 hrs. Cell viability was measured by trypan blue. Flow cytometry was used to detect EVT marker (HLA-G), dNK markers (CD45/CD56/CD16/CD103/CD9), and dNK activation by EVT coculture (CD107a/IFNγ/GM-CSF/VEGF/TNFα/perforin). After 24 hrs of coculture, we noted no difference in EVT viability (76% vs. 80%, without or with dNK) or changes in EVT marker expression (82% vs. 80%, without or with dNK). In the presence of EVT, dNK did not show differences in expression of dNK or activation markers. In conclusion, we did not see changes in either EVT or dNK with

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coculture, which aligns with in vivo events in normal pregnancy. This is the first report using maternal and placental iPSC from the same pregnancy to model dNK-EVT interactions. Future studies will include coculturing dNK and EVT derived from different pregnancy outcomes. This work will lay the foundation for further evaluation of the role of dNK-EVT interactions in both normal and complicated pregnancies.

Keywords: *dNK-EVT, Placenta, iPSC*

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DEVELOPING A PRE-VASCULARIZED ENDOTHELIAL SKIN CONSTRUCT INDEPENDENT OF ADDITIVE GROWTH FACTORS FOR WOUND HEALING

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Previous methods have successfully generated skin constructs with the development of an ordered differentiated epidermis, whose engineered skin organotypic can be used to probe the impact of different substances on cell assembly and functionality. Meanwhile, relying on additive growth factors to promote vascularization and angiogenesis is costly and unsustainable. Mesenchymal cells, however, such as fibroblasts and dental pulp stem cells, are known to secrete several pro-angiogenic growth factors. Thus, the objective of this research is to establish a microvascular network by co-culturing human umbilical vein endothelial cells (HUVECs) with mesenchymal cells in vitro without additional growth factors, to develop a series of altered mediums where both the endothelial and the epithelial cells can be co-cultured, to determine the role of an endothelial cell network in predicting vascularization and anastomosis response in-vivo, and to evaluate the impact of these constructs on wound healing. To create the 3D culture, HUVECs of 9x105 Cells/mL concentration were co-cultured with different feeder cells (Dental Pulp Stem Cells, Fibroblasts) individually, in a ratio of 2:1. EGM2 media was added into the co-culture for every other day. The extracellular matrix was comprised of collagen mixed with fibrin—formed from fibrinogen by thrombin at 1U/mg concentration—in a ratio of 1:1. Living skin equivalents were prepared according to the procedures from Margulis et al. (2005), modified with the co-culture layer on the bottom of skin constructs, cultured in the altered mediums to enhance both vascularization and epithelial differentiations. Another modified medium, which was more prone to HUVEC vascularization, was tested in the same manner as a comparison. The engineering of pre-vascularized networks within skin constructs played a vital role in the process of anastomosis, which was shown to occur as early as four days following the graft's placement on the host's wound site.

Funding Source: We acknowledge the Morin Charitable Trust for funding

Keywords: *Pre-vascularized, Skin, Construct*

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DOWNREGULATION OF SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 4 CONTRIBUTES TO IMPAIRMENT OF OSTEOGENIC DIFFERENTIATION POTENTIAL OF HUMAN BONE MARROW STEM CELLS

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Human bone marrow stem cells (hBMSCs) are a cornerstone for bone regeneration in treating human bone diseases and injuries. Achieving an adequate cell quantity for therapeutic applications necessitates the in vitro expansion of primary hBMSCs. A challenge is the rapid reduction of the osteogenic differentiation potential of hBMSCs during their in vitro expansion, which restricts their clinical utility. This study investigated the underlying mechanisms that lead to the attenuated

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osteogenic capacity during the in vitro expansion of hBMSCs. A key focus is the role of the Signal Transducer and Activator of Transcription 4 (STAT4), known for its involvement in cell migration, proliferation, and differentiation in response to various cytokines.

Primary hBMSCs were cultured and expanded to different passages with stem cell growth medium consisting of alpha-MEM and 20% FBS. We observed a progressive decline in STAT4 expression concurrent with the reduced osteogenic differentiation potential during the in vitro expansion of hBMSCs. We knocked down the STAT4 expression in early-passage hBMSCs utilizing small interfering RNA (siRNA) and subjected the cells to osteogenic induction for 2–3 weeks. We observed a significant reduction in calcium deposition and the downregulation of osteogenic markers, including alkaline phosphatase (ALP), bone sialoprotein (BSP), collagen type I alpha 1 chain (COL1A1), and osteopontin (OPN), indicating that reducing STAT4 expression in hBMSCs significantly inhibits their osteogenic differentiation potential. Conversely, we overexpressed STAT4 in different passages of hBMSCs using adeno-associated virus serotype 2 (AAV2)-mediated gene delivery. Next, we subjected the cells to osteogenic induction. We found that overexpression of STAT4 markedly enhanced calcium deposition in early-passage cells and restored the calcium deposition capabilities in late-passage cells. These findings suggest that the decrease in STAT4 expression during in vitro expansion may be a pivotal factor leading to the loss of osteogenic differentiation potential in hBMSCs. Notably, the upregulation of STAT4 expression could reverse this loss and restore the osteogenic potential of later passage cells after in vitro expansion. In conclusion, this study highlights the critical role of STAT4 in the osteogenic capability of hBMSCs during in vitro expansion. The downregulation of STAT4 expression contributes to the impaired osteogenic potential of hBMSCs. Maintaining STAT4 expression is, therefore, vital for preserving the osteogenic function of these cells during expansion. Our findings provide new insights into the molecular mechanisms behind the loss of osteogenic differentiation in expanded hBMSCs. STAT4 could be a promising target for enhancing hBMSCs-based bone regeneration therapies.

Funding Source: The National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)

Keywords: *hBMSCs, STAT4, differentiation*

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A BIOPRINTABLE MODEL OF GLIOBLASTOMA FOR DISSECTING CELLULAR MECHANISMS OF TUMOR INVASION AND DRUG RESISTANCE

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Glioblastoma multiforme (GBM), the most lethal form of primary brain cancer, employs various receptors to infiltrate the human brain and establish biological niches and resist chemotherapy. Despite the significance of CD44 and integrins in tumor invasion and drug resistance, prevailing in vitro GBM studies predominantly employ suspended tumor spheres or tumoroids without any scaffold. While this approach aids cancer stem cell maintenance, it constrains our comprehension of the extracellular matrix's role in tumorigenesis and the essential biochemical and biomechanical cues governing tumor progression and treatment resistance. Our study introduces a novel design that incorporates a bioprintable hydrogel consisting of gelatin and hyaluronic acid to maintain cancer stem cells. This carefully tailored structure, with the presence of HA, is ultimately critical for establishing a predictive in vitro platform for drug testing. It enables the growth of cancer spheroids either in isolation or in co-culture with other cell types, including brain endothelial cells. Furthermore, the viscosity of the hydrogel has been optimized to maintain the spheroids well segregated during the bioprinting process, ensuring accurate positioning within the constructs. The hydrogel can be easily mixed with 3D spheroids and crosslinked through a brief UV-light treatment. The presented biofabrication approach seamlessly integrates with a high-throughput imaging-based approach, providing spatial insights into cancer progression in response to drug treatment. Our innovative hydrogel design offers

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a versatile and customizable platform with applications in cancer research and tissue engineering. Our results showcase the effectiveness of our bioprinting approach in preserving GBM spheroids without damage and generating complex microtissues including different ECM components and cell types including brain endothelial cells and astrocytes. Microscopic characterization of our biofabricated model reveals the retention of cancer stem cell markers within this 3D structure, a phenomenon typically lost in traditional Collagen I-based scaffolds. This advancement holds promise for unraveling intricate mechanisms governing GBM behavior, providing a more physiologically relevant platform for drug testing and furthering our understanding of tumor biology.

Keywords: *Hyaluronic Acid, CD44, High-throughput*

TOPIC: DEVELOPMENTAL ORIGINS OF DISEASES: CONGENITAL MALFORMATIONS

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INVESTIGATION OF CARDIAC VALVE DISEASE MECHANISMS IN NOONAN SYNDROME WITH AN IPSC MODEL

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Noonan syndrome (NS) is primarily an autosomal dominant disorder that results from gain-of-function germline variants in the RAS/MAPK pathway. One of the main features of NS is congenital heart disease, with cardiac valve stenosis estimated to occur in over 50% of NS patients. However, progress towards a mechanistic understanding of this pathology has been hindered by a lack of access to human valve cells. To circumvent this limitation, we have developed a feeder-free human induced pluripotent stem cell (iPSC) differentiation strategy that allows us to recapitulate the steps of valvulogenesis in vitro and generate endocardial cells that can undergo endothelial-to-mesenchymal transition (EndMT) to become valvular interstitial cells (VICs), the resident cell of the cardiac valve leaflet. To investigate the valve pathology in NS, we applied this differentiation strategy to CRISPR-edited iPSCs carrying pathogenic NS variants. We found that NS-iPSCs exhibit increased specification towards a mesodermal lineage and subsequently have increased differentiation efficiency into endocardial cells. Interestingly, we found that these NS endocardial cells exhibit defective EndMT, specifically towards VICs that belong to the fibrosa layer, but not to those of the spongiosa layer. We then performed single-cell RNA sequencing on these populations and found that fibrosa NS-VICs exhibit dysregulation of numerous extracellular matrix (ECM) genes. Gene set enrichment analysis identified many putative signaling pathways that are upregulated, including RAS-MAPK, TGF-beta, and PI3K-AKT-mTOR signaling pathways. We also found that when NS-VICs are cultured in the presence of TGFb2, they exhibit significantly increased expression of ECM genes, suggesting dysregulated TGFb signaling in these cells. By applying our iPSC-derived VIC model to NS-iPSCs, we have gained valuable insights into the mechanisms driving valve stenosis in NS, which may help pave the way for developing novel therapeutics.

Funding Source: NIH/NHLBI: R35 HL135742, AHA/CHF Predoctoral Fellowship: 23PRECHF1025586

Keywords: *valve, iPSCs, cardiology*

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TEMPORAL SINGLE CELL ANALYSIS REVEALS THE REQUIREMENT FOR ODD-SKIPPED RELATED 1 IN MOUSE EMBRYONIC BLADDER DEVELOPMENT

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The bladder is a hollow, muscular sac that stores urine and expands up to 6-fold while withstanding greater tensile force than the Achilles tendon. Bladder capacity is controlled by coordinated interactions

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between three layers: the epithelium, which acts as a urine barrier and signals when the bladder is full; the extracellular matrix (ECM)-rich lamina propria that propagates epithelial signals and bears the mechanical load of filling; and the muscle layer, which contracts to expel urine. Bladder dysfunction is defined by aberrant ECM deposition and epithelial morphology; however, the cells and signaling pathways involved in these changes are largely unknown. Understanding how bladder cell types arise during development, and how they communicate to organize tissue architecture, is informative for understanding dysfunction, as well as bladder regeneration and stem cell therapies. Our lab has identified that the transcription factor Odd-skipped related 1 (Osr1) is expressed in the urogenital sinus at the onset of bladder development, is required for the development of all three bladder layers, and loss of Osr1 decreases bladder capacity. Using histological and immunofluorescent analysis, we observe that Osr1-KO embryonic bladders are smaller, fail to form a stratified epithelium, have fewer subepithelial mesenchymal cells, decreased collagen, and a thin and underdeveloped muscle layer. Using single-cell RNA seq across three embryonic time points of Osr1 KO and WT bladders, we confirm a loss of several epithelial and mesenchymal subpopulations, including progenitor cells, and cells expressing important signaling molecules like Wnts and BMPs. We will perform further computational analysis to identify key genetic networks, ligand-receptor interactions, and lineage trajectories. By creating layer-specific Osr1 knockout mouse lines, we observe that epithelial-mesenchymal crosstalk is required during bladder development. When Osr1 is knocked out in the mesenchymal cells alone, we observe muscle defects and loss of subepithelial mesenchymal cells, but also a decrease in epithelial stratification. Likewise, in the epithelial-only knockouts, we observe similar mesenchymal defects. Our research will provide a useful framework for understanding the cell types and genes important for embryonic bladder development.

Funding Source: Fonds de Recherche du Québec Santé (FRQS), Pierre-Lavoie Foundation

Keywords: *developmental biology, single-cell transcriptomics, urology*

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TIGHT JUNCTION PROTEIN 1&2 REGULATE BILE CANALICULAR FORMATION DURING HEPATOCYTE DIFFERENTIATION AND SELF-ORGANIZATION

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Newborns with deficient tight junction protein 2 (TJP2) develop a subtype of progressive familial intrahepatic cholestasis (PFIC-TJP2), which manifests as neonatal cholestasis that progresses to cirrhosis, while other organs often remain unaffected. Bile flow originates at the hepatocyte canalicular membrane, which forms the bile canaliculus (BC), a specialized bile draining "channel" between hepatocytes demarcated by tight junctions built with scaffolding proteins, TJP1&2, and transmembrane junction proteins. We found that PFIC-TJP2 patients have disrupted BC structures in liver biopsies and increased bile concentrations in both liver tissue and blood serum. Thus, we hypothesized that TJP1&2 play critical roles in BC formation during hepatocyte differentiation. To test this hypothesis, we combined novel induced pluripotent stem cell (iPSC) differentiation and culturing methods, to generate canalicular tubes between opposing induced hepatocytes (iHeps) capable of transporting bile. We then genome edited iPSCs to disrupt TJP1&2 separately, through patient relevant truncation or complete knock-out, then cultured these cells to form BC. We found that TJP1 deficiency showed cell death in the early stage of hepatocyte differentiation. TJP2 deficiency showed disrupted BC formation, resulting in shortened and rosette-like BC, but did not affect hepatocyte bile excretion into these short BC structures. We found that treating these cells with mRNA lengthens these disrupted BC, to a more WT-like phenotype. These findings indicate that TJP1&2 deficiency disrupts BC network formation which likely causes a build-up of bile in the disrupted BC, resulting in cholestatic injury and ultimate liver failure observed in PFIC-TJP2 patients. Additionally, we found that treating TJP1&2 deficient iHeps with TJP1&2 mRNA rescues disrupted BC structure can could offer a novel method for therapy this ultra-rare liver disease.

Keywords: *Liver, Junctions, Canaliculi*

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MODELING NEUROTROPHIC FACTOR-MEDIATED CAPILLARY INJURY AND REPAIR IN BRONCHOPULMONARY DYSPLASIA WITH IPSC-INDUCED VESSEL ORGANOIDS

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The importance of Neurotrophic Receptor Tyrosine Kinase 2 (NTRK2) extends beyond its role in neuronal function to also influence peripheral organs. Reduced NTRK2 expression was observed in general capillary endothelial cells (gCap ECs) in murine lungs exposed to hyperoxic injury, resembling human Bronchopulmonary Dysplasia (BPD). Yet, the role of NTRK2 signaling in pulmonary vascular regeneration after hyperoxia injury remains unclear. In murine lungs post 7-day hyperoxic injury and human lungs during BPD acute injury phase, NTRK2 expression was markedly reduced. Interestingly, in post-injury regeneration phase, an increase of truncated NTRK2 isoform lacking a tyrosine kinase domain (NTRK2-T1) was observed in a subgroup of humans and mice who failed to recover from hyperoxic injury. This isoform has a dominant-negative effect on full-length NTRK2 (NTRK2-FL). NTRK2-T1 overexpression in human pulmonary microvascular ECs impaired AKT and ERK pathways activation, leading to reduced cell proliferation and angiogenesis. Using single-nuclei ATAC-seq and gain- and loss-of-function experiments, we found the increase in NTRK2-T1 in severe BPD cases was due to increased HOXA5 and RNA splicing mediated by RBFOX2 in BPD gCap cells. Next, we generated iPSC-derived vessel organoids (VOs) to evaluate the vascular regeneration potential of NTRK2-FL. Firstly, VOs recapitulated changes of HOXA5, RBFOX2, and NTRK2 isoforms in response to hyperoxia injury. Nanoparticle delivery of NTRK2-FL in VOs promoted angiogenesis and cell proliferation after hyperoxic injury. In mice exposed to hyperoxia injury, nanoparticle delivery of NTRK2-FL promoted capillary development and rescued alveolar simplification. Collectively, we uncovered significant roles of NTRK2 signaling in lung gCap cells and in alveolar regeneration following hyperoxia injury. The iPSC-VOs served as a unique platform to study NTRK2 regulatory network and evaluate drug responses pertinent to vascular regeneration and repair.

Keywords: *BPD, Organoid, Lung*

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HUMAN KIDNEY ORGANOIDS ILLUMINATE THE ROLE OF APKC ISOFORMS AND NUCLEAR YAP IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

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Autosomal dominant polycystic kidney disease (ADPKD), resulting from PKD1 or PKD2 mutations, manifests as the development of multiple cysts and kidney enlargement, with approximately 75% progressing to end-stage renal disease (ESRD) by age 70. Atypical protein kinase isoforms (aPKCs) play a crucial role in cell signaling through phosphorylation events, and their dysregulation is implicated in ADPKD and Hippo signaling. Human kidney organoid directed

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disease modeling highlighted nuclear accumulation of YAP—a transcriptional co-activator regulating cell growth, polarity and proliferation on silencing of Hippo signaling—in advanced cysts. Multiple analyses of kidney organoid models, patient biopsies and genetic modulation of Pkd gene activity in the mouse kidney supports a role for aPKC isoform specific regulation of YAP phosphorylation and nuclear accumulation of YAP in the progressive growth of renal cysts. Together these data highlight aPKC as a therapeutic target of interest in blocking cyst progression in ADPKD.

Funding Source: C.J.S. was supported by the Amgen-USC Postdoctoral Fellowship Program

Keywords: *Kidney, Organoid, Disease-Modeling*

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MUTATIONS IN FOXF1 RESULT IN TRACHEA-ESOPHAGEAL CLEFTS

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Failure of the fetal foregut to separate into trachea and esophagus (TE) results in a spectrum of life-threatening tracheoesophageal defects (TEDs). Occurring in ~1/3,500 live births, the genetic etiology and cellular mechanisms underlying TEDs are poorly understood. One gene implicated in studies of TED patients is the pioneering transcription factor FOXF1. Patients with heterozygous FOXF1 mutations present with several defects, including tracheoesophageal fistula (TEF)—but mechanistically how FOXF1 mutations result in TEF is unknown. In Xenopus and mouse models, we identified Foxf1 as a key gene downstream of Hedgehog/Gli signaling and essential for TE morphogenesis. We show that endodermal Hedgehog signaling directly activates Foxf1 expression in the splanchnic mesoderm surrounding the fetal gut tube. While Foxf1 germline-null embryos are embryonic lethal at E9.5 and preclude analysis of later TE morphogenesis, conditional Foxf1 deletion from the E9.5-10.5 mouse foregut mesoderm (Foxg1Cre/+;Foxf1fl/fl) results in failure of foregut separation and a complete tracheoesophageal fistula. Xenopus foxf1 mutants exhibit a similar phenotype. Molecular analyses indicate that the foregut endoderm undergoes relatively normal dorsal-ventral patterning, but the mesoderm is hypoplastic and fails to constrict medially to separate the foregut tube. Preliminary single-cell RNA-sequencing studies show that loss of Foxf1 disrupts patterning of both the foregut mesoderm and endoderm. Bulk RNA-seq analysis suggests that Foxf1 regulates the expression of genes involved in remodeling the extracellular matrix, plasma membrane, and actin cytoskeleton, which may account for disrupted morphogenesis. Ongoing studies are elaborating the Foxf1-regulated GRN and testing the mechanistic role of Foxf1-targets in foregut morphogenesis. This will reveal mechanisms of aerodigestive organogenesis and inform the basis of birth defects.

Funding Source: This work is funded by NICHD P01 HD093363, NIH T32 HL0077521, and NIH T32 GM063483

Keywords: *Malformation, Disease, Model*

TOPIC: DEVELOPMENTAL ORIGINS OF DISEASES: DEGENERATIVE DISEASES

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AGE AND SEX HORMONE-BASED EFFECTS ON NEURAL STEM PROGENITOR CELL FUNCTION ACROSS DEVELOPMENTAL TIME—IMPLICATIONS FOR NEURODEGENERATION

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The adult mammalian brain harbors active neural stem and progenitor cells (NSPCs) that generate new nerve

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cells throughout life. Broadly, it is known that age- and sex-dependent processes interact to modulate the behavior and function of NSPCs. However, these fundamental dependencies remain ill defined. Our previous work, in male rats, identified a specific critical period of decline in NSPC activity during middle-age, and revealed the reduced expression of the redox-sensitive transcription factor, NRF2, as a key mediator of this process. In the current study, we utilized different groups of aging female rats (2, 6, 9, and 14 months old) to understand changes in NSC regeneration and NRF2 expression at cellular, molecular and behavioral levels. To investigate the influence of 17beta-estradiol (E2) and progesterone (P4) on NSPC function, the animals were either ovariectomized (OVX) or underwent sham surgery after which they were subjected to relevant behavioral tests, and downstream histological and molecular analyses. In terms of behavior, the rats were tested via fine olfactory discrimination, pattern separation, and platform reversal in Morris Water Maze tasks to specifically assess functions relevant to brain NSPC niches in the forebrain subventricular zone (SVZ) and hippocampal subgranular zone (SGZ). Early data indicate that OVX compromises olfactory discrimination, pattern separation and reversal learning particularly at 6 and 9 months of age, with more prominent changes noted in especially in the 9 months old animals. In addition, western blot and immunohistochemical analyses showed alterations in NRF2 and estrogen receptor expression, as well as neurogenesis, associated with the OVX-induced behavioral changes. Ongoing studies are further assessing the relationship between E2/P4, NRF2 and neurogenesis in the SVZ and SGZ, and will shed light on age-related vulnerabilities in NSPC plasticity that may set the stage for nascent neurodegenerative processes.

Funding Source: NSF IOS 2207023 (LM), University of Arizona BIO5 postdoc fellowship and NIH T32 AG044402 (AI)

Keywords: *NSPCs, Female, Nrf2*

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HUMAN INDUCED PLURIPOTENT STEM CELL MODEL OF ALZHEIMER'S DISEASE-ASSOCIATED FRACTALKINE RECEPTOR POLYMORPHISM DEMONSTRATES MICROGLIAL DYSFUNCTION

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Dysfunctional microglial activity has recently been identified as a potential mechanism leading to accumulation of amyloid beta and pTau and subsequent neurodegeneration in Alzheimer's Disease (AD). The CX3CR1/fractalkine axis serves as a mechanism for bi-directional communication between microglia and neurons, respectively, to promote a resting, anti-inflammatory state in microglia. Previous studies have demonstrated that deficiency in CX3CR1 signaling leads microglia to a more pro-inflammatory phenotype and phagocytosis deficits and increases susceptibility of neurons to cell death. Additionally, the CX3CR1-V249I polymorphism was recently identified as a potential risk allele for AD with worsened Braak staging in post-mortem AD patients. However, the role of fractalkine dysfunction in human cells and the mechanisms by which microglia with the CX3CR1-V249I SNP contribute to neurodegeneration remain unclear. Thus, to address this shortcoming, we utilized human induced pluripotent stem cells and CRISPR/Cas9 technology to elucidate the effects of the V249I SNP on microglia-like cells compared to an isogenic control cell line. We demonstrate effective differentiation from both isogenic control and CX3CR1-V249I backgrounds into human microglia-like cells, which express characteristic microglial markers and are functionally phagocytic. Microglia bearing the homozygous V249I allele demonstrated decreased phagocytosis of amyloid

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beta in vitro compared to isogenic controls as well as increased stress-induced cell death and altered proliferation. These findings suggest that the CX3CR1-V249I SNP may cause a dysfunctional microglia phenotype that is further associated with a pro-inflammatory, reactive microglial state, subsequently contributing to neuronal dysfunction and death. Ongoing work will expand upon the transcriptome and secretome profile of CX3CR1-V249I microglia and elucidate how this gene variant contributes to AD-related neurodegeneration.

Funding Source: Funding for this study was provided by the National Institutes for Aging (RF1AG069425 and RF1AG069425-S1), as well as a training grant from the NIA (T32AG071444).

Keywords: *Alzheimer's, Microglia, iPSC*

TOPIC: FUNDAMENTAL MOLECULAR MECHANISMS OF EARLY DEVELOPMENT

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DIFFERENTIAL SPATIO-TEMPORAL REGULATION OF DOPAMINERGIC NEURONS IN MOUSE OLFACTORY BULB AND MID-BRAIN

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Dopaminergic (DA) neurons in the Olfactory bulb (OB) are involved in odor detection and discrimination. Transcription factor (TF) regulatory network responsible for their fate specification remains poorly understood and the spatial regulation of DA neurons remains elusive. Here, we demonstrate a novel role for Zinc finger transcription factor of Cerebellum (ZIC) 3, in determining the DA neuronal differentiation by Tyrosine Hydroxylase (TH) regulation in stem cell-based model systems. Stringent co-expression analysis showed ZIC3 and TH dual positive neurons in OB. Genetic manipulation showed ZIC3 to be both essential and sufficient to drive TH expression in OB DA neurons. Mechanistic investigation showed that ZIC3 interacts with ER81 and binds to region encompassing ER81 binding site on Th promoter and is indispensable for TH expression in OB. Interestingly, ZIC3 also governs the expression of TH in midbrain (MB) DA neurons both in vitro and in vivo. However, to circumvent for the absence of ER81 in MB, ZIC3 switches its molecular partner and binds to Pitx3 promoter—a DA fate determinant. Under ectopic expression of ER81 in MB DA neurons, propensity of ZIC3 binding to Pitx3 promoter is compromised and its occupancy on Th promoter encompassing ER81 binding site is established, finally culminating in desired TH expression. Together, these findings reveal a unique ZIC3 mediated bimodal regulation of TH in OB and MB to ultimately facilitate DA differentiation.

Keywords: *Olfactorybulb, midbrain, Neurons*

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SELF-ASSEMBLED GENERATION OF MULTI-ZONAL LIVER ORGANOIDS FROM HUMAN PLURIPOTENT STEM CELLS

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The liver is the pivotal organ that maintains metabolic homeostasis with functions ranging from protein and lipid synthesis to xenobiotic metabolism. These diverse functions are carried out in specific zones of the hepatic cords that make up the liver lobule. In vitro replication of zonal hepatocytes with interconnected assembly is an unmet challenge. Here we found that extracellular bilirubin triggers cytoskeletal rearrangement to

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self-organize multi-Zonal Human Liver Organoids (mZ-HLOs) from human pluripotent stem cells. By synthetically generated ascorbate gradient, mZ-HLOs are tailored to contain CPS1+ and GLUL+ hepatocyte-like cells, wherein snRNAseq analysis identifies interzonal like hepatocytes as a transitional state with functional glutathione synthesis activity. Epigenetic and transcriptomic analysis showed the zonal divergence is orchestrated by differential EP300 binding to HIF1A and TET1. Transplantation of the mZ-HLOs improved the multiple symptoms and overall survival caused by bile duct ligation in Il2rg- and Rag1-deficient rats. Overall, the mZ-HLOs serves as an in vitro human model to study the functional ensemble across multi-zonal hepatocytes in development and disease.

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Keywords: *Liver, Zonation, Metabolism*

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SWEAT GLAND DEVELOPMENT REQUIRES EDEN, AN ECCRINE DERMAL NICHE, AND COUPLES TWO EPIDERMAL PROGRAMS

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Eccrine sweat glands are indispensable for human thermoregulation and like other mammalian skin appendages form from multipotent epidermal progenitors. Limited understanding of how epidermal progenitors specialize to form these vital organs has precluded therapeutic efforts towards their regeneration. Herein, we applied single nucleus transcriptomics to compare the expression content of wildtype, eccrine-forming mouse skin to that of mice harboring a skin-specific disruption of Engrailed 1 (En1), a transcription factor that promotes eccrine gland formation in humans and mice. We identify two concurrent, but disproportionate, epidermal transcriptomes in the earliest eccrine anlagen: one that is shared with hair follicles, and one that is En1-dependent and eccrine-specific. We demonstrate that eccrine development requires the induction of a dermal niche proximal to each developing gland in humans and mice. Our study defines the signatures of eccrine identity and uncovers the eccrine dermal niche, setting the stage for targeted regeneration and comprehensive skin repair.

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Keywords: *eccrine, niche, development*

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SOX21 NEGATIVELY REGULATES SOX2 TO CONTROL THE FETAL PROGENITOR DYNAMICS AND DIFFERENTIATION DURING HUMAN STOMACH ORGANOGENESIS

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The transcriptional mechanisms that govern human stomach progenitor dynamics during organogenesis are not well understood. The transcription factor SOX2 is important in foregut patterning and stem cell pluripotency, but its temporal functions in the developing glandular stomach are not well characterized. The aim of this study is to test the hypothesis that fetal SOX2 is crucial for the establishment and maintenance of the fetal progenitor pool in the mammalian stomach and mechanisms

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that restrict SOX2 function are required for growth termination and differentiation. To address this hypothesis, we utilized genetic murine models and human pluripotent stem cells differentiated into 3-dimensional human gastric organoids (hGOs). Temporal ablation of SOX2 in the developing mouse significantly decreased gastric growth via premature cell cycle exit and differentiation. Tetracycline-inducible control of SOX2 levels correlated with the number of fetal progenitors in hGOs. The transcriptional repressor SOX21, a direct SOX2 transcriptional target, acts to counteract SOX2 function to limit the number of fetal progenitors. SOX21 is expressed throughout the developing stomach and its expression is maintained throughout adulthood. SOX21 gain- and loss-of-function in hGOs is inversely correlated with fetal progenitor number through its regulation of SOX2. SOX21 is necessary for the temporal expression of cell cycle inhibitors in the gastric epithelium. SOX21 plays a role in cell fate decisions by repression of the endocrine and acid-secreting cell lineages and promoting mucus differentiation. Mechanistically, SOX2 and SOX21 bind similar regions throughout the human genome and directly compete for common genomic loci controlling gastric patterning, signaling, cell cycle progression and differentiation in hGOs. Together, these results indicate that SOX2 and SOX21 levels dictate the molecular switch between proliferation and differentiation during mammalian stomach organogenesis.

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Keywords: *SOX, stomach, organoids*

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DEFINING HUMAN PLACENTA TROPHOBLAST STEM CELL FROM EARLY 3D HUMAN EMBRYOS IN SILICO TO ISOLATE INDUCED TROPHOBLAST STEM CELLS FROM NAÏVE PLURIPOTENT STEM CELLS IN VITRO

Puckett, Kenisha A.[1], Stockman, Courtney[1], Wang, Sicong[1], Viruet-Quintero, Olivia[2], Desai, Tushar[1], Nakauchi, Hiromitsu[1], Nusse, Roeland[1], Sebastiano, Vittorio[3]

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The human placenta is the first organ established and the primary site of nutrient exchange during pregnancy. A specialized population of stem cells called trophoblasts forms the architecture of the placenta. Current methods for culturing human trophoblast stem cells (TSC) in vitro have limited reproducibility and create heterogeneous trophoblast populations, causing any TSC to terminally differentiate. The distinct molecular features of TSC during development from a biologically relevant context remain undefined. A recent spatial transcriptomic study highlighted the insufficiency of current trophoblast markers (i.e., GATA3, TP63, TFAP2C) to pinpoint TSC because they are present across multiple trophoblast subtypes. Therefore, we defined when TSC originates in human development by utilizing publicly available single-cell transcriptomic sequencing data of three-dimensional human embryos from post-fertilization days six to fourteen. We analyzed biologically relevant stem cell pathway Wingless-integrated (Wnt), which is required for placenta organogenesis, to evaluate transcriptomic signatures of known pathway stem cell markers (i.e., TBX3, FOXM1, AXIN2) to identify TSC. Next, we used the in-silico examination to optimize a protocol for chemical induction of TSC from naïve pluripotent stem cells in vitro. Finally, we tested a list of surface markers (i.e., GREM2, S1PR2, NRP1) to purify induced TSC via a fluorescently activated cell sorting (FACS) isolation strategy. Our results show a population of FOXM1+AXIN2+ TSC arise on post-fertilization day seven. We examined four distinct clusters of trophoblast

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subtypes, including syncytiotrophoblast progenitors. We performed FACS isolation experiments to validate a list of novel TSC surface markers and trophoblast subtypes. In conclusion, a high-throughput bioengineering tool of TSC in vitro is vital for disease models to uncover potential mechanisms of placenta dysfunction and pave the way for future therapies in Maternal-Fetal Medicine to prevent adverse pregnancy outcomes.

Funding Source: California Institute for Regenerative Medicine

Keywords: *Trophoblasts, Placenta, Wnt*

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CHARACTERIZATION OF HUMAN PLURIPOTENT STEM CELL CHARACTERISTICS WITH NANO-RESOLUTION MICROSCOPY

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Human pluripotent stem cells (hPSCs) are fast becoming a key instrument in regenerative medicine. One of the main hurdles remaining unsolved is understanding their characteristics and regulation of pluripotency. Investigating hPSCs is essential for further understanding stem cell biology in detail, thus enabling personalized medicine. Until now, research shows limited research on what characteristics make hPSCs unique. To delve into the characteristics of hPSCs, the cells were cultured and fixed in specific terms to be imaged under different nano-resolution microscopy techniques. First, hPSCs were visualized by Transmission Electron Microscopy (TEM) to characterize their biological components. Then hPSCs were imaged with Scanning Electron Microscopy (SEM) to determine their cellular surface features. As a result of our total proteome and cell surface proteomic data with Stale Isotope Labelling by Amino Acids in Cell Culture (SILAC), we verified that hPSCs have specific proteins on the cellular surface. Imaging these proteins with nano-resolution microscopy techniques and measuring them provides tremendous significance in understanding the biology of hPSCs.

Keywords: *Imaging, Nano-resolution, Morphology*

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STRADA-MUTANT HUMAN VENTRAL FOREBRAIN ORGANOIDS MODEL MEGALENCEPHALY AND DELAYED INTERNEURONAL MATURATION IN PMSE SYNDROME

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Genetic diseases involving overactivation of the mechanistic target of rapamycin (mTOR) pathway are termed “mTORopathies,” characterized by malformations of cortical development, epilepsy, and cognitive impairment. Prior studies focused on the effects of mTOR hyperactivity on dorsal forebrain development, but how excessive activation of mTOR pathway causes ventral forebrain (VF) development remains unclear. This is important because the inhibitory interneurons, important in epilepsy and autism arise from the VF. We study PMSE syndrome (Polyhydramnios, Megalencephaly, Symptomatic Epilepsy), a rare mTORopathy caused by biallelic mutations in the STE20-related kinase adaptor alpha (STRADA) gene, an upstream inhibitor of mTOR. Using a STRADA-mutant iPSC model, we developed separately patterned human cortical organoids (hCOs) of PMSE with dorsal hCOs mostly containing excitatory neurons and ventral hCOs (vhCOs) generating inhibitory interneurons. Successful ventralization was confirmed by QPCR, immunostaining, and bulk RNA-seq. We examined morphology and size for the first 5 weeks of organoid growth. Starting from the second week, PMSE vhCOs enlarged more rapidly than controls, consistent with our prior findings on dorsal hCOs. This augmented expansion of MGE might be attributable to increased cell proliferation, as shown by increased Ki-67 level in PMSE vhCOs compared to controls. We further investigated cell fate specification at weeks 2 and 5. We found an

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upregulation of doublecortin and SOX2 expression with concomitantly downregulated Tuj1 and MAP2ab in PMSE vhCOs, indicating delayed neurogenesis in the VF. Together, these findings suggest that mTOR hyperactivity results in increased cell proliferation and delayed interneuron maturation in the VF, consistent with our prior conclusions from dorsal hCOs. These findings elucidate the mechanistic framework by which mTORopathies have altered excitatory and inhibitory neurogenesis at early stages of neurodevelopment.

Funding Source: This work was supported by NIH NS109289 from the Department of Pediatrics at Michigan Medicine (LTD)

Keywords: *mTOR, organoids, neurogenesis*

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PIONEER TRANSCRIPTION FACTOR FOXA AND PRDM1 GUARANTEE LINEAGE FIDELITY BY COORDINATING REPRESSIVE CHROMATIN STATE

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The pioneer transcription factors (TFs) regulate cell fate by establishing transcriptionally primed and active state. The repression of alternative lineage gene at specific timing is also important for proper differentiation. However, the regulatory mechanism for alternative lineage repression is poorly understood. In this study, we focused on the pioneer TF Forkhead box A (FOXA), which is required for endodermal lineage specification. We established two FOXA loss-of-function systems in human pluripotent stem cells (hPSCs): CRISPR interference (CRISPRi) for FOXA1/2/3 triple knockdown (TKD) and CRISPRd for blocking FOXA binding at its target enhancer. We differentiate these hPSCs into foregut endoderm (FG) and performed RNA-seq, ChIP-seq/qPCR, CUT&Run, and proteomics analysis to evaluate epigenetic mechanisms underlying FOXA-mediated gene regulation. We found that [1] FOXA prevents alternative-lineage and precocious liver gene expression during human FG differentiation; [2] FOXA cooperates with PR domain zinc finger 1 (PRDM1) to repress these genes by recruitment of repressive epigenetic complexes (Nucleosome Remodeling and Deacetylation [NuRD] complexes and Polycomb repressive complexes [PRC]) and establish bivalent enhancers; [3] FOXA binding is directly involved in the recruitment of PRDM1 and epigenetic repressors for gene repression. We propose that FOXA and PRDM1 coordinate to safeguard cell fate through epigenetic repression mechanisms.

Funding Source: Trustee Award, Pediatric Genomics Pilot Award, P30 DK078392, 1R01GM143161, JSPS, Postdoctoral Fellowship and Uehara Memorial Foundation, Postdoctoral Fellowship

Keywords: *Pionner-TF, Epigenome, CRISPR*

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EARLY DIFFERENTIAL IMPACT OF MECP2 MUTATIONS ON FUNCTIONAL NETWORKS IN RETT SYNDROME PATIENT-DERIVED HUMAN CEREBRAL ORGANOIDS

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Human cerebral organoids derived from induced pluripotent stem cells have attracted attention for their ability to recapture early human developmental processes. Mutations in the X-linked methyl-CpG binding protein 2 (MECP2) gene are associated with Rett syndrome and are widely believed to downregulate and upregulate numerous downstream genes, resulting in abnormal neuronal activity in mouse models. However, the early developmental changes and regression associated with MeCP2 mutation typically proceed in the absence of remarkable anatomical alterations. Here, we focused on neuronal activity during early developmental processes in Rett syndrome patient-derived organoids, analyzing two types of MeCP2 mutations (V247X and R306C) using calcium sensors with three-photon microscopy. To analyze functional connectivity in organoids, topological mathematical analysis based on graph theory was deployed in Ca2+ imaging data. In addition,

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we compared network structure. We found that highly abnormal neuronal activity in Rett organoids compared to isogenic controls and altered network structures are defined by local clustering coefficients and small-world propensity. Furthermore, transcriptome analysis revealed HDAC2-associated mitochondrial impairment in R306C organoids and decreased inhibitory receptors GABA in V247X organoids compared to each isogenic control pair. In addition, to recapture mosaicism of Rett syndrome, we engineer mosaic organoids by simply mixed with mutant and wildtype cells. Both functional and transcriptome assessments revealed differential impacts depending on the mutations and mosaicism, which may determine to disease severity. Our finding and analysis pipeline with brain mosaic organoid, imaging and metaethical analysis could have implications for understanding the underlying mechanisms of the Rett syndrome according to mutation-by-mutation and mosaicism and could open a new avenue to study the pathology of Rett syndrome.

Funding Source: NIH grant R01MH085802 and the Simons Foundation Autism Research Initiative (SFARI) through the Simons Center for the Social Brain (SCSB)

Keywords: *Organoid, Neuroscience, Imaging*

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GENERATION OF GASTRIC AND ESOPHAGEAL ORGANOIDS CONTAINING TISSUE RESIDENT MACROPHAGES TO MODEL DEVELOPMENT AND INFLAMMATORY DISEASES

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Organs of the gastrointestinal tract contain tissue-resident immune cells that function during development, homeostasis, and disease. Organoid models lacked an immune component but recently studies have now shown immune cells can be introduced into these in vitro systems. This opens the possibility to the study of the interactions of different cell populations with immune cells, including macrophages, during organ development. The embryonic development of tissue-resident macrophages within the human gastric organoids (HGOs) and esophageal organoids (HEOs) has not been described. This study aims to develop HGOs and HEOs model containing mesenchymal cells and functional tissue-resident macrophages to generate a better models for understanding development of tissue residency and inflammation for future therapeutics. We generated HGOs and HEOs by combining epithelium, splanchnic mesenchymal (SM) cells and macrophages separately derived through the directed differentiation of human pluripotent stem cells. Following two weeks of co-culture, the organoids were used for transcriptional profiling and functional analysis of macrophages. As a results, SM cells and mature macrophages were established into developing HGOs and HEOs and persisted for 2 weeks in vitro. The SM was necessary to incorporate macrophage without exogenous growth factors. These macrophages had a transcriptional signature that resemble those of the human fetal intestine, indicating that they were acquiring the features of tissue-resident macrophages. In conclusion, we generated an HGO and HEO system containing SM cells and tissue-resident macrophages, which can be used to investigate the molecular mechanisms involved in inflammatory disease, including gastritis.

Keywords: *macrophages, HGO, HEO*

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MODELING NEURODEVELOPMENT IN NURDOPATHIES USING FOREBRAIN ORGANOIDS DERIVED FROM PATIENTS WITH GATAD2B-ASSOCIATED NEURODEVELOPMENTAL DISORDER (GAND)

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The Nucleosome Remodeling and Deacetylase (NuRD) complex is a chromatin remodeling complex that regulates gene transcription during neurodevelopment. NuRD is composed of numerous paralogous subunits separated into a Histone Deacetylase Subcomplex (HDAC-core) and the Chromatin Remodeling Subcomplex (CRS), with GATAD2B acting as a

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protein bridge linking the two. NuRDopathies are neurodevelopmental disorders (NDDs) associated with NuRD deficiency caused by the haploinsufficiency of several different CRS paralogs. GATAD2B-associated Neurodevelopmental Disorder (GAND) is a NuRDopathy characterized by global delays, intellectual disability, apraxia of speech, macrocephaly, and distinct facies. GATAD2B is a keystone of NuRD complex structure, which makes GAND an ideal candidate for studying NuRDopathies. Our work investigated patient-derived GAND and control IPSC lines during neuronal differentiation. GAND-IPSCs were able to generate neural progenitor cells (NPCs) and cortical neurons that expressed haploinsufficient levels of mRNA and protein. Transcriptome analysis of GAND- versus CTL-IPSCs and -NPCs indicated GAND-NPCs had altered expression of 650 differentially expressed genes (DEGs) involved in gene ontogeny (GO) groups associated with neural cell proliferation, migration, and differentiation. GAND-neurons had altered in vitro temporal and quantitative expression of cortical laminar markers. Dorsal forebrain cerebral organoids (DFOs) were also evaluated for NPC, neuronal, and cortical laminar marker expression to characterize the expression and structural organization. Single-cell RNA sequencing (scRNA-seq) will also investigate the relative expression of various genes in these DFOs. Patient-derived IPSC-based cerebral organoids will enable the generation and perturbation of complex functional neurons in vitro, providing an unprecedented opportunity to decipher NuRD's roles in these processes. We anticipate our FO system will complement previous clinical GAND and Gand mouse research and further advance our understanding of how NuRDopathies affect neurodevelopment.

Funding Source: Support provided by the California Institute for Regenerative Medicine (CIRM) Scholar Grant [EDUC4-12751], Helping Hands for GAND Foundation Grant, and the Fashion Industries Guild Endowed Fellowships

Keywords: *NuRD, Neurodevelopment, Organoids*

TOPIC: INFECTIOUS DISEASES

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DISPARATE EFFECTS OF EBOLA AND LASSA VIRUSES ON PURIFIED HUMAN STEM CELL-DERIVED HEPATOCYTES

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Ebola and Lassa viruses are among the deadliest viruses on Earth and require biosafety level 4 (BSL4) containment. Many fundamental questions surround these Risk Group 4 viruses. What are their effects on human cells, and do different viruses exert distinct effects? Due to a paucity of physiologically relevant human models, cancer cell lines are extensively employed in BSL4 virology. To explore the effects of Risk Group 4 viruses on physiologically relevant human cells, we developed a hepatocyte model system compatible with BSL4 containment. To this end, we differentiated human pluripotent stem cells (hPSCs) into nearly-pure hepatocytes by devising a novel "metabolic selection" approach to selectively kill non-liver cells. Purified hPSC-derived hepatocytes more closely approximate primary human hepatocytes than liver cancer cell lines employed in BSL4 virology. Then, we showed that Ebola virus (a filovirus) and Lassa virus (an arenavirus) could infect human hepatocytes in vitro and non-human primate hepatocytes in vivo. Surprisingly, however, they led to starkly different mechanistic effects. Ebola virus extensively replicated and triggered the integrated stress response (ISR) and WNT pathways in human hepatocytes. By contrast, Lassa virus elicited transient effects and did not induce ISR or WNT signaling. This represents the first direct comparison of how different families of Risk Group 4 viruses affect their human target cells, thus addressing an important question in comparative virology. More broadly, our human hepatocyte model provides a new stem cell toolkit for BSL4 virology and could facilitate the discovery of viral mechanisms and candidate therapies.

Funding Source: Additional Ventures, California Institute for Regenerative Medicine, Robert Koch Institute intramural funds, Stanford Maternal and

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Child Health Research Institute, The Thomas & Stacey Siebel Foundation

Keywords: *Virus, differentiation, Metabolism*

TOPIC: MORPHOGENETIC MECHANISMS OF EARLY DEVELOPMENT

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IN-VIVO SINGLE NUCLEI RNA SEQUENCING CHARACTERIZATION OF HPSC-DERIVED PALLIAL INTERNEURONS POST-TRANSPLANT INTO ANIMAL MODELS

Zhou, Hongjun R., Bershteyn, Marina, Fuentealba, Luis, Subramanyam, Geetha, Sezan, Meliz, Maury, Yves, Banik, Gautam, Priest, Catherine, Nicholas, Cory

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Inhibitory GABAergic pallial interneuron cell therapy represents a promising strategy for the treatment of chronic neurological disorders characterized by local neural circuit hyperexcitability, like temporal lobe epilepsy (TLE). Pallial interneurons are derived from medial and caudal ganglionic eminences (MGE and CGE, respectively). MGE-derived pallial interneurons (MGE-pIN) are comprised of somatostatin (SST) and parvalbumin (PV) subclasses, both of which are lost and/or dysfunctional in the epileptic brain and represent promising targets for the development of cell replacement therapy; while some CGE-derived pINs may potentiate seizure activity. Thus, precise ON-target cell patterning and characterization of the graft composition are critical for assuring therapeutic safety and efficacy. We developed a GABAergic MGE-pIN cell therapy candidate derived from human embryonic stem cells (hESCs). Since human interneuron maturation is protracted over several years from pre- to post-natal brain development, we leveraged the growing number of public endogenous interneuron single cell transcriptomes to evaluate the MGE-pIN cell composition pre- and post-transplantation into animal models. Several independent manufacturing batches of cryopreserved hESC-derived MGE-pINs were transplanted into the mouse brain for up to 18 months to investigate grafted cell composition and subtype fate using Single nuclei RNA sequencing (snRNA-seq). The grafted human cells comprised SST and PV subtypes, without contamination from OFF-target cell lineages. The grafts persisted long-term with stable composition. Transcriptomic evidences of synapse formation and neurochemical maturation were found as early as the first month after transplantation. When compared with published human datasets, the hESC-derived MGE-pINs closely resemble endogenous MGE-type cortical interneurons. This study provides a deeper understanding of hESC-derived MGE pIN subtype composition, maturation, and fate fidelity.

Funding Source: CIRM Awards DISC2-10525; TRAN1-11611

Keywords: *cell-therapy, interneurons, epilepsy*

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INVESTIGATING HEPATIC HILAR DUCT ORGANOGENESIS AS NAVIGATED BY MAB21L2-EXPRESSING MESENCHYME THROUGH THE INHIBITION OF BMP4

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During early embryogenesis, the ventral foregut endoderm becomes specified into adjacent domains of the liver and extrahepatic biliary system (EHBD), which are connected at the hilar region. Hepatic specification and morphogenesis of hepatic endoderm is sustained by inductive interactions with splanchnic mesoderm. However, the exact mesodermal subtypes and their mechanistic role in supporting EHBD morphogenesis particularly at the level of hepatic hilum have yet to be established. Here we explored the developing EHBD system in mouse and human to study how regionalized mesenchyme exclusively pattern and distinguish the extrahepatic biliary system from the emerging liver with a future goal of developing a tubularized hepatic hilar duct from pluripotent stem cells. In a collaborative effort with Zorn and Wells lab, we performed single-cell

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transcriptomics of the mouse embryonic foregut and identified four unique mesenchymal cell populations associated with the hepatic-biliary (HB) region and predicted mesenchymal-epithelial foregut signaling pathways that coordinate HB organogenesis. We focused on the mesenchymal cell population expressing Mab21l2 which has been implicated in specifying the extrahepatic biliary system region from the developing liver in vivo. Our single-cell sequencing data showed that Mab21l2-expressing cells are a subpopulation of Msx1+ mesenchyme and lack BMP4 expression that is essential for liver progenitor specification while expressing TGFB that is necessary for biliary specification. Furthermore, we found that Mab21l2 overlaps with genes regulating cell contraction (Pitx2, Ltbp1, Yap1) suggesting that Mab21l2-expressing mesenchyme condenses to promote biliary cell differentiation and eventual tubularization into the hilar duct. These data suggest that Mab21l2+ mesenchyme may play an influential role in hilar duct structural development. Understanding this process will have major implications for the advancement of humanized models such as liver organoids that lack a hilar duct structure ultimately toward the understanding of cholangiopathies and the development of regenerative therapeutics.

Funding Source: This work was supported by Cincinnati Children's Research Foundation grant, NIH Director's New Innovator Award (DP2 DK128799-01), and the Falk Transformational Awards Program

Keywords: *morphogenesis, hepatobiliary, mesenchyme*

TOPIC: MOVING INTO THE FUTURE WITH EMERGING TECHNOLOGIES

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THE INDUSTRIALIZED SCALED PRODUCTION OF IPSC-DERIVED PANCREATIC ISLET-LIKE AGGREGATES

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Pluripotent stem cells (PSC) are a promising source for in vitro generation of the specialized cell types needed for the emerging field of regenerative medicine. However, to reach this potential scaled production of runs consisting of 1E9–1E11 cells per batch are needed. A specialized cell type of much clinical interest is the insulin producing beta-cell of the pancreas which has been shown to successfully reverse the clinical implications of type 1 diabetes (T1D). Using an unbiased systems biology approach, High-Dimensional Design of Experiments (HD-DoE), we extracted the critical process parameters of a novel differentiation protocol able to generate pancreatic endocrine cells. To deliver clinically meaningful numbers of cells, scaled production was attempted. Where protocol development mainly focuses on the biological aspects of the cells, scaled production requires process understanding related to the reactor system used. Seeking to attain reproducibility, robust differentiation and purity, careful consideration of in-process and out of process quality measurements which function as pass/fail criteria need to be determined. For instance, process parameters measured in- process need not only to be indicative of the current state of the biological product, but equally importantly are predictive of the final product's quality attributes. This is a hallmark of directed differentiation where early deviations in a compound matter impact the final output. As a learning principle, we emphasize that careful definition of intermediary quality attributes as they relate to the final product should be considered at every step of the process of manufacturing a TEMP product.

Keywords: *Scale-up, Beta-cell, Bioreactor*

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GCKR-RS1260326:C>T VARIANT DISRUPTS THE METABOLIC ADAPTATION AND RESTORATION AGAINST FASTING-REFEEDING TREATMENT IN HUMAN LIVER ORGANOIDS

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Intermittent fasting (IF) holds promise as a treatment for Metabolic disorder Associated Steatotic Liver Disease (MASLD). Cellular responses to IF encompass metabolic adaptation and restoration (MAR) in the nutrient fluctuations. MAR is thought to underlie the mechanism of IF effects, yet inter-individual variations in MAR have been reported. Glycolysis is rate-limited by the interaction of glucokinase and GKRP, coded by GCKR gene. Besides GCKR-rs1260326:C>T contributes to MASLD pathology, our transcriptome analysis unveiled a difference in cellular response to starvation between GCKR-CC and TT genotypes. However, the impact of this variant on the reaction to IF through alterations in MAR remains unclear. Here, we aimed to replicate MAR in human liver organoids (HLOs) and ask whether IF could be beneficial for individuals with the GCKR variant. HLOs were generated from iPSCs harboring the GCKR variant through haplotype editing and cultured for 25 days in hepatocyte culture medium (HCM). On day 26, the medium was switched to a fasting medium, comprising HCM diluted with PBS and supplemented with lactate and pyruvate. After 24 hours, the medium was reverted to HCM to refeed the HLOs. Steatotic lipid accumulation was induced by 3 days of culturing with sodium oleate. RNA was extracted from HLOs at baseline, after 24 hours of fasting, and after 24 hours of refeeding. Confocal imaging was conducted using Bodipy lipid dye and CellRox oxidative stress dye. Fasting upregulated mRNA expression of genes related to gluconeogenesis and fatty acid oxidation, while downregulating genes related to glycolysis and lipogenesis in GCKR-CC HLOs. Refeeding restored these changes to baseline. Lipid particles persisted in steatotic GCKR-CC HLOs, mimicking fasting-induced steatosis, but refeeding reduced them. Conversely, fasting and refeeding failed to induce similar gene expression changes in GCKR-TT HLOs. Moreover, even after refeeding, GCKR-TT HLOs maintained an equivalent amount of lipid compared to the fasting condition, accompanied by increased CellRox intensity. Our findings suggest that GCKR-CC HLOs exhibit physiological MAR, whereas GCKR-TT HLOs do not. This implies that individuals with the GCKR-TT allele might not benefit from IF. Further studies using GCKR humanized mice are planned to verify this hypothesis.

Keywords: *MASLD, Fasting, Personalization*

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GROWTH FACTOR PRODUCTION FOR ENHANCED GROWTH OF FISH INDUCED PLURIPOTENT STEM CELL CULTIVATION

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The growth factor fibroblast growth factor 2 (FGF2) regulates a variety of cellular functions, including cell proliferation, differentiation, and migration. As such, FGF2 is commonly used to maintain and promote the growth of induced pluripotent stem cells (iPSCs) in culture. FGF2-G3, a recently developed mutation of the native FGF2 sequence, further improves FGF2's stability and potency at typical cell culture temperatures, reducing protein usage and need for medium changes in cell passaging schedules. But while human FGF2 has been extensively studied in the context of mammalian cell culture, its performance in the culture of fish-derived cells has not, and nor has the comparative performance of FGF2 native sequences derived from fish species. Such data would be valuable in enabling fish stem cell culture for basic research, with clear applications in veterinary medicine and cell-cultivated meat. We hypothesized that FGF2 proteins derived from various species of fish will better facilitate the growth of fish stem cells while decreasing off-target differentiation, compared with native human FGF2. FGF2 protein was expressed recombinantly in E. coli, purified, and evaluated for thermostability

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(via a SYPRO Orange thermal shift assay) and function (via 3T3 cell proliferation). The resulting data highlight the effect of fish sequence change on protein stability and performance in mammalian cell culture, with further studies pending that utilize proliferating fish cells to evaluate changes in functional effect.

Keywords: *FGF2, Protein, cell-cultivated*

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A CUBE-IN-A-CHIP PLATFORM TO LOCALISE SIGNALLING MOLECULE SOURCE, MIMICKING THE NOTOCHORD DURING FORMATION OF NEURAL TUBE ORGANOID

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Interdisciplinary integration of engineering into stem cell and organoid research brings us closer to recapitulating the complex human system in vitro. Microfluidic devices simulate physiological flow of blood, 3D printing and fabrication enables the recreation of intricate tissue structures, and biomaterials provide the scaffolding on which cells grow and organise. However, there is often a mismatch in expectations of ease of use and outcome between biologists and engineers the solutions designed by engineers are not always easy to use by non-specialists and may not be compatible with cells that are very sensitive to external stimulation. Here, we introduce the concept of creating complexity from a simple CUBE culture device, which comprises a hard frame and a supporting hydrogel. As the cells are contained in the CUBE, they can be safely and easily transferred to a fluidic chip device where the external environment can be controlled. Gradients of signalling molecules are important cues that guide the differentiation and self-organisation of cells into forming a tissue or organ. We have previously shown that a single iPSC spheroid in the CUBE can be differentiated to two separate localized regions by culturing the CUBE in a gradient chip with two separate differentiation media on opposing sides of the CUBE. In this work, we present the control of morphogen signal source position by limiting the diffusion of signalling molecule to a specific region of cells. We then utilised this platform to recapitulate the formation of neural plate to a neural tube organoid with dorsal-ventral axis via controlled Shh signalling from a localized source (mimicking the notochord). The platform developed here provides a simple and modular method to control the differentiation of complex organoids with relevant body axis information. This can not only lead to the generation of more relevant organoids, but also help in understanding the formation mechanisms involved in development.

Funding Source: JSPS Grant-in-Aid for Early-Career Scientists

Keywords: *Gradient, Notochord, NeuralTube*

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A FULLY AUTOMATED HIGH-THROUGHPUT PLATFORM FOR EFFICIENT DEVELOPMENT AND MAINTENANCE OF HUMAN LIVER ORGANOIDS (HLO) FOR DRUG TOXICITY ASSESSMENT

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Drug-induced liver injury (DILI) poses a significant challenge in drug development, contributing to clinical trial failures and market withdrawals. Identifying agents with the potential to cause DILI before launch is crucial for the pharma- and medical community. Previous efforts demonstrated that Human Liver Organoids (HLOs) faithfully replicate the intricate architecture and (patho)physiology of the human liver. Despite these advancements, HLO-based screening models face limitations due to the need for multiple plate replicates and the use of typical 96-well dishes, resulting in heterogeneous HLO development. The biological complexity of organoid cultures further hampers automation and large-scale image-based screening applications, hindering their broad implementation in industrial-scale compound testing. In this study, we introduce a fully automated, high-throughput screening (HTS)-compatible workflow for generating, maintaining, and optically analyzing HLOs in 96-well hydrogel-based microcavity arrays (Gri3D, Sunbiosciences). Our centralized automation system features an

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automated CO2 incubator (StoreX STX110, LiCONIC), a liquid handler (BioMek i7, Beckman Coulter), and a confocal high-content imager (ImageXpress Confocal, Molecular Devices). By combining guided aggregation of single-cell progenitors in Gri3D microcavity arrays with fully automated workflows, we achieve highly homogeneous HLO cultures in terms of morphology and size with significantly increased throughput compared to standard HLO generation methods. In addition, our real-time image processing, facilitated by deep learning-based algorithms developed a priori, streamlines data analysis. The integration of Gri3D plates with high-throughput automation-based HLO development enables rapid, repeated HLO generation, facilitating screening studies. This scalable HLO culture technology, coupled with a fully automated HTS workflow, allows for the assessment of drug effects at the single-organoid level. This includes multiplexed evaluation of liver health and function through high-content image analysis and quantification of soluble liver injury biomarkers. Overall, this innovative approach brings us closer to realizing the full potential of organoids in drug development and precision medicine.

Keywords: *Organoid, Liver, Automation*

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IN VITRO ORGANOID MODELING OF COMPLEX INTERACTIONS INFLUENCING EPITHELIAL CELL FATE IN HUMAN LUNG ALVEOLI

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The alveoli perform the critical function of gas exchange in the human lung that ensures the survival of all our tissue. The alveolar region consists of unique cells such as the alveolar type II pneumocytes (AT2) that are multifunctional surfactant-producing stem cells, and alveolar type I pneumocytes (AT1), which are long, thin cells that exchange gas with capillary endothelial cells. During normal lung homeostasis, and injury-repair, AT2 proliferate and differentiate into AT1 to populate the alveoli, however, the cell-cell interactions and cues that govern the AT2-to-AT1 transition are poorly understood. Current in vitro organoid models of the human alveoli do not adequately allow us to interrogate cell-cell interactions and signaling events that maintain homeostasis or support regeneration, as they have limited co-culture compatibility, and do not reflect the aerated, oxygen rich environment of the alveolus. To overcome these limitations, I have advanced several approaches that will allow me to manipulate alveolar complexity in a modular fashion to interrogate cell-cell interactions, extracellular matrix, biochemical signaling, and biophysical cues. These include developing co-culture systems, and interrogating new growth environments, such as epithelial-immune co-culture, epithelial-endothelial co-culture, and hanging-drop organoid models of the alveolus. My preliminary data shows increased AT2 maturation in all three culture systems compared to controls, and increased markers of AT1 fate in endothelial and hanging drop culture methods, along with AT1 morphology in the hanging drop method. This work lays the foundation to interrogate complex interactions in the human alveoli, and represents a substantial step towards a more comprehensive in vitro model of the alveolus.

Funding Source: NIH T32 GM145470

Keywords: *organoid, lung, co-culture*

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