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Welcome

Dear Friends and Colleagues,

On behalf of the Children’s Hospital of Philadelphia and University of Pennsylvania Research Hubs of the NHLBI Progenitor Cell Translational Consortium (PCTC), we warmly welcome you to the 4th Annual PCTC meeting. While we had hoped to host you in-person to explore our campus and the city of Philadelphia, we have organized this virtual meeting to provide an ongoing forum to promote collegiality and collaborative interaction, as PCTC investigators review the past year’s progress and establish future plans in these truly historic times.

We hope the meeting will provide a stimulating environment for all investigators, post-doctoral fellows and trainees to have lively discussions that will catalyze progress and future collaborations. This year’s agenda is a day and a half, with a Trainee’s session organized by John Leach (University of Pennsylvania), Terren Niethamer (University of Pennsylvania), Leonardo Cardenas, (University of Pennsylvania), Asher Kahn-Krell (University of Alabama), Danielle Pretorius (University of Alabama), Eric Zhang (University of Alabama,) and Yang Zhou (University of Alabama), where trainees will have the opportunity to present and interact with one another. The main meeting session will allow additional trainees and junior investigators to present their research in the Plenary sessions, followed by breakout sessions comprising 35 abstracts. We will also welcome two exceptional guest speakers, Drs. Katherine High and Alan Flake, who will discuss the translational aspects of their research foci and successfully bringing their projects to the FDA and to industry. Highlighting the translational nature of our consortium, we will close the day with a special session on COVID-19 research from PCTC investigators.

We are extremely grateful to Michael Terrin and his team at the University of Maryland School of Medicine for their tireless efforts in getting this meeting organized, pre-recording presentations, and becoming expert Zoom navigators. We very much hope to see you in person for our 2021 meeting!

Best wishes,

Stella Chou, MD
Associate Professor of Pediatrics
University of Pennsylvania School of Medicine
The Children’s Hospital of Philadelphia

Deborah French, PhD
Director, Human Pluripotent Stem Cell Core
The Children’s Hospital of Philadelphia
Professor, Pathology and Lab Medicine
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Paul Gadue, PhD
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Edward Morrissey, PhD
Professor of Medicine, Cell & Developmental Biology
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University of Pennsylvania School of Medicine

https://translationalcells.org
Agenda

Trainees Only Session
September 21, 2020, 1:00-5:00 PM, EDT

Link: https://us02web.zoom.us/j/81864257520?pwd=djM3VW9ZalZsdHBwdTFIYoFGQ2RCZ209
Meeting ID: 818 6425 7520
Passcode: 016059

Monday, September 21, 2020

1:00 PM  Welcome/Opening Remarks
  • Leonardo Cardenas, John Leach, Terren Niethamer

Research Reports

1:05 PM  Abstract #1: Chapman Research Hub 01
  • Regulation of Type 2 Alveolar Epithelial Cell Reprogramming by Transforming Growth Factor Beta
    o Max Cohen

1:25 PM  Abstract #2: Chou Research Hub 02
  • A Doxycycline-inducible GATA1 Expression System in Human iPSCs
    o Sit Ying Ting

1:45 PM  Abstract #3: Daley Research Hub 03
  • CellComm: A Cell Communication and Signaling Platform to Unravel HSC Emergence
    o Caroline Kubaczka

2:05 PM  Abstract #4: Snoeck Research Hub 04
  • Imaging Human Lung Organoids with Light Sheet Microscopy
    o Remy Schneider

2:25 PM  BREAK

2:55 PM  Abstract #5: Thomson Research Hub 05
  • Direct Induction of Hemogenic Endothelium and Blood from Human iPSCs Using ETV2 Modified RNA
    o Kran Suknuntha

3:15 PM  Abstract #6: Whitsett Research Hub 06
  • Patient-Specific iPSCs Carrying an SFTP C Mutation Reveal the Intrinsic Alveolar Epithelial Dysfunction at the Inception of Interstitial Lung Disease
    o Kostas Alysandratos

3:35 PM  Abstract #7: Zhang Research Hub 07
  • Mimicking the Myocardium One Layer at a Time: A Thick, Bi-Layered Stem Cell-Derived Patch for Cardiac Tissue Regeneration
    o Danielle Pretorius

Special Talk/ Collaborative Opportunities

3:55 PM  Panel presentations and discussion on career development and laboratory start-up with COVID-19 response restrictions
  • Brittany Taylor, Ph.D.
  • Samantha Brugmann, Ph.D.
  • Jarod Zepp, Ph.D.

4:55 PM  Concluding Remarks
  • Leonardo Cardenas, John Leach, Terren Niethamer
Abstracts
ABSTRACT #1

Regulation of Type 2 Alveolar Epithelial Cell Reprogramming by Transforming Growth Factor Beta

Max L. Cohen*, Jaymin Kathiriya, Alexis Brumwell, Tsung-che Ho, Harold A. Chapman
University of California, San Francisco, CA

In organoid co-culture with mesenchymal cells, human lung type 2 alveolar epithelial cells (AEC2s) maintain stable cell fate when co-cultured with embryonic fibroblasts. However, when co-cultured with primary adult lung fibroblasts, human AEC2s reprogram to a basal-cell-like state, characterized by a loss of surfactant protein C (SPC) expression and by new expression of cytokeratin 5 (KRT5). The dependence of AEC2 reprogramming on the source of co-cultured fibroblasts demonstrates a critical effect of mesenchymal signaling. We hypothesize that transforming growth factor beta (TGFβ) may be an important regulator of this reprogramming, as TGFβ is known to be important in alveolar epithelial responses to injury as well as broadly in development and tissue homeostasis. To test this hypothesis, the phenotype of AEC2s was examined during organoid coculture in the presence of TGFβ signaling blockade. SB431542 is a small-molecule inhibitor of ALK4, ALK5 and ALK7, which are required for TGFβ receptor intracellular signaling. While inhibition of TGFβ signaling with SB431542 did not impair induction of KRT5 expression in AEC2s, it did attenuate the loss of SPC expression otherwise seen in this system (see accompanying figure; scale bar is approximately 50 μM). This result indicates that TGFβ has an important role in regulating AEC2 fate. During reprogramming towards basal-cell fate, adoption of basal-cell phenotype (such as expression of KRT5) may be independent of TGFβ signaling, but TGFβ signaling may have a crucial role in controlling exit from the AEC2 cell phenotype. Given the central importance of AEC2s in lung homeostasis, recovery from injury, and chronic fibrotic lung disease, the mechanisms underlying this finding may be of broad significance.
ABSTRACT #2
A Doxycycline-inducible GATA1 Expression System in Human iPSCs

Ying Ting Sit*, Rachel Helton, Hyun Hyung An, Deborah L. French, Paul Gadue, Stella T. Chou
Children's Hospital of Philadelphia, Philadelphia, PA

Introduction: GATA1 is a key transcription factor for normal development of megakaryocyte erythroid progenitors (MEP), and the terminal maturation of erythrocytes and megakaryocytes. In murine embryonic stem cells, a doxycycline (dox)-regulated shGATA1 system demonstrated that GATA1 knockdown led to an expansion of MEPs that retained the capacity to differentiate into functional erythroid and megakaryocytes after re-expression of GATA1 during hematopoietic differentiation. In this study, we used CRISPR/Cas9-mediated genome editing to construct a doxycycline-regulated GATA1 expression system in human induced pluripotent stem cells (iPSC). Our goal was to determine whether GATA1 knockout would result in MEP proliferation from human iPSCs, and whether re-introduction of endogenous GATA1 in these progenitors could produce mature megakaryocytes and erythrocytes.

Methods: A LoxP-Stop-LoxP transgene with a splice acceptor (SA) and hygromycin (Hygro) selection cassette was inserted into intron 3 of the GATA1 locus via CRISPR/Cas9 technology (Figure), and a dox-regulated Cre recombinase expression cassette was introduced into the AAVS1 locus of the iPSCs. In the absence of doxycycline, a non-functional GATA1 protein would be expressed, whereas doxycycline treatment would induce excision of the stop codon and allow endogenous GATA1 expression. Hematopoietic differentiation of targeted clones by embryoid body formation was performed with and without the addition of doxycycline.

Results: Hematopoietic differentiation of targeted clones demonstrated GATA1 knockout decreased the absolute progenitor cell number and abolished erythroid potential. In contrast, GATA1 knockout progenitors displayed a 5-fold increase in megakaryocytes compared to untargeted cells in the presence of thrombopoietin and stem cell factor. Doxycycline treatment starting on days 3, 5, or 8 during embryoid body differentiation restored endogenous expression of GATA1. Addition of doxycycline at D3 also restored progenitor cell production and erythroid potential, whereas doxycycline treatment starting on D5 or D8 showed decreased progenitor cells and erythroid maturation similar to GATA1 knockout in dose-dependent manner.

Conclusion: We successfully constructed a dox-inducible GATA1 expression system in human iPSCs. Our findings suggest that GATA1 disruption biased hematopoietic differentiation potential toward megakaryopoiesis, providing a potential strategy to enhance megakaryocyte production.
ABSTRACT #3

CellComm: A Cell Communication and Signaling Platform to Unravel HSC Emergence

Caroline Kubaczka*, Edroaldo Lummertz da Rocha, Wade Sugden, Mohamad Najia, Trista North and George Daley
Boston Children’s Hospital, Boston, MA

While the generation of hematopoietic progenitor cells from pluripotent stem cells (hiPSCs) can readily be accomplished in a dish, transgene free generation of long term reconstituting hematopoietic stem cells (HSCs) remains a major challenge to the field. In order to recapitulate definitive hematopoiesis in vitro, we need a better understanding of the developmental processes occurring during HSC emergence in vivo. Despite having identified the origin of HSCs to be specialized endothelium, termed hemogenic endothelium, we have yet to fully characterize all distinct cell types which may play a role during the process of endothelial-to-hematopoietic transition. Here, we have utilized single cell RNA sequencing to profile the aorta-gonad-mesonephros (AGM) region on embryonic day (E)10.5, just prior to the first emergence of HSCs. In addition to capturing endothelial and nascent hematopoietic stem cells, we also included cells of the surrounding embryonic tissue, allowing us to computationally predict cell-cell interactions based on ligand and receptor expression using our novel algorithm CellComm. This approach faithfully identified previously described regulators of HSC emergence, i.e. the recently published surface antigen CD44, while also predicting additional signaling pathways, which have not been implicated in HSC development so far. Using a morpholino based knockdown screening approach in zebrafish, we tested the effect of the predicted target ligand and receptor interactions on the presence of runx1+/cmyb+ cells in the aorta. Following this approach, we were able to identify novel modulators of HSC emergence in the AGM with conserved roles in zebrafish, mouse and human hematopoiesis. In addition, CellComm is able to predict putative transcriptional regulators downstream of cell surface receptors expressed on type 1 pre-HSCs.

In conclusion, the implementation of the transcriptional and cell-cell communication findings will aid enhancement of hiPSC-derived hematopoietic differentiation protocols toward the production of functionally competent HSCs.
ABSTRACT #4

Imaging Human Lung Organoids with Light Sheet Microscopy

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Organoids are in vitro three-dimensional (3D) organ-like cultures derived from pluripotent or adult stem cells that at least partially recapitulate anatomical and functional features of in vivo tissues. Organoid-based models have proven valuable for studying the dynamics of organ development and to model disease, in a controlled and tunable environment, modelling human development with greater precision than mouse genetic models in vivo. The Snoeck lab reported previously the generation from hPSCs of lung bud organoids (LBOs) in suspension culture that contain mesoderm and pulmonary endoderm and develop into branching airway and early alveolar structures after xenotransplantation and in Matrigel 3D culture that allowed the modeling of pulmonary fibrosis associated with Hermansky-Pudlak syndrome as well as infection with human respiratory viruses. Lung organoids undergo branching morphogenesis and can grow larger than 3 mm. Identifying the precise location of cells and of their effect on maturation cannot be reliably assessed using conventional fluorescence or confocal microscopy. We therefore developed an approach to clear and image intact lung organoids using light sheet fluorescence microscopy (LSFM). LSFM allows 3D imaging of larger structures with a low dwell time of the light sheet at each plane. This reduces the number of photons required and therefore limits phototoxicity. To achieve LSKM imaging, lung organoids are embedded in an acrylamide-based hydrogel to retain the morphology of fragile branches, depleted of light-scattering lipids, and refractive index-matched to attain translucency. Proximodistal specification was for example observed by evaluating expression of collagen IV surfactant protein C, a marker of alveolar epithelial type II cells (figure), and SOX2/SOX9 (not shown). This methodology will greatly enhance our ability to study human lung development and model lung disease.
Direct Induction of Hemogenic Endothelium and Blood from Human iPSCs Using ETV2 Modified RNA


University of Wisconsin-Madison, Madison, WI

Previously, we demonstrated that overexpressing transcription factors ETV2 and GATA2 is sufficient to induce a pan-myeloid program in human induced pluripotent stem cells (hiPSCs), which proceeds through a hemogenic endothelium (HE). Although we have found that constitutive overexpression of ETV2 using lentiviral vectors induces predominantly non-HE, we also noted that ETV2 induces GATA2 expression in hPSCs and very few HE with macrophage potential. In addition, our recent studies suggest that molecular mechanisms upstream of GATA2 are sufficient to specify hematoendothelial program in hPSCs, while GATA2 is required for endothelial-to-hematopoietic transition. Given these findings, we explored whether transitional expression of ETV2 with modified mRNA (mmRNA) alone is sufficient for hematoendothelial programming in hiPSCs. Here, we showed that a single delivery of ETV2 synthetic mmRNA is sufficient to generate endothelial cells that have limited expandability and blood-forming activity from hPSCs. Hematopoietic cells derived from ETV-induced HEs reveal typical immunophenotype of CD34+, CD117+ hematopoietic stem/progenitor cells and are able to form cells producing hematopoietic colonies in a methylcellulose medium. We also found that culture of ETV2-induced HE in the presence of GM-CSF, FGF2, and UM171 led to continuous production of generous amounts of CD34+CD33+ myeloid progenitors that could be harvested every 8–10 days for up to 30 days of culture. Subsequently, myeloid progenitors were differentiated into neutrophils in the presence of G-CSF and the retinoic acid agonist Am580 or macrophages in presence of M-CSF and IL3 or IL-1β and M-CSF. Neutrophils obtained in these conditions displayed a typical somatic neutrophil morphology, produced reactive oxygen species, formed neutrophil extracellular traps, and possessed phagocytic and chemotactic activities. Generated monocyte/macrophages possessed typical macrophage phenotype and morphology and demonstrated invasive migration in gelatin. Overall, this technology is suitable for generating functional neutrophils and macrophages from iPSCs to interrogate the role of genes in a myeloid cell development and function when coupled with genetic engineering technologies.
ABSTRACT #6

Patient-Specific iPSCs Carrying an SFTPC Mutation Reveal the Intrinsic Alveolar Epithelial Dysfunction at the Inception of Interstitial Lung Disease


Boston University School of Medicine, Boston, MA

The incompletely understood pathogenesis of pulmonary fibrosis (PF) and lack of reliable preclinical disease models have been major hurdles in developing effective therapies. An emerging literature now implicates alveolar epithelial type 2 cell (AEC2) dysfunction as an initiating pathogenic event in the onset of a variety of PF syndromes, including idiopathic pulmonary fibrosis (IPF). Mutations in surfactant protein C (SFTPC), an AEC2-restricted gene, have been associated with both sporadic and familial PF. Elucidating the molecular pathogenesis of AEC2 dysfunction caused by these mutations is likely to inform the broader mechanisms by which AEC2 dysfunction leads to IPF. As access to primary AEC2s is limited and these cells are difficult to maintain in culture, we sought to engineer an in vitro disease model utilizing patient-specific induced pluripotent stem cells (iPSCs). We generated iPSC lines expressing the most common SFTPC mutation (SFTPCI73T) and their gene-edited, corrected counterparts. Through directed differentiation to alveolar epithelium, we derived mutant and corrected AEC2s (iAEC2s) which were able to expand >10^20 fold in vitro. SFTPCI73T expressing iAEC2s accumulated large amounts of misprocessed pro-SFTPC protein which mistrafficked to the plasma membrane, similar to the donor patient’s in vivo AEC2s. These changes resulted in a time-dependent late block in autophagic flux, accumulation of dysfunctional mitochondria with consequent metabolic reprogramming of mutant iAEC2s from oxidative phosphorylation to glycolysis, and marked alterations in self-renewal capacity. Treatment of SFTPCI73T expressing iAEC2s with hydroxychloroquine, a medication commonly prescribed to these patients, resulted in aggravation of autophagy perturbations and metabolic reprogramming. Thus, PSC-derived AEC2s provide a patient-specific preclinical platform for modeling the intrinsic epithelial dysfunction associated with the inception of pulmonary fibrosis.
**ABSTRACT #7**

*Mimicking the Myocardium One Layer at a Time: A Thick, Bi-Layered Stem Cell-Derived Patch for Cardiac Tissue Regeneration*

Danielle Pretorius*, Asher M. Kahn-Krell, Wesley C. LaBarge, Xi Lou, Ramaswamy Ramaswamy, Andrew Pollard, Vladimir Fast, Joel Berry, Alan W. Eberhardt, Jianyi “Jay” Zhang

University of Alabama at Birmingham, Birmingham, AL

**Introduction:** To avoid mechanical mismatch in tailor-made engineered tissues, in-depth characterization of these tissues and materials is vital. Generating a functional myocardium, for use in tissue regeneration post-myocardial infarction or to use as a drug-testing platform, requires certain minimal considerations. In the current study, we present a novel method for fabrication of thick (~2 mm) viable cardiac tissues using human induced pluripotent stem cells (hiPSCs), along with the characterization of these tissues.

**Materials and Methods:** Easily scalable (2x1 cm²) cardiac patches were fabricated via layer-by-layer deposition technique, using hiPSC-derived cardiomyocytes (iCMs) and -endothelial cells (iECs). Cells were suspended in a fibrin extracellular matrix (ECM) and deposited into the desired alternating layers. Patches were cultured for 7–28 days, during which time histology, viability and changes in cellular expression were monitored. Stress relaxation testing in compression was done using a Low Force Testbench (TA Instruments, New Castle, DE) with a 250 g load cell, where a 10% strain (ε) was applied and held for 3 minutes on each sample. Stress relaxation responses were characterized using a generalized Maxwell model of order 4 in MATLAB (). Fresh mouse left ventricle (LV; n = 5) samples were used as controls and all samples were tested under submerged conditions in PBS.

**Model:**

\[
\sigma(t) = \varepsilon E_0 + \varepsilon E_1 \left( e^{\frac{-t}{\tau_1}} \right) + \varepsilon E_2 \left( e^{\frac{-t}{\tau_2}} \right) + \varepsilon E_3 \left( e^{\frac{-t}{\tau_3}} \right) + \varepsilon E_4 \left( e^{\frac{-t}{\tau_4}} \right)
\]

Eq. 1

**Instantaneous Young’s modulus (Pa):**

\[
E_{\text{instant}} = E_0 + E_1 + E_2 + E_3 + E_4
\]

Eq. 2

**Equilibrium Young’s modulus (Pa):**

\[
E_{\text{eq}} = E_0
\]

Eq. 3

**Viscosity (Pa·s):**

\[
\eta_1 + \eta_2 + \eta_3 + \eta_4
\]

Eq. 4

**Results and Discussion:** The generalized Maxwell model of order 4 yielded high R²-values for all samples (0.7 < R² < 0.98). Patch elasticity moduli reported in this study ranged between ~4.7-5.8 kPa, while the control mouse samples were ~6.4 kPa. Minimal necrosis was noted (< 5.4%).

**Conclusions:** The use of a novel, bottom-up fabrication method allowed for production of thick, functional cardiac patches that can be used not only for tissue regeneration, but also as drug-testing platforms. Mechanical testing confirmed that the engineered tissue demonstrated viscoelastic properties, and that culturing conditions allowed for maximal cell vitality even within extremely thick structures. Increased fluid-like properties, vascularization and culturing conditions may be key contributing factors allowing for enhanced transport capabilities in these engineered tissues. Future studies will include *in vivo* testing in severe combined immune deficiency (SCID) mice to assess the long-term effects of these engineered myocardial tissues.