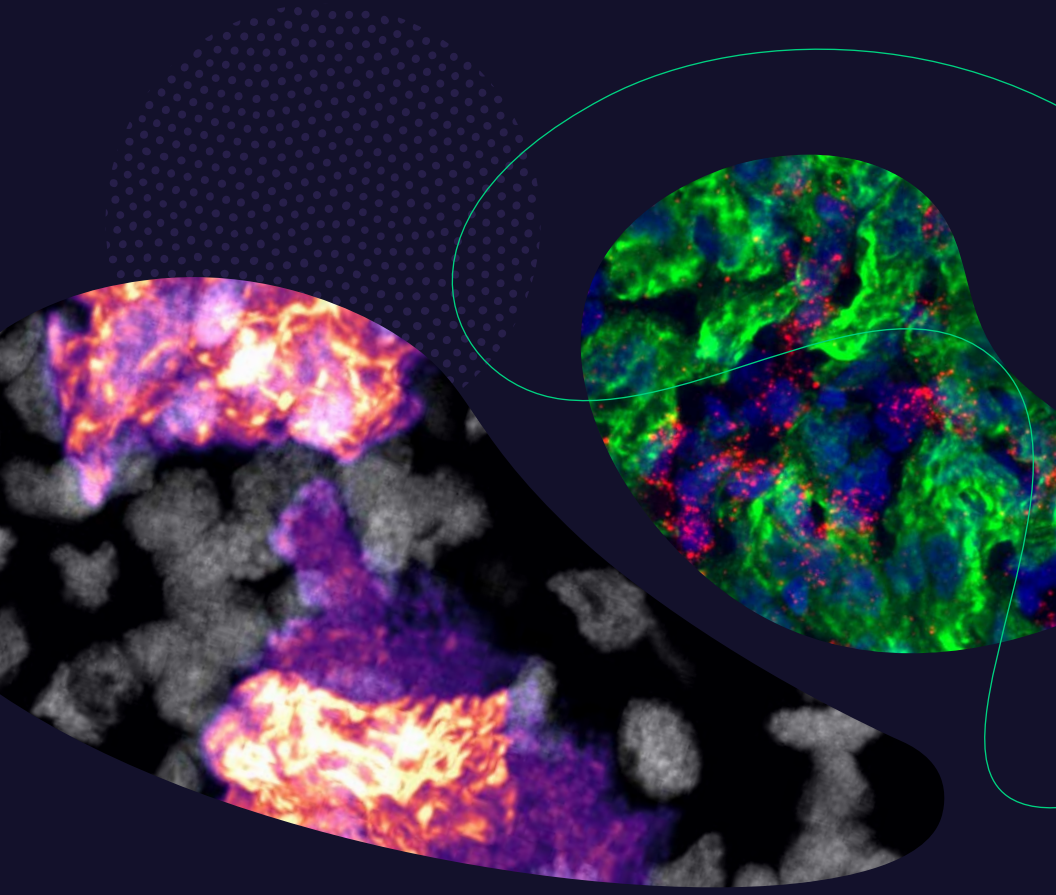
Stowers Research Conferences

Stowers Institute

Stem Cells, Tissue Plasticity, and Regenerative Biology

OCTOBER 11 - 13 | STOWERS INSTITUTE | KANSAS CITY, MO



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Conference Program

Invited Speakers and Trainees
Speakers Chosen from Abstracts

All talks will be presented in the Stowers Auditorium

WEDNESDAY, OCTOBER 11

2:00 PM - 3:00 PM	**CONFERENCE REGISTRATION, ORIENTATION & POSTER SET-UP**
3:00 PM - 3:15 PM	**OPENING REMARKS**

Session 1: STEM CELL DYNAMICS

3:15 PM - 4:10 PM	**KEYNOTE:** **Allan Spradling & Haolong Zhu (Graduate Student)** **Carnegie Science** *"Ovary and Gut: A dynamic Duo"*
4:15 PM - 4:35 PM	**Adam Gracz** **Emory University** *"Sox9 promotes biliary maturation in liver development and injury induced transdifferentiation"*
4:40 PM - 5:20 PM	**Timothy Wang & Quin Waterbury (Graduate Student)** **Columbia University** *"A Novel Model of Intestinal Stem Cell Hierarchy – Isthmus Cells Contribute to homeostatic cellular turnover and support regeneration following intestinal injury"*
5:45 PM - 7:00 PM	**WELCOME PARTY: LINDA HALL LIBRARY**

THURSDAY, OCTOBER 12

Session 2: STEM CELLS AND REGENERATION

8:30 AM	**OPENING ANNOUNCEMENTS**
8:35 AM - 9:15 AM	**Mayssa Mokalled & Vishnu Saraswathy (Postdoc)** **Washington University School of Medicine in St. Louis** *"Comparative approaches reveal regenerative mechanisms in the spinal cord"*
9:20 AM - 9:40 AM	**Vidyanand Sasidharan** **Stowers Institute for Medical Research** *"Extracellular-vesicle mediated cell-cell communication is required for planarian tissue maintenance"*
9:45 AM - 10:05 AM	**Ram Kumar** **University of Kansas Medical Center** *"METTL3 Orchestrates the Self-Renewal and Differentiation Potential of human Trophoblast Stem/Progenitor Cells"*
10:05 AM - 10:30 AM	**BREAK: COFFEE/BREAKFAST SNACKS**
10:30 AM - 11:10 AM	**Andy Groves & Ishwar Hosamani (Graduate student)** **Baylor College of Medicine** *"Can we restore hearing by reprogramming the cochlea?"*

11:15 AM - 11:35 AM	**Fang Tao** **Children's Mercy Research Institute** *"Stem Like T Cells in Anti-cancer Immunosurveillance Against Therapy-resistant Pediatric Cancer"*
11:40 AM - 12:00 PM	**Kunal Jindal** **Washington University School of Medicine** *"Single-cell lineage capture across genomic modalities with CellTag-multi reveals fate-specific gene regulatory changes"*
12:00 PM - 1:30 PM	**LUNCH: STOWERS LIBRARY**

Session 3: STEM CELL REGULATION AND DISEASE

1:40 PM - 2:20 PM	**Sandra Pinho** **The University of Illinois at Chicago** *"Regulation of leukemic stem cell proliferation by the bone marrow microenvironment"*
2:25 PM - 2:45 PM	**Ruochen Dong** **Stowers Institute for Medical Research** *"Spatial Transcriptomics Reveals Distinct Hematopoietic Stem Cell Niches in Mouse Fetal Liver"*
2:50 PM - 3:10 PM	**Levi Arnold** **University of Kansas Medical Center** *"DCLK1-Mediated Regulation of Invadopodia Dynamics and Matrix Metalloproteinase Trafficking Drives Invasive Progression in Head and Neck Squamous Cell Carcinoma"*
3:10 PM 3:30 PM	**BREAK: SNACKS & REFRESHMENTS**
3:30 PM - 4:10 PM	**Simón Méndez-Ferrer & Livia Lisi-Vega (PhD Student)** **University of Cambridge** *"Disentangling the microenvironment regulation for improved treatment of myeloid malignancies"*
4:15 PM - 4:35 PM	**John Perry** **Children's Mercy Research Institute** *"Induced pluripotent stem cell models for leukemia development and discovery of targeted therapies"*
4:35 PM - 5:35 PM	**POSTER SESSION 1**
5:40 PM - 6:35 PM	**KEYNOTE:** **David Scadden** **Harvard University** *"Regenerating the adult thymus"*
6:45 PM	**DINNER: STOWERS LIBRARY**

FRIDAY, OCTOBER 13

8:30 AM	**OPENING ANNOUNCEMENTS**

Session 4: STEM CELLS AND THEIR ENVIRONMENT

8:35 AM - 9:15 AM	**Sara A. Wickström & Clémentine Villeneuve (Postdoc)** **Max Planck Institute for Molecular Biomedicine; University of Helsinki** *"Coordination of cell states and morphogenesis by mechanical force"*

9:20 AM - 9:40 AM	**Colton Lysaker** **University of Kansas Medical Center** *"Unraveling the Role of APOE Genetic Variation in Metabolic Function: Insights from iPSC-Derived Models"*
9:45 AM - 10:05 AM	**Heather Le Bleu** **University of Oregon** *"Voltage-gated calcium channels restrain outgrowth to restore zebrafish regenerated fin size"*
10:05 AM - 10:30 AM	**BREAK: COFFEE/BREAKFAST SNACKS**
10:30 AM - 11:10 AM	**Richard Locksley & Victor Cortez (Postdoc)** **University of California San Francisco** *"Lessons from Helminths"*
11:15 AM - 11:35 AM	**Shinghua Ding** **University of Missouri** *"Targeting bone marrow hematopoietic stem/progenitor cells to systematically increase bioenergetics for neurodegenerative disease therapy"*
11:40 AM - 12:00 PM	**Daniela Muench** **Stowers Institute for Medical Research** *"Immune and Sensory Organ Regeneration Programs Differ in Response to Distinct Types of Cell Death"*
12:00 PM - 1:30 PM	**LUNCH: STOWERS LIBRARY**

Session 5: STEM CELLS, AGING, AND THE BRAIN

1:40 PM - 2:20 PM	**Ana Martin-Villalba** **German Cancer Research Center** *"The molecular secrets of stemness in the young, injured and aging brain"*
2:25 PM - 2:45 PM	**Seppe De Winter** **KU Leuven** *"Deciphering the gene regulatory mechanisms underlying human neural tube development using organoids, single-cell multiomics and machine learning"*
2:50 PM - 3:10 PM	**Carlo Donato Caiaffa** **Dell Pediatrics Research Institute, The University of Texas at Austin** *"The Effects of Dolutegravir on the Development of Brain Organoids"*
3:10 PM - 4:10 PM	**POSTER SESSION 2**
4:15 PM - 5:10 PM	**KEYNOTE:** **Jürgen Knoblich & Ramsey Najm (Postdoc)** **Institute of Molecular Biotechnology** *"Modelling neurodevelopmental disorders in stem cell derived brain organoid culture"*
5:15 PM - 6:30 PM	**CONFERENCE CLOSING RECEPTION: STOWERS LIBRARY**

Poster Sessions

Poster Session 1	Poster Session 2
Thursday, October 12 4:35 PM - 5:35 PM	Friday, October 13 3:10 PM - 4:10 PM
Odd Numbered Posters	Even Numbered Posters

Author	Title	Poster #
Francis Karanu	Conversion of iPSCs to Insulin-Producing Organoids for Treatment of Diabetes	1
Yi Pan	AT1R as the core bond between AML and CVD	2
Megan Hamilton	Controlled Release Hydrogel Microspheres to Deliver Multpoint Stem Cells for Treatment of Knee Osteoarthritis	3
Jon Bell	foxg1a is required for hair cell development and regeneration in the zebrafish lateral line	4
Jeremy Sandler	A Gene Regulatory Landscape that Drives Sensory Hair Cell Regeneration in Zebrafish	5
Mark Lush	Regulation of mechanosensory hair cell regeneration via different cyclin D family members	6
Aldyn Wildey	Reduction of Activin A Gives Rise to Comparable Expression of Key Definitive Endoderm and Mature Beta Cell Markers	7
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Xinjian Mao	Single-cell Multiome-seq Analysis Prospectively evaluates Human Hematopoietic Stem Cell Function	10
Shinghua Ding	Targeting bone marrow hematopoietic stem/progenitor cells to systematically increase bioenergetics for neurodegenerative disease therapy	11
Julia Peloggia de Castro	Fluctuating environmental conditions drive adaptive differentiation of ionocytes to maintain zebrafish lateral line function	12
Payel Bhanja	Myeloid microbiome axis in intestinal stem cell regeneration	13
Thomas Andl	Comparative Stem Cell Biology of Squamous Epithelia: Insights into Cancer and Longevity in Mammals	14

Author	Title	Poster #
Paloma Meneses-Giles	Deciphering the role of islet-1 in zebrafish hair cell regeneration	15
Elizabeth Ellis	Characterization of the role of Cxcl12 signaling during hair cell regeneration	16
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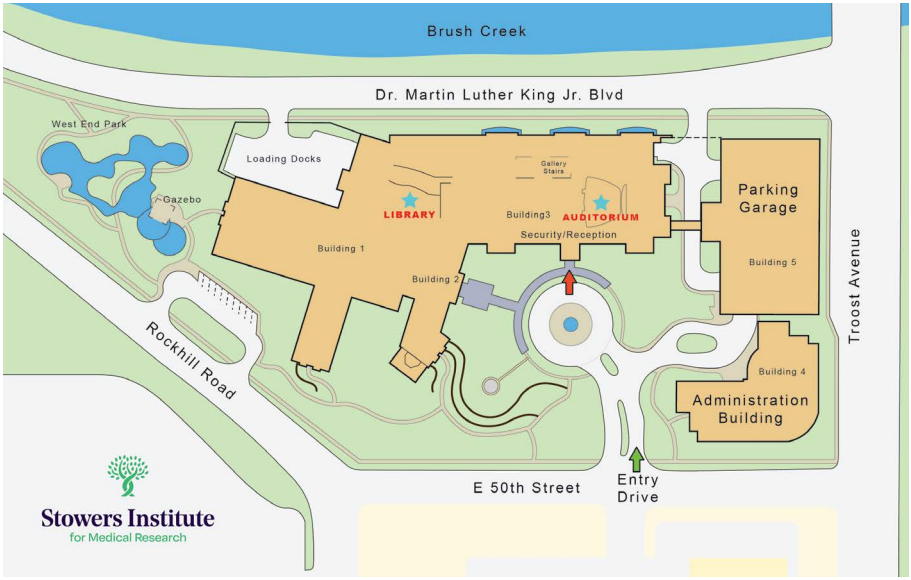
Abhik Saha	GATA3-RUNX1 Modulates Trophoblast Development in Trisomy 21	18
Charles Ayemoba	Megakaryocyte-Derived PF4 Signals Via LDLR To Inhibit Leukemia Stem Cell Proliferation	19
Alena Laier	Elucidating the Interwoven Molecular Nexus of Interferons in Antiviral Defense and Stem Cell Function	20
Purbasa Dasgupta	Essential Role of Arginine methylation protein PRMT1 in trophoblast lineage development	21
Julie Semon	pH Neutral Bioactive Glass for the Fabrication of Human Microphysicological Systems	22
Stephanie Nowotarski	Characterizing stem cells and muscles of *Schmidtea mediterranea* at high resolution using CLEM and SBF-SEM	23
Mol Mir	Middle Juice: Designing a Book to Share the Secrets of Planarian Cells	24

Campus Map


Brush Creek
Dr. Martin Luther King Jr. Blvd
West End Park
Loading Docks
Gazebo
LIBRARY
Gallery Stairs
Building3
AUDITORIUM
Security/Reception
Building 1
Building 2
Parking Garage
Building 5
Troost Avenue
Rockhill Road
Building 4
Administration Building
E 50th Street
Entry Drive
Stowers Institute
for Medical Research

Shuttle Schedule

A shuttle will run between the Courtyard Country Club Plaza (4600 Mill Creek Parkway Kansas City, MO 64112) and the Stowers Institute for Medical Research (1000 E. 50th Street, KC, MO 64110) at the times listed below:

WEDNESDAY, OCTOBER 11

2:15 PM / 2:30 PM / 2:45 PM	Pick up at hotel, drop off at Stowers
5:20 PM	Pick up at Stowers, drop off at Linda Hall Library for Welcome Party
7:00 PM	Pick up at Linda Hall Library, drop off at hotel
7:00 PM	Pick up at Linda Hall Library, drop off at Stowers

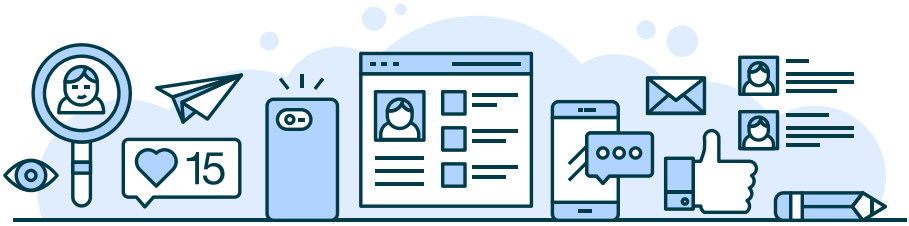
THURSDAY, OCTOBER 12

7:45 AM / 8:00 AM / 8:15 AM	Pick up at hotel, drop off at Stowers
7:45 PM / 8:00 PM / 8:15 PM	Pick up at Stowers, drop off at hotel

FRIDAY, OCTOBER 13

7:45 AM / 8:00 AM / 8:15 AM	Pick up at hotel, drop off at Stowers
6:15 PM / 6:30 PM / 6:45 PM	Pick up at Stowers, drop off at hotel

SRC Social Media Guidelines



The Stowers Research Conference organizers **encourage the use of social media to share information and network with other attendees.**

We remind you to **remain courteous and respectful in your comments and posts.**

Avoid sharing speaker or poster presentation content that's not your own, copyrighted or trademarked, or material protected by other intellectual property rights.

Follow and tag **@stowersinstitute** on Instagram

Follow and tag **@ScienceStowers** and **@Stowers_SRC** on X (Twitter).

Use hashtag **#SRCKC23**

Featured Speakers

David Scadden, Harvard University

David Scadden is the Gerald and Darlene Jordan Professor of Medicine at Harvard University. He is a practicing hematologist/oncologist who focuses on bringing stem cell biology to patient care. Scadden founded and directs the Center for Regenerative Medicine at the Massachusetts General Hospital. Together with Douglas Melton, he co-founded and co-directs the Harvard Stem Cell Institute (HSCI).

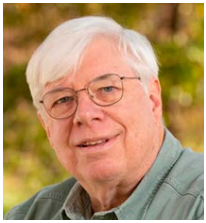
Scadden is a member of the Institute of Medicine of the National Academies of Science, the Board of External Experts for the National Heart, Lung and Blood Institute and a former member of the National Cancer Institute's Board of Scientific Counselors. He has received multiple honorary degrees, awards, and memberships in honorary societies. His work emphasizes targeting the stem cell niche to attain novel therapies for blood diseases.

Jürgen Knoblich, Institute of Molecular Biotechnology

Jürgen Knoblich is a developmental neuroscientist studying human brain development and psychiatric disorders. His laboratory is interested in the development of the human brain and the mechanisms of neuro-developmental disorders. To analyze this process, they have developed cerebral organoids, a 3D culture method that recapitulates the early steps of human brain development starting from pluripotent stem cells.

By growing organoids from disease patients, they were able to model microcephaly and demonstrate for the first time that human neurodevelopmental disorders can be studied in 3D culture. Using the new model system, they have developed in vitro models for the long-distance migration of human interneurons between brain areas.

They were also able to recapitulate brain tumor formation and show that in vitro grown human brain tumors can be used for testing anti-tumor drugs. Their goal is to address more complex neuro-developmental disorders like epilepsy and autism and recapitulate long-range connections between functionally distinct brain areas.



Allan Spradling, Carnegie Science

Allan Spradling is a Howard Hughes Medical Institute Investigator and former director of the Department of Embryology. The Spradling Lab is interested in the biology of reproduction, particularly oogenesis — the process of egg formation.

Working in Drosophila and mice, Spradling and his team focus on multiple aspects of oogenesis, including germline cyst formation, oocyte, and nurse cell specification, the germline chromatin cycle, oocyte storage in vivo, and environmental and nutritional influences. Their work has been informed by areas of evolutionary conservation between Drosophila and mammalian oogenesis. In addition, the team is interested in developing better tools and resources for these studies to share with the scientific community.

Invited Speakers

Images featured left to right and top to bottom.

Richard Locksley, University of California San Francisco

Sara A. Wickström, Max Planck Institute for Molecular Biomedicine; University of Helsinki

Ana Martin-Villalba, German Cancer Research Center

Andy Groves, Baylor College of Medicine

Timothy Wang, Columbia University

Simón Méndez-Ferrer, University of Cambridge

Sandra Pinho, The University of Illinois at Chicago

Mayssa Mokalled, Washington University School of Medicine

Abstracts

GATA3-RUNX1 Modulates Trophoblast Development in Trisomy 21

Abhik Saha1, Avishek Ganguly1, Ananya Ghosh1, Soma Ray1, Namrata Roy1, Ram P. Kumar1, Purbasa Dasgupta1, Rajnish Kumar1, Angela Martin2, Charles Gibbs2 and Soumen Paul1.

1Department of Pathology and Laboratory Medicine and Institute for Reproduction and Perinatal Research, 2Department of Obstetrics and Gynecology, University of Kansas Medical Center, 3901 Rainbow Boulevard Kansas City, KS 66160, USA

Trisomy 21 is the most prevalent genetic abnormality associated with pregnancy. The outcome of pregnancy with Trisomy 21 is heterogenous. Many of those pregnancies need medical termination, whereas babys born with trosomy 21 suffer from Down Syndrome, associated with physical and mental disabilities and shorten life expectancy. However, how Trisomy 21 affects trophoblast development/function and placentation is almost unknown. The Trisomy 21 of a developing fetus is often tested via chorionic villous sampling (CVS). In this study, we used a novel strategy, in which we isolated CTBs from residual tissues from CVS and established patient-derived human trophoblast stem cell (TSC) lines. Unexpectedly, our initial analysis of these TSC lines compared to that of normal TSC line, revealed that Trisomy 21 affects both self-renewal capacity and proliferate very slow. Further careful analysis of these cell lines indicated that the markers for EVT differentiation ACL2, HLA-G MMP2 are getting affected. Gene expression analyses show that Trisomy 21 induces expression of human leucocyte antigen-A (HLA-A) in human trophoblast cells. Trisomy 21 also induces the expression of CD25 in T cells and the expression of CD107 in Natural Killer cells, thereby making them a target for maternal immune system. GATA3, one of the genes involved in the development of TSCs, is downregulated at both mRNA and protein level. Interestingly, Runtrelated transcription factor 1 (RUNX1), a gene present in the Down Syndrome Critical Region (DSCR) on Chromosome 21, expression is relatively high in Trisomy 21 TSCs, indicating the presence of RUNX1-GATA3 axis in the development of a trophoblast cell.

Sox9 promotes biliary maturation in liver development and injury induced transdifferentiation

Adam Gracz1, Hannah Hrncir1, Fransky Hantelys2, Kendall Kanakanui3, Sergei Bombin2, Brianna Goodloe2

1Graduate Program in Biochemistry, Cell and Developmental Biology; Emory University, 2Division of Digestive Diseases; Emory University, 3Graduate Program in Genetics and Molecular Biology; Emory University

BACKGROUND: The adult liver exhibits a remarkable capacity for post-injury regeneration, involving reciprocal plasticity between biliary epithelial cells (BECs) and hepatocytes. This plasticity mirrors development, when bipotential hepatoblasts specify hepatocytes and the BECs that form a complex network of intrahepatic bile ducts (IHBDs). Data from our lab demonstrate that the transcription factor Sox9 regulates BEC identity by promoting both BEC maturation during IHBD development and hepatocyte-to-BEC transdifferentiation in adult cholestatic liver injury.

RESULTS: Conditional deletion of Sox9 in hepatoblasts (Sox9cKO) decreases BEC and IHBD numbers in adult mice. Whole-organ light sheet imaging reveals a specific loss of small ductules and extensive disorganization of IHBDs in Sox9cKO livers. Bulk and scRNA-seq demonstrate upregulation of TGFß signaling and embryonic hepatocyte and BEC gene signatures in a subpopulation of Sox9cKO BECs. Notably, small ductule BECs in Sox9cKO livers express high levels of Inhba, the precursor to TGFß family ligand Activin A. Treating wild-type BEC organoids with Activin A recapitulates morphological defects observed in Sox9cKO organoids. Inhibiting TGFß signaling in vitro rescues Sox9cKO phenotypes in organoids and inhibiting Activin A in vivo partially restores 3D IHBD morphology in postnatal Sox9cKO mice. To determine if Sox9 is also required for plasticity in injury/regeneration, we ablated Sox9 and simultaneously induced lineage-tracing in a hepatocyte-specific manner by administering AAV8.TBG.Cre to Sox9fl/fl:R26LSL-tdTomato mice (Sox9HepKO). Sox9HepKO livers exhibit reduced numbers of hepatocyte derived K19+ BECs following 6 weeks of DDC injury. Surprisingly, we also observe a significant increase in A6+ metaplastic hepatocytes in Sox9HepKO livers at the same timepoint, suggesting that Sox9 is dispensable for the initiation of hepatocyte-to-BEC transdifferentiation but required for its completion.

CONCLUSION: Our data demonstrate that Sox9 is required for full acquisition of BEC identity during development and regeneration. We propose a “two step model” of Sox9-independent BEC specification followed by Sox9-dependent BEC maturation.

Reduction of Activin A Gives Rise to Comparable Expression of Key Definitive Endoderm and Mature Beta Cell Markers

Aldyn Wildey, BASc1, Stephen Harrington, PhD1, Lisa Stehno-Bittel, PhD2, Francis Karanu, PhD1

1Likarda LLC, 2Likarda LLC, University of Kansas Medical Center

New price pressures have entered the pharmaceutical marketplace that require consideration of the cost of production early in the therapeutic's development phase. In the case of differentiating induced pluripotent stem cells (iPSC) into insulin-producing islet like cells, it was determined that Activin A was one of the more expensive growth factor components used in published protocols of beta cell differentiation. Human Activin A (AA) is a member of the TGF-ß protein family and is critical in the differentiation of human stem cells into definitive endoderm (DE) and subsequently into insulin-producing cells. Unfortunately, published AA small molecule replacements inducer of definitive endoderm 1 (IDE1) and IDE2 failed to lead to efficient DE differentiation. However, reduction of the concentration of AA from the standard 100 ng/ml to 75 ng/ml and 50ng/ml did not statistically alter the viability and cell counts. Expression of DE and islet like cells tested by immunohistochemistry staining, quantitative reverse transcription- polymerase chain reaction (qRT-PCR), and static glucose-stimulated insulin secretion (GSIS) showed no significant differences with reduced AA concentrations. Our results show that a reduction of the concentration of AA to 75 ng/ml and 50ng/ml did not adversely affect the quality of DE nor did it affect the downstream differentiation process into stem cell derived islet like cells (SC-ILCs). Further testing needs to be completed to examine in-vivo functioning of the SC-ILCs at the lower concentrations of AA.

Elucidating the Interwoven Molecular Nexus of Interferons in Antiviral Defense and Stem Cell Function

Alena Laier1,2, Damián Carvajal Ibáñez1,2, André Macedo1, Maxim Skabkin1, Eugenia Guerrero Aruffo1, Jooa Hooli1,2,3, Manuel Göpferich1,2, Ana Martin-Villalba1

1 Molecular Neurobiology, German Cancer Research Center (DKFZ), Heidelberg, Germany; 2 Faculty of Biosciences, University of Heidelberg, Heidelberg, Germany, 3 Institute of Applied Mathematics, University of Heidelberg, Heidelberg, Germany

Background: We have recently shown that in neural stem cells (NSCs) interferons do not only play a role in protecting these vulnerable cells from viral infection but are also needed to regulate stem cell dynamics. Increased interferon expression, which is highest in the aged brain leads to a decrease in the rate of activation in NSCs. Among others, interferon stimulated genes (ISGs) sense viral RNA by their low cap methylation state (Cap0) opposed to eukaryotic RNA which usually is 2′-O methylated on the first two transcribed nucleotides.

Results: We find, that ISGs bind a subset of cellular RNAs that are involved in neurogenesis. This indicates similarities between cellular and viral transcripts making them susceptible for ISG binding and regulation. A potential mechanism is via recognition of viral-like lowly methylated cap structures (Cap0) that could arise from cytoplasmic recapping, a recently found mechanism of transcript storage and reuse upon stress. Studying mRNA capping and recapping in a physiological setting is so far challenging due to a lack of high throughput methods designed for small RNA input. To overcome this, we have developed a method to separately sequence capped and phosphorylated transcripts. Upon treatment of NSCs with interferon followed by Cap and P-Sequencing we find an overall shortening of 5′UTRs in interferon related transcripts.

Conclusion: Optimization of our method will now enable in vivo investigation of this phenomenon, using an RNA input of just 25 NSCs isolated from the brain by flow cytometry. Ultimately, our method enables to study the capping landscape of NSCs and their lineage in the natural environment of the brain during homeostasis, aging and disease. Upon interferon treatment we find altered transcription start sites and shortened 5′UTRs speaking for post-transcriptional modifications triggered by ISG binding. In summary, we find that interferons have a dedicated stem cell function.

Extracellular-vesicle mediated cell-cell communication is required for planarian tissue maintenance

Vidyanand Sasidharan1, Alejandro Sanchez Alvarado1

1Stowers Institute for Medical Research

After amputation, planarians exhibit stem cell proliferation throughout their body, followed by migration and localized neoblast proliferation near the wound. These steps are critical in blastema formation making intercellular communication imperative during wound healing and tissue regeneration. Yet, little is known about the mechanisms that ultimately help localize stem cell proliferation near the wound. Extracellular Vesicles (EVs) are bi-layered structures known to play key roles in cell-cell communication. EVs carry a wide variety of biomolecules, such as proteins, mRNAs, small RNAs, lipids, metabolites, surface molecules, etc., reflecting the nature of the cells from which they are originated. In order to characterize EVs in planarians, we isolated them from regenerating animals using established protocols, followed by proteomics and small RNA sequencing to define their cargo. To test the potential functional role the identify cargo may have during regeneration, we performed RNAi on the identified and uncovered a number of phenotypes affecting the viability and regeneration ability of treated animals. Additionally, small RNA sequencing revealed that planarians use EVs as a mode of transportation for dsRNA-derived siRNAs. To test whether EVs are responsible for the broad effect of RNAi in planarians, we transplanted EVs isolated from dsRNA-fed animals to healthy animals. We found that EV-transplanted animals exhibited gene-specific RNAi phenotypes, suggesting that planarians pack dicer-processed siRNAs from dsRNA into EVs for extracellular transportation. We conclude that EVs play key functional roles in planarian cell communication and that their study will help elucidate the molecular and cellular mechanisms underpinning animal regeneration.

The Effects of Dolutegravir on the Development of Brain Organoids

Caiaffa, CD1, Tukeman, G2, Ambekar YS3, Zevallos-Delgado C3, Scarcelli G4, Larin KV3, Cabrera R2, Finnell RH2

1Dell Pediatric Research Institute, University of Texas at Austin, 2Center for Precision Environmental Health, Baylor College of Medicine, 3Department of Biomedical Engineering, University of Houston, 4Fischell Department of Bioengineering, University of Maryland

The formation of the brain and spinal cord is initiated in the earliest stages of mammalian pregnancy in a highly organized process known as neurulation. Environmental or genetic interferences can impair neurulation, resulting in neural tube defects during embryonic development. In 2018, preliminary data from a surveillance study in Botswana suggested an increased risk of neural tube defects among newborns whose mothers were taking the HIV integrase inhibitor, Dolutegravir, at the time of conception or early during pregnancy. Following these findings, the World Health Organization and the U.S. Food and Drug Administration issued recommendations and guidelines emphasizing the importance of considering the potential risks regarding the use of dolutegravir during pregnancy. Stem cell derived brain organoids are three-dimensional, miniaturized models of the human brain that can be used to study early neural development, and to assess the potential neurotoxicity of drugs and other compounds. Here, we associate gene expression information from RNA sequencing, with structural information from Optical Coherence Elastography, Optical Coherence Tomography and Brillouin microscopy, to understand the development of brain organoids in the presence of Dolutegravir. Our sequencing data indicate that Dolutegravir can modulate the expression of the gene networks required for brain organoid development. The Brillouin frequency shift observed in the surface of brain organoids indicates increased stiffness levels after exposing brain organoids to Dolutegravir, while Optical Coherence Elastography measurements indicate decreased organoid volumes and a global stiffness decrease. Considering the developmental timeline for brain organoids, at 20 days in culture, FOLR1 is highly expressed in the presence of Dolutegravir, which induces increased stiffness levels at the organoid surface and impaired growth development.

MEGAKARYOCYTE-DERIVED PF4 SIGNALS VIA LDLR TO INHIBIT LEUKEMIA STEM CELL PROLIFERATION

Charles E. Ayemoba1,3, Sen Zhang1, Anna M. Di Staulo1, Thomas G. Henderson2, Mary Menhart2, Alex M. Dittmar1, Constantinos Chronis2, and Sandra Pinho1,3

1Department of Pharmacology and Regenerative Medicine, University of Illinois at Chicago, Chicago, USA. 2Department of Biochemistry and Molecular Genetics, University of Illinois at Chicago, USA. 3Cancer Biology Research Program, University of Illinois Cancer Center, Chicago, USA.

Hematopoietic stem cells (HSCs) reside in and are regulated by highly specialized bone marrow microenvironments, known as niches. Due to the accumulation of mutations, hematopoietic stem and progenitor cells can give rise to leukemia initiating cells or leukemic stem cells (LSCs), whose expansion is associated with impaired hematopoiesis. Despite advances, acute myeloid leukemia (AML) remains associated with high rates of morbidity and mortality, often due to non-eradication of LSCs. Understanding LSC interactions with their niches is crucial to eliminating LSCs and preventing AML relapse.

Previous work from our lab has revealed that platelet factor 4 (PF4), a chemokine secreted by megakaryocytes, can induce quiescence in healthy myeloid-biased HSCs. Here, we investigate the regulation of LSCs by PF4. Our results show that PF4 directly regulates LSC proliferation in the MLL-AF9 AML model. In vivo cell cycle analyses revealed that LSCs proliferation is significantly inhibited 24h post-PF4 administration. Accordingly, in vivo depletion of megakaryocytes led to poor leukemic mice survival due to accelerated disease progression. To assess whether PF4 can alter the course of AML relapse in a preclinical setting, we treated leukemic mice with the chemotherapeutic drug Cytarabine followed by PF4. Remarkably, PF4 treatment reduced the frequency of LSCs during the relapse phase. Mice transplanted with MLL-AF9 cells treated with Cytarabine and PF4 showed improved survival compared to the Cytarabine control group. Finally, single-cell RNAseq and functional studies revealed that PF4 signals through Low-Density Lipoprotein Receptor (LDLR) to block LSC proliferation, by limiting LDL uptake. Altogether, our studies reveal an unknown function for PF4 on AML and highlight its potential as an adjuvant therapy in preventing AML relapse by inhibiting the recurrence of therapy-resistant LSCs.

Unraveling the Role of APOE Genetic Variation in Metabolic Function: Insights from iPSC-Derived Models

Colton Lysaker1, Vivien Csikos Drummond2, Brittany Hauger3, Taylor Strope1, Cole Birky4, Caleb Gilmore4, Daniel Chen5, Heather Wilkins1

1University of Kansas Medical Center Department of Neurology; University of Kansas Alzheimer's Disease Research Center; University of Kansas Medical Center Department of Biochemistry and Molecular Biology, 2University of Kansas Medical Center Department of Neurology; University of Kansas Alzheimer's Disease Research Center; University of Kansas Medical Center Department of Cell Biology and Physiology, 3University of Kansas Alzheimer's Disease Research Center, 4University of Kansas Lawrence, 5University of Kansas Medical Center Physician Scientist Training Program

Background: Metabolic dysfunction and altered mitochondrial function represent emerging pathological hallmarks in Alzheimer's disease (AD). The apolipoprotein E (APOE) gene is the strongest genetic risk determinant for sporadic AD (sAD), with three primary alleles, ε2, ε3, and ε4. Specifically, the ε2 allele decreases an individual's risk for sAD while the ε3 allele is considered neutral, and the presence of the ε4 allele increases risk. The main sources of APOE production in the body are the liver and brain. APOE plays a crucial role in metabolic processes like lipid transport and amyloid beta clearance from the brain, yet its direct impact on mitochondrial function is not well understood. Here, we used isogenic APOE induced pluripotent stem cells (iPSCs) from the Jackson Laboratory with APOE2, APOE3, and APOE4 alleles. iPSCs were differentiated into neurons, astrocytes, and hepatocytes for downstream experiments. We assessed mitochondrial function using Seahorse XF analysis and mitochondrial fluorescence-based indicator assays.

Results: Seahorse XF metabolic analysis revealed decreased mitochondrial respiration in APOE4 iPSC-derived neurons and astrocytes when compared to their APOE3 counterparts. Similar changes were also seen in iPSC-derived hepatocytes. We also observed significant increases in mitochondrial membrane potential, superoxide, and H2O2 levels in APOE4 neurons, astrocytes, and hepatocytes when compared to APOE2 and APOE3 cells. Additionally, APOE4 neurons and astrocytes displayed increased intracellular and mitochondrial calcium (Ca2+) levels relative to APOE3 cells.

Conclusion: This research has revealed that natural APOE polymorphisms can influence mitochondrial health in iPSC-derived cells. We observe an overall decrease in mitochondrial respiration in APOE4 cells and increased levels of reactive oxygen species. However, additional work is required to elucidate mechanisms underlying these changes.

Immune and Sensory Organ Regeneration Programs Differ in Response to Distinct Types of Cell Death

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Successful tissue regeneration following injury requires the concerted response of multiple cell types, including immune cells and stem cells. The cellular and molecular components of regeneration programs induced by tissue damage can be affected by the severity of the injury, the identity of the injured tissue, as well as the type of cell death. Determining the individual contribution of these parameters to regeneration has remained challenging, largely due to the mechanical, variable nature of many injury paradigms. Our recent work has provided an in-depth molecular characterization of zebrafish lateral line hair cell (HC) regeneration, identifying key transcriptional programs of both macrophages and lateral line cells following HC necrosis using single cell RNA-seq. Here, we established a comparative approach of regeneration programs, inducing either necrosis or apoptosis in HCs, respectively. Keeping the identity and quantity of the ablated cells consistent between both paradigms, this approach allows us to specifically interrogate the influence of the cell death modality on regeneration. High resolution live imaging not only revealed intricate morphological details of each type of HC death, but also led us to visualize the rapid recruitment of tissue-resident macrophages. While cell debris were efficiently cleared by macrophages following both paradigms, phagocytic behavior differed dramatically, providing evidence for distinct cellular responses depending on the injury. At the molecular level, HC necrosis triggered a robust injury response in lateral line cells, while it was greatly diminished following apoptosis. Despite these differences in the early response to injury, both paradigms eventually converge on similar genes important for hair cell regeneration. In sum, our data provide evidence for distinct molecular and cellular responses to different forms of cell death in a regenerating sensory organ, allowing us to investigate molecular triggers of regeneration. Taken together, this study provides invaluable insights into the context-dependent nature of regeneration programs.

Characterization of the role of Cxcl12 signaling during hair cell regeneration

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Hair cell death in mammals is permanent, resulting in deafness as mammals are incapable of regenerating damaged or dead hair cells. Similar to those of the inner ear, zebrafish possess mechanosensory hair cells in lateral line sensory organs, called neuromasts, which are present on the head of the fish in the anterior lateral line (aLL) and along the trunk in the posterior lateral line (pLL). In contrast to mammals, zebrafish are capable of regenerating lateral line hair cells through proliferation and differentiation of support cells. Therefore, elucidating the molecular mechanisms of hair cell regeneration in zebrafish may help identify strategies to trigger regeneration in the mammalian cochlea. Cxcl12 is a chemokine involved in cell adhesion, proliferation and survival. Signaling through the receptor Cxcr4, it has been shown to play a significant role in the regeneration of multiple tissues, such as the liver, skeletal muscle and the optic nerve. Analysis of scRNA-sequencing of lateral line neuromasts revealed upregulated expression of the receptor cxcr4b in support cells following hair cell death (Baek et al., 2022). Here, we investigate the role of Cxcl12/Cxcr4 signaling in hair cell regeneration using cxcl12a mutants. While a mutation in the cxcl12a gene disrupts formation of the pLL, the aLL forms normally, allowing for the analysis of hair cell regeneration. To specifically ablate lateral line hair cells, zebrafish were incubated with the aminoglycoside antibiotic neomycin. Using an EdU incorporation assay, a significant increase in proliferation was observed in aLL neuromasts of cxcl12a mutants. These results suggest that Cxcl12 may negatively regulate proliferation of support cells. Further investigation of Wnt, Notch, and Fgf signaling pathways may clarify the role of Cxcl12 signaling in hair cell development and regeneration.

Stem Like T Cells in Anti-cancer Immunosurveillance Against Therapy-resistant Pediatric Cancer

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Leukemia is the most commonly diagnosed pediatric cancer. Acute lymphoblastic leukemia (ALL) has an 85% 5-year-survival rate; yet 20% of patients experience relapse driven by therapy-resistant cells and do not survive long-term. The Wnt/β-catenin and PI3K/Akt signaling pathways interact to confer resistance to cancer therapies. We previously found low dose doxorubicin (DXR) specifically targets therapy-resistant leukemic stem cells (LSCs) by inhibiting immune check point genes on LSCs in a T-cell ALL (T-ALL) mouse model. However, the cellular and molecular mechanisms of how low DXR functions to achieve this anti-cancer immunity are not well understood. Wnt signaling was shown to arrest effector cell differentiation and generate memory stem cells, which preserves anti-tumor potential. As DXR inhibits the cooperative interaction of Akt and β-catenin, we hypothesized that low DXR facilitates immune reactivation against resistant LSCs by regulating stem-like T cells. In this study, we investigated multiple populations of CD8+ T cells, especially the stem-cell like T cells in T-ALL. We found low dose doxorubicin increased the naïve T cell and stem-like T cell pool. Moreover, our flow cytometry and single-cell sequencing analysis of LSCs and blast cells revealed upregulation of stem-like T cell gene signature after low DXR treatment, but not after conventional chemotherapy. Meanwhile, we characterized T cells in patients with minimal residual disease (MRD). We found that MRD negative patients were presented with more naïve T cells, and enhanced polyfunctionality in their T cells. These cells may ultimately be pharmacologically stimulated by low DXR while genetically engineered to express chimeric antigen receptors, allowing application of adoptive immunotherapy based on the tumor-specific stem-like T cells and preventing relapse in patients treated with immunotherapy.

Conversion of iPSCs to Insulin-Producing Organoids for Treatment of Diabetes

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Patients with Type 1 diabetes have struggled with blood glucose levels fluctuating between hyper and hypoglycemic conditions. Cell transplants have improved the quality of life for diabetic people that can afford them or lucky enough to enroll in a clinical trial. However, there is a lack of donor cells to treat all of the people with Type 1 diabetes. Therefore, scaled stem cells differentiated into insulin-producing cells are needed. We began with induced pluripotent stem cells (iPSCs) as a scalable cell source and tested for their capacity to form islet-like cells (SC-ILCs) in vitro. A clinically-compliant cell line was chosen and a unique differentiation protocol established, resulting in SC-ILCs characterized by RT-PCR, flow cytometry, and immunocytochemistry. SC-ILCs were injected interparitoneally and shown to correct the blood glucose.
To block immune rejection of the SC-ILCs and maintain cell viability, microencapsulation was explored. Microencapsulants offer a wide range of tailorable properties with the goals of 1) immunoprotection, 2) protection during cryopreservation and thawing, and 3) maintenance in a target location in the body, thus reducing the required dose. Alginate has traditionally been used as a microencapsulant however, conventional alginate microspheres result in a notable foreign body response. A new microencapsulation technology called Core-Shell Spherification (CSS) was developed to overcome these issues.
When microencapsulated SC-ILCs were implanted in chemically-induced diabetic dogs at a therapeutic dose, the blood glucose normalized and exogenous insulin was no longer required. Importantly, without systemic immunosuppression, the SC-ILCs maintained a normal blood glucose for over 9 months. In a safety study, dogs were purposefully overdosed to 4X levels and no reported hypoglycemic events were recorded. In addition, blood chemistry and hematology showed no adverse effects of the transplants. Thus, encapsulated SC-ILCs show promise in reversing insulin-dependent diabetes.

Voltage-gated calcium channels restrain outgrowth to restore zebrafish regenerated fin size

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How regenerating organs and tissues adopt the correct size and shape is a fundamental question in the field of regenerative biology. Adult zebrafish fins regenerate to their initial proportions irrespective of the extent or type of damage, making them a compelling and tractable system to interrogate "organ scaling" mechanisms. Existing zebrafish mutants with abnormally sized fins have centrally suggested a role for bioelectricity in fin development and regulation. However, the links between ion signaling and their effectors to specific cell behaviors determining the extent of fin outgrowth is limited. Perturbed ion signaling, notably by elevated voltage-gated K+ channel activity and inhibited Ca2+-dependent calcineurin signaling, leads to dramatic overgrowth of regenerating zebrafish fins. A unique distal pool of mesenchymal cells within the fin's regenerative blastema sustains fin outgrowth. Our lab discovered the classic longfint2 mutant phenotype is caused by ectopic expression of the Kcnh2a potassium channel within intra-ray mesenchymal- lineage cells. Ectopic Kcnh2a activity enhances fin outgrowth in late regeneration rather than at early blastema establishment. Kcnh2a likely blocks Ca2+- dependent calcineurin signaling with both acting uniquely during late stages of regeneration. The mechanisms that ensure adequate Ca2+ signaling in fin regeneration have not been explored. Here, we show, that dynamic Ca2+ signaling within mesenchymal cells depends upon voltage-gated Ca2+ channel activity. Our single-cell transcriptomics identified candidate upstream voltage-gated Ca2+ channels. We found distinct L-, N, and T-type voltage- gated Ca2+ channel expression patterns within mesenchymal cells derived from mature fibroblast cells. We mutated the genes encoding each channel, generating the first Ca2+ channel long finned zebrafish model. We found the overgrown phenotype of these mutant fins is due to a prolonged period of outgrowth. We now hypothesize Ca2+ signaling within fin mesenchyme, modulated by a cadre of Ca2+ channels, activates calcineurin to promote cell state transitions in distal mesenchymal cells for fin growth cessation.

A Gene Regulatory Landscape that Drives Sensory Hair Cell Regeneration in Zebrafish

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Regeneration in response to damage is found across the phyla, but regenerative capacity is variable between species and tissues. Sensory hair cells in the vertebrate ear are responsible for hearing through the transduction of vibrations into nerve impulses. Unfortunately, hair cells do not regenerate when damaged or killed, leading to permanent hearing loss. Mammalian and zebrafish lateral line sensory hair cells are functionally and genetically homologous, however zebrafish hair cells rapidly regenerate after damage. Previous studies in our lab have characterized transcriptional changes in the lateral line in a fine time scale using scRNA-seq, and we identified three core modules that drive the regeneration of hair cells. A key missing perspective, however, is the epigenetic and regulatory landscape during regeneration. Therefore, we completed an ATAC-seq and ChIP-seq time course of the epigenetic regulatory environment and combined this information with scRNA-seq data. Chromatin accessibility and regulatory histone marks rapidly change matching the expression dynamics of genes during regeneration time points. Co-regulated enhancers form ten "regulatory groups" across time. Motif enrichment analysis reveals that each enhancer group is defined by a unique core set of transcription factors necessary at steps of regeneration/regulatory modules in the time series. Early injury/stress response genes, such as fos and jun have enriched binding sites in enhancers of hair cell regeneration genes. Thus, there is a direct regulatory link between the injury response from dying hair cells and the genes necessary to regenerate new hair cells. We identified enhancers and show that they drive reporter gene expression. Functional analyses show that enhancer deletion causes hair cell defects, demonstrating that these regulatory links are essential for regeneration. By understanding the regulatory landscape and how links and binding motifs have changed between zebrafish and mammals, our data provide key insight and gene targets for hair cell regeneration in mammals.

Spiny mice display a pro-regenerative response to acute retinal damage

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Background: The capacity for retinal regeneration varies widely, however, to date no mammal has been shown to have this ability. Studies of Mus musculus have expanded our understanding of retinal function, development, and disease; however, valuable insight could be gained by investigating mammals that demonstrate a propensity for tissue regeneration. Identifying such a mammal could provide valuable mechanistic insights and inform therapeutic approaches to treat blinding retinal diseases. The common spiny mouse, Acomys cahirinus, has been shown to have remarkable regenerative ability in the skin, kidney, muscle, heart, and spinal cord. Here, we present an overview of Acomys retinal anatomy and an assessment of its response to acute retinal damage as compared to the non-regenerator Mus.

Results: Acomys retinal cell type abundance and morphology were largely similar to Mus, however Acomys retinas displayed lower cone and horizontal cell densities, as well as altered laminar location of some bipolar cell bodies. In response to acute retinal damage via intraocular injection of 100mM NMDA, Acomys retinas contained more proliferating cells across the retina compared to Mus. Subsets of these proliferating cells co-localized with markers of Müller glia. Acomys also displayed an increased and prolonged microglial response compared to Mus, in response to retinal injury. Additionally, Acomys (but not Mus) displayed increased expression of retinal regeneration genes in response to damage. At ten days post injury, recovery of nuclei in the ganglion cell and inner nuclear layers was evident in Acomys but not Mus retinas.

Conclusions: Our results show that A. cahirinus mounts a pro-regenerative response to inner retinal damage that includes increased cellular proliferation, induction of regenerative gene expression, and recovery of neurons. To our knowledge, these data represent the first description of a mammal with the potential for retina regeneration.

Induced pluripotent stem cell models for leukemia development and discovery of targeted therapies

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Cancer can be viewed as a subversion of normal regenerative or developmental mechanisms. This is particularly well-illustrated by infant acute lymphoblastic leukemia (iALL). Interestingly, iALL originates in utero with the most aggressive cases being associated with an early embryonic or fetal origin. However, little is known regarding the cell(s) of origin or how early blood development (hematopoiesis) is subverted into leukemia development.

iALL is a high-risk subtype of pediatric leukemia with dismal survival outcomes despite intensive therapies. Rearrangement of the histone modifier, KMT2A (KMT2A-r), is the most frequent cytogenetic abnormality in iALL. Unfortunately, KMT2A-r is associated with refractoriness to therapy, early relapse, and poor survival. KMT2A-r generates a driver fusion oncogene, most commonly KMT2A::AFF1, resulting in epigenetic dysregulation of target gene transcription. iALL expressing this fusion oncogene is an unusual cancer in that other somatic mutations are uncommon and there are no known genetic risk factors. Unfortunately, existing models fail to recapitulate the disease phenotype. This indicates an essential, outstanding need for the development of more representative model systems for KMT2A-r leukemias.

Using CRISPR gene editing technology, our team of basic, translational, and clinical scientists has created a highly controlled human induced pluripotent stem (iPS) cell model system to understand the genomic and epigenetic landscape and cellular evolution of KMT2A::AFF1 iALL. Our studies demonstrate our ability to engineer inducible KMT2A-r fusion oncogenes into iPS cells and generate functional human hematopoietic stem and progenitor (HSPCs) from iPS cells. Notably, this model at least partially recapitulates hematopoietic ontogeny. Our iPS cell-based model system provides the opportunity to investigate a range of critical and outstanding questions of KMT2A-r disease initiation, progression and treatment. Future studies include a comparison of this model to single cell transcriptomic datasets from pediatric leukemia patients and comparison with developmental datasets from human embryonic and fetal hematopoiesis.

foxg1a is required for hair cell development and regeneration in the zebrafish lateral line

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Mechanosensory hair cells, located in the inner ear, mediate the sensations of hearing and balance. If damaged, mammalian inner ear hair cells are unable to regenerate, thus loss of hair cells can result in permanent sensory deficits. In mice, the transcription factor Foxg1 functions to promote normal development of inner ear hair cells and other tissues. Foxg1 mutations in humans result in severe neurological and developmental disabilities. Unlike mammals, aquatic vertebrates like zebrafish (Danio rerio) have a specialized class of mechanosensory hair cells found in the lateral line system, allowing them to sense changes in water current. Lateral line hair cells are robustly able to regenerate following damage. foxg1a is expressed in lateral line sensory organs in zebrafish larvae, but its function lateral line development and regeneration has not been investigated. The zebrafish foxg1aa266 mutant was generated using CRISPR-Cas9 genome editing (Thyme et al. 2019). Examination of foxg1aa266 mutants found that mutants develop significantly fewer hair cells compared to heterozygous controls. In addition, foxg1aa266 zebrafish show a similarly reduced complement of hair cells after regeneration following ablation with neomycin. foxg1aa266 larvae show a significant reduction in proliferating cells during development and regeneration. These findings suggest that Foxg1 function is critical to the development and regeneration of mechanosensory hair cells. Investigating the role of foxg1a in the zebrafish lateral line will increase our understanding of the cellular and genetic mechanisms driving the development and regeneration of hair cells. Funding provided by NIGMS 1R16GM146690-01 (HFM).

Fluctuating environmental conditions drive adaptive differentiation of ionocytes to maintain zebrafish lateral line function

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Organisms adjust their physiology to cope with environmental fluctuations and maintain fitness. These adaptations occur via genetic changes over multiple generations or through acclimation, which are reversible phenotypic changes that confer resilience to the individual. The zebrafish sensory lateral line contains hair cells (HCs) that detect water movement. The fine regulation of the ionic microenvironment of HCs is essential for their proper function, but how they maintain their function through seasonal fluctuation of water salinity and pH is unknown. We discovered a new cell population in the lateral line, called Nm ionocytes, derived from basal skin cells. Ionocytes are specialized cell types that control ion homeostasis and osmoregulation. Detailed analyses of Nm ionocyte dynamics show that following changes in water salinity and pH, these cells invade lateral line organs in pairs and modulate hair cell function. Specifically, Nm ionocyte progenitors are rapidly induced after salinity decrease, undergo one cell division and differentiate in a foxi3a/bdependent manner. We demonstrate that a sequential activation of different Notch pathway components controls ionocyte determination downstream of foxi3a/b. Tissue explants, as well as zebrafish mutants lacking sensory neurons, show that salinity is not sensed systemically by neurons in the skin or olfactory system but directly by Nm ionocyte progenitors. To identify which signals trigger foxi3a/b and Nm ionocyte progenitor activation, we manipulated the concentrations of different ions in the media. Depletion of Ca2+ions induces new Nm ionocytes, while depletion of K+ is insufficient. In summary, we have identified how environmental changes activate a signaling cascade that triggers basal skin cell progenitors to differentiate into Nm ionocytes and invade lateral line organs. We discovered an important acclimation mechanism that allows for fine tuning of HC function in seasonal changes of water conditions.

Planarian Outreach Program

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The Planarian Outreach Program (POP) is designed to bridge the gap between the field of stem cell regeneration and high school education by utilizing planarian as a basic model organism. POP is structured around the high school curriculum to incorporate classroom topics with hands-on learning experiences to promote student interest in biological research. Connecting the laboratory to the classroom provides guidance and mentorship to encourage scientific curiosity.

In collaboration with scientists and teachers, we have developed a focused curriculum that aligns with Advanced Placement Biology and Zoology courses. The POP is structured in two lectures and a laboratory workshop in which students learn the principles of stem cell biology and the remarkable regenerative capabilities of planarians. POP lectures incorporate engaging activities, such as demonstrations of planarian anatomy, amputations techniques, fission assays, RNA interference technology and resulting phenotypes. To build upon what is learned within the lectures, the laboratory workshop leads students to design experiments investigating the factors they hypothesize would influence planarian regeneration. The students carry out the experiments, collect and analyze data, and formulate conclusions based on their observations. The students present their findings in poster presentations. In this way, POP allows students to have a hands-on research experience from experimental design to sharing their scientific knowledge to their peers. These presentations are evaluated by laboratory members that provide feedback on the students' scientific research.

Since launching in 2021, POP has reached two Kansas City school districts, seven classrooms, and more than 250 students. By actively incorporating high school students in exploring planarian regeneration, we are inspiring a new generation of scientists. POP has made lasting impacts on students and will have a wider impact on the scientific community. At this presentation, I will describe our program design and examples of curriculum to give opportunity to propagate this approach.

pH Neutral Bioactive Glass for the Fabrication of Human Microphysicological Systems

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Background: Using growth factors in tissue engineered constructs can have negative effects in some populations. An alternate approach is to use dissolvable bioactive glasses (BGs) doped with therapeutical relevant ions. Borate bioactive glasses have been shown to have regenerative effects in vivo and have: (1) been administered safely with multiple applications to speed the healing of dermal wounds in > 90% of elderly patients, (2) had complete healing of dermatological wounds with no signs of inflammation or infection, and (3) resulted in little to no scarring. Yet, borate BGs increase local pH by rapidly releasing alkaline ions and, thereby, create a toxic environment for cells. A bioactive glass that degrades in a pH-neutral manner while not affecting local pH and still releasing therapeutic ions is needed. The goal of this study is to create a novel bioink using pH-neutral borophosphate bioactive glasses (BPBGs), adipose stem cells (ASC), and endothelial cells (EC). Methods: We evaluated a series of BGs with different borate-to-phosphate ratios for degradation and ion release. We also tested the series on ASCs and ECs for viability and changes in phenotype, and we examined the angiogenic abilities of the series in a chick chorioallantoic membrane (CAM) model. Results: The pH-neutral BPBGs supported EC proliferation/migration and stimulated more blood vessel formation in the CAM model. Conclusions: The results indicate these pH-neutral bioactive glasses can be used safely in cell culture studies and are promising angiogenic biomaterials for human microphysiological systems in tissue engineering.

Single-cell lineage capture across genomic modalities with CellTag-multi reveals fate-specific gene regulatory changes

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Complex gene regulatory mechanisms underlie differentiation and reprogramming. Contemporary single-cell lineage-tracing (scLT) methods use expressed, heritable DNA barcodes to combine cell lineage readout with single-cell transcriptomics. However, reliance on transcriptional profiling limits adaptation to other single-cell assays. With CellTag-multi, we present an approach that enables direct capture of heritable random barcodes expressed as polyadenylated transcripts, in both single-cell RNA sequencing and single-cell Assay for Transposase Accessible Chromatin using sequencing assays, allowing for independent clonal tracking of transcriptional and epigenomic cell states. We validate CellTag-multi by applying it to an in vitro model of hematopoiesis, characterize functional lineage priming of chromatin landscapes across progenitor populations, and quantify fate predictability from transcriptional and epigenomic states of progenitor cells using machine learning. Next, we apply CellTag-multi to a less defined system of fate conversion - Transcription Factor (TF) mediated direct reprogramming of Mouse Embryonic Fibroblasts to induced Endoderm Progenitors (iEPs) - identifying core regulatory programs underlying on-target and off-target fate specification. This analysis also reveals distinct sets of TFs driving fate- specific gene programs in both on-target and off-target destined cells, suggesting lineage dependent re-wiring of gene regulatory networks during the early stages of reprogramming. Finally, we experimentally validate the function of one such TF, Zfp281, in driving off-target cell identity. Together, these results establish CellTag-multi as a novel lineage tracing method compatible with multiple single-cell modalities and demonstrate its utility in revealing fate specifying gene regulatory changes across multiple paradigms of cell fate conversion.

DCLK1-Mediated Regulation of Invadopodia Dynamics and Matrix Metalloproteinase Trafficking Drives Invasive Progression in Head and Neck Squamous Cell Carcinoma

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Head and neck squamous cell carcinoma (HNSCC) poses a significant threat to patients despite therapeutic advancements. Understanding the progression from early confined disease to invasive malignancy is crucial for identifying therapeutic targets. Locoregional invasion, facilitated by invadopodia, represents an early step in metastasis, where cellular protrusions promote extracellular matrix (ECM) infiltration through microtubule- mediated processes. Kinesin motor proteins play a pivotal role in transporting matrix metalloprotease (MMP)-laden vesicles for secretion, yet the regulation of kinesins within invadopodia remains unclear. Notably, doublecortin-like kinase 1 (DCLK1), a microtubule-binding kinase implicated in cancer stem cells and axonal transport, emerges as a potential key player at the invasive front of HNSCC.

Through comprehensive analysis, we investigated the role of DCLK1 in invadopodia-mediated invasion in HNSCC. High DCLK1 levels were observed at the tumor-stromal interface in patient samples, particularly in cases with elevated histological grade and lymph node involvement. Knockdown of DCLK1 using short hairpin RNA (shRNA) disrupted invadopodia formation and substantially reduced transwell invasion. Proteomic and phosphoproteomic analysis highlighted the disruption of cellular movement, cytoskeletal organization, and cargo transportation upon DCLK1 knockdown. DCLK1 co-localized with key invadopodia markers such as MMP14, TKS5, TKS4, and cortactin. Proximity ligation assays and immunoprecipitation confirmed DCLK1's associations with TKS4, RAB40B, MMP9, and KIF16B. Furthermore, conditioned media experiments substantiated DCLK1's influence on MMP secretion.

This study uncovers a novel invasive mechanism involving DCLK1 in HNSCC. The presence of DCLK1 at the invasive front and its associations with invadopodia-related proteins suggest its role in promoting MMP transportation and ECM invasion. These findings underscore the potential of targeting DCLK1 as a therapeutic strategy to impede early metastatic dissemination in HNSCC, offering new insights into combating this aggressive disease.

Regulation of mechanosensory hair cell regeneration via different cyclin D family members

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Regeneration is limited in mammals but can be robust in other vertebrates. For instance, zebrafish can fully regenerate lateral line mechanosensory hair cells. The lateral line is composed of neuromasts which contain mechanosensory hair cells surrounded by multiple support cell types. Neuromast hair cells regenerate via proliferation and differentiation of support cells. In mammals there is little proliferative regeneration in response to hair cell death. To uncover genes that are important for hair cell regeneration we performed single-cell RNAseq (scRNAseq) analysis of regenerating neuromasts. Two cyclinD family members were identified to have unique expression patterns. ccnd2a and ccndx are expressed in proliferative support cells and hair cell progenitors respectively. Intriguingly ccndx is only found in anamniotes and has been lost in amniotes. ccnd2a mutant neuromasts have fewer support and hair cells, but proliferative regeneration still occurs. In contrast, in ccndx mutants, EdU analysis and time-lapse imaging show that hair cell progenitors fail to proliferate during development and regeneration, and that new hair cells form instead via direct differentiation. This finding is surprising as it was thought that mitosis was required for neuromast hair cell regeneration. scRNAseq analysis of ccndx mutants found no difference in hair cell gene expression, suggesting hair cells develop normally in the absence of progenitor proliferation. ccndx and ccnd2a double mutants show limited support cell proliferation, suggesting additional genes can compensate for their loss. We identified two enhancers near ccndx that are sufficient to drive neuromast expression. We have identified transcription factor motifs within these enhancers that we are currently functionally testing. These results show that hair cell regeneration in zebrafish is robust and can occur in the absence of proliferation. ccndx is an exciting candidate gene to induce proliferative regeneration in the mammalian ear.

Controlled Release Hydrogel Microspheres to Deliver Multipoint Stem Cells for Treatment of Knee Osteoarthritis

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Osteoarthritis (OA) is the most common form of joint disease affecting articular cartilage. Current treatments are insufficient, aimed at mitigating symptoms. Multipotent Stromal Cell (MSC) therapy is proposed as a treatment capable of preventing cartilage destruction and treating symptoms. While many studies have investigated MSCs for treating OA, therapeutic success is often inconsistent due to low MSC viability and retention in the joint. To address this, biomaterial-assisted delivery is of interest. Particularly hydrogel microspheres which can be easily injected into the joint. Microspheres composed of hyaluronic acid (HA) have advantages due to HA's inherent biocompatibility and biodegradability. Accordingly, this study investigated HA- based hydrogel microspheres as MSC delivery vehicles. Microrheology measurements indicated that the microspheres had structural integrity alongside sufficient permeability. Additionally, encapsulated MSC viability was found to be above 70% over one week of encapsulation. Gene expression analysis of MSC-identifying markers showed no change in CD29 levels, increased expression of CD44, and decreased expression of CD90. Analysis of chondrogenic markers showed increased expression of aggrecan and SRY-box transcription factor 9, and decreased expression of osteogenic markers, runt-related transcription factor 2 and alkaline phosphatase. In vivo analysis revealed that HA microspheres remained in the joint for up to 6 weeks. Rats underwent destabilization of the medial meniscus resulting in overt OA followed by treatment with 1) control vehicle, 2) empty HA microspheres, 3) MSCs alone, 4) MSC-laden microspheres. Pain measurements taken before and after the treatment illustrated temporarily decreased pain in group treated with encapsulated cells. Finally, histopathological scoring of each group illustrated significantly less OA damage in those treated with encapsulated cells compared to controls. Overall, these studies demonstrate the potential of using HA-based hydrogel microspheres to enhance the therapeutic efficacy of MSCs in treating OA.

Middle Juice: Designing a Book to Share the Secrets of Planarian Cells

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Middle Juice is an illustrated book exploring high resolution cellular anatomy of the planarian flatworm Schmidtea mediterranea using SBF-SEM data. The title "middle juice" is a translation of the word "mesenchyme" – a reference to the messy, seemingly-unordered appearance of the ultrastructure of these animals. The primary goal of this work is to visually and verbally communicate to a general audience how animals are made of cells.

Secondary goals include: (1) teaching how to interpret EM images, especially as a section of a 3D structure that has been paused at a moment in time, and (2) to serve as a reference for planarian experts of different cell types, including 3D morphology.

This project is a work-in-progress and we would be glad to hear your feedback.

Deciphering the role of islet-1 in zebrafish hair cell regeneration

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Isl1 is a transcription factor critical in proliferation, differentiation, and survival of progenitor cells in multiple tissues. Its overexpression in concert with other transcription factors, in mouse, aids hair cell production during regeneration, but its mechanism of action and how it's regulated are poorly understood. Zebrafish possess sensory hair cells within mechanosensory organs called neuromasts, that are homologous to hair cells in the mammalian inner ear, however zebrafish robustly regenerates hair cells throughout life. We previously identified central support cells as the direct hair cell progenitors in zebrafish hair cell regeneration. isl1 is specifically expressed in central support cells with a dynamic response to hair cell death. We found that deleting isl1 results in a disproportionately higher number of hair cells in regeneration, showing that isl1 affects cell type specification. Time lapse imaging analysis revealed that this increase is driven by overproliferation. Transcriptomic analyses and in situ hybridization of candidate genes showed that isl1 acts upstream of the Wnt signaling pathway, while Fgf and Notch signaling remain unaffected. isl1 loss results in the upregulation of multiple central support cell genes, suggesting that it plays a repressive role in this population. Epigenetic analysis revealed that deletion of isl1 in regeneration increases chromatin accessibility, both in promoter and enhancer regions. This supports the idea that isl1 is acting as a repressor in the neuromast. It's yet to be determined which of these genes are isl1 direct targets. Additionally, we found that several upregulated genes in the isl1 mutant are specific to regeneration and inflammation/injury response. These genes fail to be regulated at the right time when isl1 is deleted, suggesting that isl1 is a crucial player in orchestrating the gene expression dynamics during regeneration.

This work was supported by institutional funds from the Stowers Institute for Medical Research.

Myeloid microbiome axis in intestinal stem cell regeneration

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1KUMC

Gut microbiome plays an important role in gastrointestinal homeostasis and repair. Although the role of gut microbiome in tissue homeostasis has been studied extensively, involvement of tissue microenvironment in the regulation of microbiome population and function has not been examined. We have shown previously that Macrophages (M_ϕ) play a significant role in the repair and regeneration of intestinal stem cells (ISC) following radiation injury. In the present study, we have examined the involvement of M_ϕ in the regulation of gut microbiome population in response to irradiation (IR). We demonstrated that mucosal Mφ regulate the commensal bacteria critical for ISC regeneration. Mucosal Mφ primarily consists of Ly6ChiCX3CR1lo newly recruited monocytes and Ly6CloCX3CR1hi tissue resident Mφ. In injured tissue, recruitment of circulating Ly6Chi monocytes replenish intestinal Mφ. However, recent evidence suggested that CX3CR1hi (Tim4+CD4+) tissue resident Mφ are maintained independent of monocyte recruitment. It was also observed that gut microbiome is localized in macrophage enriched area. CCR2/CCL2 chemokine axis plays a significant role in Ly6C+ inflammatory monocyte recruitment. Studies showed that recruitment of inflammatory monocytes mediated inflammatory cascades are involved in ROS overproduction. Our current study demonstrate that radiation induces Ly6C+ inflammatory monocyte recruitment in mouse intestine. However, deletion of CCR2 in these monocytes significantly inhibit monocyte recruitment in irradiated intestine. Inhibition of Ly6C+ve monocyte recruitment in CCR2-/- mice inhibits ROS production. Moreover, at 48h post-whole-body-irradiation, metagenomic analysis of mouse fecal material demonstrates significant increase in A. muciniphila (AM) population in CCR2-/- mice. Using Lgr5-eGFP-IRES-CreERT2; Rosa26-CAG-tdTomato mice and ex-vivo organoid, we showed that AM promotes regeneration of ISCs and activates tetrahydrofolate-pathway, potentially be involved in survival of intestinal epithelium. In conclusion, our study clearly showed that modulation of CCR2/CCL2 chemokine axis alters gut microbiome populations in favor of mucosal epithelial radiation resistance with alteration of inflammatory mucosal myeloid population.

Essential Role of Arginine methylation protein PRMT1 in trophoblast lineage development

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The placenta is an indispensable organ for the growth and development of fetus and mother in mammals, facilitating nutrients and gas exchange. Pregnancy complications like fetal growth restriction and pre-eclampsia are associated with abnormal placentation. In a post-implantation mouse embryo trophoblast stem and progenitor cells (TSPCs) reside within the extraembryonic ectoderm (ExE)/ ectoplacental cone (EPC), whereas, in a post-implantation human conceptus, mononuclear cytotrophoblast (CTBs) constitute the trophoblast progenitor cell compartment. CTBs differentiate to either multi-nucleated Syntiotrophoblasts cells (STBs) or invasive extra villous trophoblast cells (EVTs).

Here, we show that Protein arginine methyl transferase 1 (PRMT1), a conserved epigenetic regulator, is one of the essential regulators for trophoblast progenitor self-renewal and differentiation. PRMT1 is the most predominant enzyme to catalyze mono-methylation and asymmetric demethylation of arginine side chains in Histone H4 (H4R3me2a) and other proteins to regulate cellular processes like gene transcription.

PRMT1 is abundantly expressed in mouse TSPCs and in CTBs and EVTs within a developing human placenta. Mouse embryos lacking Prmt1 (Prmt1-KO) die at early post-implantation stage, (~embryonic day (E)7.0), a developmental stage equivalent to the first trimester of human pregnancy. E7.0 conceptus analyses revealed that loss of PRMT1 resulted in defective development of both the embryo proper and the ExE/EPC region, which contains the TSPCs. Furthermore, loss of PRMT1 in human trophoblast stem cells (hTSCs), which were derived from CTBs, impairs hTSC self-renewal ability and their differentiation to EVTs. Thus, our study uncovers PRMT1 as an important regulator for trophoblast development during mammalian placentation.

METTL3 Orchestrates the Self-Renewal and Differentiation Potential of human Trophoblast Stem/Progenitor Cells

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N6-methyladenosine (m6A) is the most abundant RNA modification which is methylated by the core catalytic subunit Methyltransferase-like 3 (METTL3) of methyltransferase complex (MTC complex) in the nucleus during transcription. The m6A methylation affects the stability, export, degradation, and translation of varieties of RNA. Thereby, METTL3 plays an essential role during mammalian development including maintenance of stem cell fate and differentiation in multitude of cellular contexts including cancer. However, its specific functions in trophoblast stem cells and pregnancy-related diseases remain unclear, highlighting the need for further investigation to explore potential implications in pregnancy complications.

Remarkably, our novel findings reveal that dysregulated METTL3 expression levels within trophoblast lineages, whether excessively low or high, are intimately associated with severe pregnancy-related complications such as pre-eclampsia, IUGR, preterm birth and recurrent pregnancy loss. Our investigations further demonstrate that the loss of METTL3 or inhibition of its kinase function using the STM2457 inhibitor, as well as METTL3 overexpression in human trophoblast stem cells (hTSC), result in a complete loss of their self-renewal potential, preventing the formation of both 2D monolayer and 3D organoid hTSC. METTL3-deficient hTSC spontaneously differentiates into syncytiotrophoblast and fail to differentiate into extravillous trophoblasts. Importantly, METTL3 fRIP-seq and m6A RNA-Cut&Run-seq corroborate the fact that numerous known essential genes required for self- renewal and differentiation are m6A-modified RNA molecules bound by METTL3, and these genes are downregulated in METTL3 knockdown hTSC.

These findings underscore the pivotal role of METTL3 and its m6A methylation activity in hTSC in a self-renewing state and guiding lineage differentiating state, and thereby ensures faithful maintenance of pregnancy. Understanding the molecular mechanisms underpinning METTL3- dependent gene regulation in placental development and identifying METTL3-interacting regulators hold significant implications for both fundamental research and potential therapeutic interventions in pregnancy-related disorders.

Spatial Transcriptomics Reveals Distinct Hematopoietic Stem Cell Niches in Mouse Fetal Liver

Ruochen Dong1, Xi He1, Hua Li1, Jonathon Russell1, Seth Malloy1, Xinjian Mao1, Zhe Yang1, Kate Hall1, Kaitlyn Petentler1, Alison Scott1, Cathy McKinney1, Sarah Smith1, Yongfu Wang1, Anoja Perera1, Sean McKinney1, Brian Slaughter1, Jay Unruh1, Linheng Li1

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Background: The hematopoietic stem cell (HSC) microenvironment, termed the niche, supports the proliferation, self-renewal, and differentiation abilities of HSCs. The definitive HSCs emerge from the hemogenic endothelium in the aorta-gonad-mesonephros (AGM) region after E10.5, and then migrate to the fetal liver after E12.5 for expansion. In this study, we use cutting-edge spatial transcriptomics to investigate the interactions between fetal liver HSCs and the niche cells.

Results: By using single-cell RNA seq, we first revealed the transcriptomics of HSCs and potential niche cells, including hepatoblasts, endothelial cells, macrophages, megakaryocytes, and mesenchymal stromal cells (MSCs) in E14.5 and E16.5 mouse fetal liver. Interestingly, we found that MSCs and hepatoblasts were characterized by enriched N-cadherin expression. Both slide-seq and 10x Visium showed that the majority of fetal liver HSCs are near N-cadherin-expressing MSCs and endothelial cells in the portal vessel area, indicating a supportive role of N-cadherin-expressing MSCs and endothelial cells in HSC maintenance. Subsequent CellPhoneDB (CPDB) analysis demonstrated that the N-cadherin-expressing MSCs are major niche-signaling senders with an enriched expression of niche factors, such as CXCL12 and KITL, and stemness pathway-related ligands, indicating N-cadherin-expressing MSCs could be the major niche cells in supporting HSCs in the fetal liver. We then generated a N-cadCreER;Cxcl12 f/f strain to conditionally knock out the well-studied niche factors, CXCL12, in N-cadherin-expressing cells. Interestingly, conditional knockout of Cxcl12 in N-cadherin-expressing cells resulted in an increase in the number of HSCs, along with a depletion of lymphoids, resulting in a myeloid-biased differentiation. Subsequential slide-seq showed that fetal liver HSCs were not enriched near MSCs in portal vessel regions due to loss of Cxcl12.

Conclusion: We postulated that N-cadherin-expressing cells, especially MSCs, maintain fetal liver HSCs. The knockout of Cxcl12 in N-cadherin-expressing cells leads to the migration of HSCs away from MSCs, which may induce HSC expansion and biased differentiation.

Developmental Consequences of Mutating the Ceramide Transfer Protein (CERT)

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The ceramide transfer protein (CERT) extracts ceramide from the endoplasmic reticulum for movement to the trans-Golgi. Previous studies show that knock-out of CERT leads to developmental defects and shortened lifespan. Several specific alleles have been identified in human patients that link CERT function to neural deficits, though the precise nature of the mutations is still under investigation. To better understand the developmental role of CERT protein, we have established a zebrafish model using CRISPR-Cas9 genome editing to simultaneously target exons 1 and 2 of the zebrafish gene cert1a and generate a null mutant allele. Analysis of the F2 generation shows recessive inheritance of a cert1a mutant phenotype including small body, eyes, and brain. Embryos homozygous for the cert1a mutation rarely survive past 5dpf. We sought to phenocopy our cert1a mutant phenotype by targeting ceramide transfer with a pharmacological competitive inhibitor, HPA-12. Inhibitor-treated embryos exhibited a similar phenotype to the cert1a mutants, including developmental delays in the forebrain, further corroborating results found here. Using our cert1a mutant line, we will begin to address how CERT functions during embryonic development and how specific human mutant alleles disrupt these processes. Funding was provided by the UMKC Funding for Excellence grant.

Deciphering the gene regulatory mechanisms underlying human neural tube development using organoids, single-cell multiomics and machine learning.

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Background
The vertebrate central and peripheral nervous system originate from transient embryonic structures, the neural tube and neural crest respectively. These structures have highly conserved gene regulatory mechanisms, with cis-regulatory elements (CREs) orchestrating cell specific gene expression patterns. Due to challenges associated with high-throughput experimental identification of transcription factor (TFs) binding sites, we lack gene regulatory networks (GRNs) that include CREs as nodes. Furthermore, profiling gene regulatory changes at high temporal resolution is challenging. In this work, we propose computational approaches for inferring GRNs with CREs as nodes and apply these approaches to organoids, designed to mimic the early stages of vertebrate nervous system development.

Results
We applied single-cell multiomics at multiple timepoints during neural tube organoid differentiation from human pluripotent stem cells within a 3D synthetic hydrogel culture. Leveraging the expression profiles of evolutionarily conserved marker genes, distinct neural crest states, neural progenitor populations, and differentiating neuronal fates were identified. Notably, the neural progenitor population exhibits a clear dorso-ventral patterning, highlighting the fidelity of this organoid model. Next, we developed a computational framework called SCENIC+ to infer enhancer driven GRNs. With this framework we could identify TFs specific to the different cell types along with their target genes and genomic target regions. The edges of the resulting GRN are consistent with previous meta-analysis based on data of multiple species. Finally we made use of deep learning models to gain insights into enhancer architecture. Using this we could accurately pinpoint the exact binding sites of TFs in the genome and infer TF cooperativity.

Conclusion
This study addresses the intricate regulatory processes underlying development of the vertebrate nervous system, making use of computational approaches to infer GRNs and gain insight into the functional mechanisms of enhancers.

Targeting bone marrow hematopoietic stem/progenitor cells to systematically increase bioenergetics for neurodegenerative disease therapy

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Background
Nicotinamide adenine dinucleotide (NAD+) is an essential cofactor involved in cellular energy homeostasis. Reduction of NAD+ levels will therefore greatly affect metabolic activity. The salvage pathway is the predominant pathway for NAD+ biosynthesis in mammalian cells, where nicotinamide phosphoriboslytransferase (Nampt) is the rate-limiting enzyme, Decrease in Nampt protein and MAD+ levels have been observed in many neurodegenerative diseases (NDs) as well as in normal aging. Thus, physiological means to systemically maintain elevated NAD+ levels through Nampt overexpression (OE) may represent a promising intervention from NADs. Red blood cells (RBCs) are released from bone marrow (BM) after differentiation and maturation from hematopoietic stem/progenitor cells (HSPCs) and by far the most abundant cell type in the body of human and mice.

Results
In current study, we used two-vector system of integrating and helper-dependent adenovirus (HD-Ad5/355++), i.e., HD-Ad5/35++-Nampt and HD- Ad5/35++-SB100x vectors with beta-globin promotor to transduce HSPCs. Because the vector system includes an activity-enhanced Sleeping Beaty (SB) transposases (SB100x), the transgene can be integrated into genome of HSPCs, leading to its permanent transgene OE. Moreover, because beta-globin promoter was used, Nampt was specifically expressed in peripheral RBCs. Our results showed that Nampt OE increased NAD and NADH in the BM, RBCs, plasma as well as in the brain. Using metabolic cage, we found Nampt OE significantly increased O2 consumption, CO2 production, energy expenditure and wheel running counts of mice. In addition, Nampt OE did not affect hematology.

Conclusion
Our results demonstrated that Nampt OE in RBCs can augment metabolic activity at cellular and organismal levels but did not cause effects on hematopoiesis. Our study suggests that RBCs-enabled NAD+ pathway my restore systemic metabolic homeostasis and brain cell function in NDs and RBCs can serve as an invaluable tool for ND therapy through inter-organ cross talk.

Characterizing stem cells and muscles of Schmidtea mediterranea at high resolution using CLEM and SBF-SEM

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Planarian flatworms like Schmidtea mediterranea (Smed) are consummate regenerators, with adult animals able to regenerate relatively complex tissues and whole organs from fragments of their bodies on the order of 10,000 cells. Full understanding of the regenerative capabilities of Smed requires a high-resolution characterization of organs, tissues, and the adult stem cells necessary for regeneration in vivo. Alone, in situs and light microscopy only describe molecular identity limited by probes and markers. Electron microscopy of single sections in standard transmission techniques are limited by the inability to understand the 3D environment, and by the field of view able to fit onto a support grid. Here we expand upon correlative light and electron microscopy (CLEM) techniques used to identify neoblasts by incorporating View RNA insitu probes as well as antibody staining on array tomography, allowing a more full understanding of molecular identity and morphology with limited 3D capability. Using our increased morphological understanding of neoblasts from CLEM we identify and characterize neoblasts in both homeostatic and regenerating tissue in serial block-face scanning electron microscopy (SBF-SEM) volumes. In addition to starting to describe neoblasts in vivo, we also use deep learning to segment muscle fibers and better describe a fiber alluded to by Libbie Hyman in 1951.

Comparative Stem Cell Biology of Squamous Epithelia: Insights into Cancer and Longevity in Mammals

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Background: Within the class of mammalia, few species exhibit a maximum life span above 100 years. Humans belong to this small group with extreme longevity. The molecular basis of longevity in mammals is still not well understood, but it is believed that all organs, tissues, and tissue stem cells in a long-lived organism must be improved, with the weakest contributing to morbidity. A contributor to morbidity is cancer which is primarily occurring in self-renewing epithelia and is associated with stem cell proliferation rates.

Results: To better understand adaptations of stem cell to longevity on the tissue level, we compared the organization of cell proliferation, differentiation, and cytoprotective protein expression in the squamous epithelium of the esophagus of mammals with varying lifespans. Adhering to the statements by stem cell pioneers Pottten and Loeffler that stem cells are best studied in their native state and any manipulation may distort and change their behavior, we performed in situ analyses. Our findings, obtained through multi- and ultra-plexed immunostainings show that the expression of DNA repair and NRF2/ferroptosis pathway proteins is higher in the human basal stem cell layer compared to that in short-lived mice. Additionally, humans are the only species with a quiescent basal stem cell layer that is clearly physically separated from parabasal transiently amplifying cells. Furthermore, the parabasal cells seem to originate most often from delaminating basal cells rather than asymmetric stem cell division. In humans, the combination of an elevated cytoprotective signature and novel tissue organization may enhance resistance to aging and prevent cancer. These features are lost during tumorigenesis, suggesting a tumor suppressor function for both.

Conclusion: Our results point to enhanced cellular cytoprotection and a tissue architecture which separates stemness and proliferation as potential factors contributing to the increased fitness of human squamous epithelial stem cells to support longevity.

Characterizing the Immunosuppressive Niche of Cancer Cell

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Immunotherapy has shown limited efficacy in colon rectal cancer (CRC), primarily due to the immune-excluded tumor phenotype where immune cells, including CD8+ T cells, accumulate around the tumor periphery rather than infiltrating the tumor site. Enhancing the infiltration of cytolytic lymphocytes into CRC tumors is a significant research focus. Our previous studies have highlighted the role of cancer stem cells (CSCs) and the tumor microenvironment, specifically myeloid-derived suppressor cells (MDSCs), in creating an immunosuppressive niche. The RPS19-C5AR1 signaling module has been identified as a mediator of immunosuppressive signaling. To investigate this further, the MC38 mouse model of colon cancer was employed, treating mice with an anti-C5AR1 neutralizing antibody. Remarkably, a single treatment resulted in a 50% reduction in tumor mass. Various techniques were utilized to explore CSC-niche interactions, including imaging, single-cell secretome analysis, scRNA-sequencing, and spatial transcriptomics. The analysis revealed a significant increase in immune cell infiltration, such as Macrophages, CD8+ T cells, and NK cells, from the tumor periphery to the central region. Isoplexis single-cell Secretome analysis further confirmed downregulation of immunosuppressive signals (Tgfb and IL-10) and upregulation of inflammatory signals (TNFa and IFNg). In summary, treatment with the anti-C5aR1 antibody facilitated the transition from an immune- excluded to an immune-inflamed tumor phenotype in the MC38 mouse model of CRC. This was supported by the activation of IFNg signaling and downregulation Cd8T cells exhaustion. Additionally, treatment-induced upregulation of MHC-I expression in tumor cells may enhance the recognition and targeting of tumor cells by CD8+ T cells. Taken together, these data suggest that using Anti-C5AR1 to block the interaction between CSCs and MDSCs could enhance the immune response to CRC by targeting key immunosuppressive signaling pathways.

Single-cell Multiome-seq Analysis Prospectively evaluates Human Hematopoietic Stem Cell Function

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The limited number of human hematopoietic stem cells (HSCs) remains a barrier of HSC transplantation in the clinic. Therefore, ex vivo expansion of HSCs has significant clinical implications. However, all the reported methods fail to expand long-term (LT) HSCs that are indispensable for long-term graft success, and commonly lead to deficient multi-lineage potential of HSCs. Retrospective transplantation is still the only way to evaluate functional HSCs. Prospective evaluation on HSC functionality remains unattainable, impeding the quality control of the ex vivo cultured HSCs for clinical application. Although recent scRNA-seq analysis revealed the transcriptional landscape of HSCs before and after ex-vivo culture, their epigenetic change is uncharacterized.

Here we established a culture method that recapitulates both intrinsic (low expression of YTHDF2) and extrinsic factors (hypoxia, three-dimensional microenvironment (3D), N-cadherin peptides) found in adult bone marrow and successfully expands human cord blood and bone marrow derived LT- HSCs ex vivo. These expanded HSCs were functionally evaluated using serial competitive repopulation assays, which exhibited robust self-renewal capacity and balanced multi-lineage potential. Specifically, HSCs cultured in 3D and N-cadherin peptide condition, with or without YTHDF2 inhibition, exhibited enhanced lymphoid lineage potential than uncultured HSCs and HSCs cultured in 2D. The molecular identity of these HSCs was evaluated using single-cell multiome sequencing (sc-Multiome-seq, which measures gene expression and chromatin accessibility in the same cells at single-cell level). We delicately compared the transcriptional and epigenetic landscapes of HSCs cultured under different conditions. By connecting the molecular profiles of these expanded HSCs to the corresponding functional outcomes, we show that both the self-renewal and multi-lineage potential of HSCs can be prospectively evaluated using sc-Multiome-seq analysis.

The culture approach and evaluation system enhance our understanding of HSC biology and open new avenues for the clinical applications of HSCs.

AT1R as the core bond between AML and CVD

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1University of Missouri

Acute Myeloid Leukemia (AML) and cardiovascular disease (CVD) are the two leading causes of death in the world. Prior studies have reported that AML and CVD are exceptionally close-related to each other, indicating potential shared targets between AML and CVD. To test the hypothesis, we analyzed RNA-seq data from multiple AML and CVD databases and published data. Using UMAP/WGCNA-based method to analyze gene co-expression patterns among AML and CVD patients, we identified AT1R as one of the leading expression genesamong a algorism-identified sub-population containing both AML and CVD patients. While AT1R is a famous target in CVD, our primary patient sample analysis revealed AT1R is a potential target in AML, as we found AT1R is significantly enriched in AML blasts. Indeed, our AT1R-shRNA PDX model study using 11 primary human samples showed that AT1R is essential for AML development. To evaluate whether AT1R is a safe target, we deleted AT1R in mice hematopoietic system to study the role of AT1R in both normal hematopoiesis and AML. We found AT1R deletion does not affect normal hematopoiesis. Our MLL-AF9 and AML1-ETO9a genetic mouse study also shows AT1R deletion reduces leukemic burden and prolongs mice survival. Such effects are mediated by Notch1-regulated stemness and cell cycle change, as supported by RNAseq analysis and our in vivo and in vitro assays.

While cardiotoxicity is common in cancer chemotherapy, its incidences are even higher in AML, especially those harbor certain mutations. The fact we identified AT1R as the shared target makes it a perfect target for cardiotoxicity. Indeed, AT1R inhibitor treatment not only alleviated chemotherapy- induced cardiotoxicity, but also significantly prolongs mice survival when co-administered with chemo-drug.

In conclusion, AT1R is a promising target that can treat AML and CVD simultaneous, especially during chemotherapy.

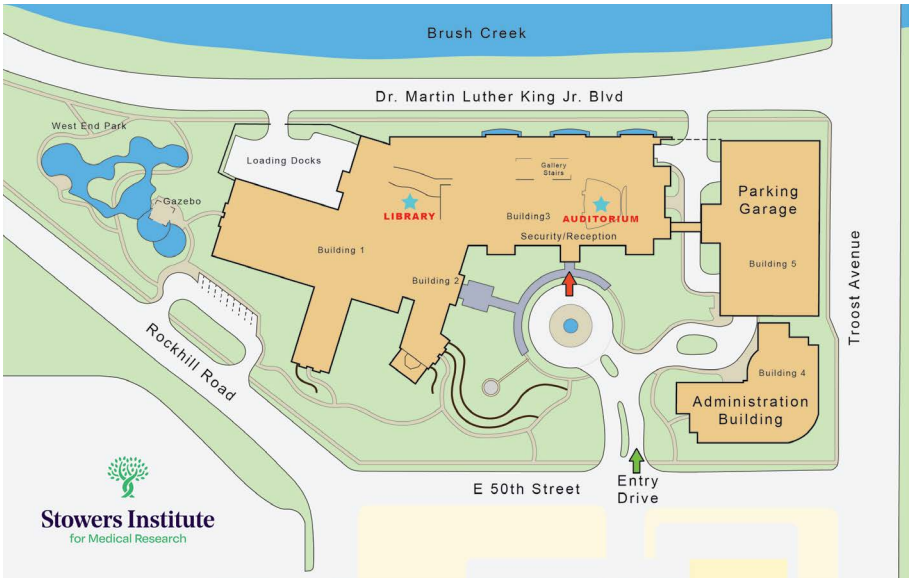
Attendee Resources

Location: Stowers Institute for Medical Research – 1000 E. 50th Street, Kansas City, MO 64110
General Phone Number: 816-926-4000
Driving directions from Kansas City International Airport:
(approximately 30 minutes under normal driving conditions)

1. Follow the signs on International Circle to the airport exit.
2. Take Cookingham Drive to I-29 South (right ramp), heading toward Kansas City.
3. Continue to follow I-29 South as it merges into I-29 South 71 (merge in left lane), then I-35South (merge in left lane again).
4. Cross the Paseo Bridge and get in the lane for I-70 South 71 (Exit 3)
5. Merge right and follow sign to South 71 Highway (Exit 2M).
6. Take the Emmanuel Cleaver II Boulevard Exit and turn right onto Emmanuel Cleaver IIBoulevard.
7. Follow Cleaver II to Troost Avenue and turn left on Troost.
8. Turn right on 50th Street and take another immediate right at the Stowers Institute's entrance(1000 E. 50th Street, Kansas City, MO 64110).

For turn-by-turn directions to the Institute from where you are, click here.

Campus Map



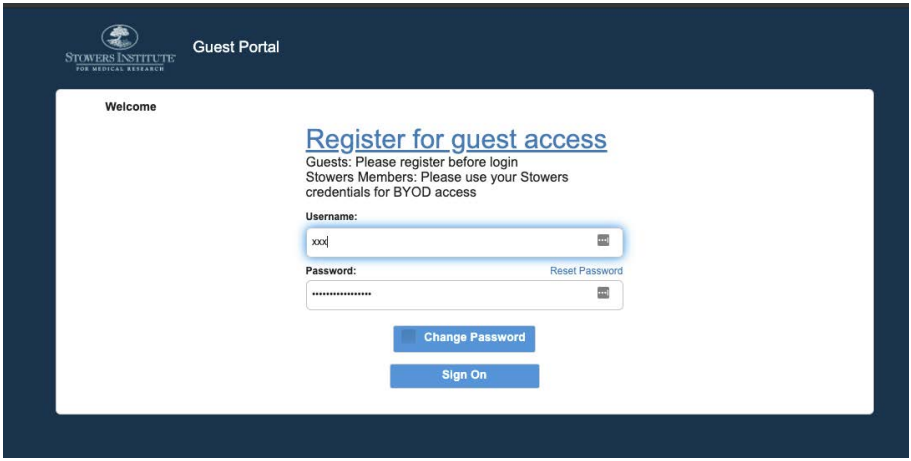
Parking: Visitor parking is available in the parking structure located between the Administration Building (to your right as you enter the campus) and the Research Building (to your left). Please park on the 5th floor of the parking garage and take the elevator down to the 1st floor to enter the Research Building.

Restrooms: located by the two large planters on the 1st floor of the Research Building. All gender restrooms are available at the base of the stairs on the B1 level.

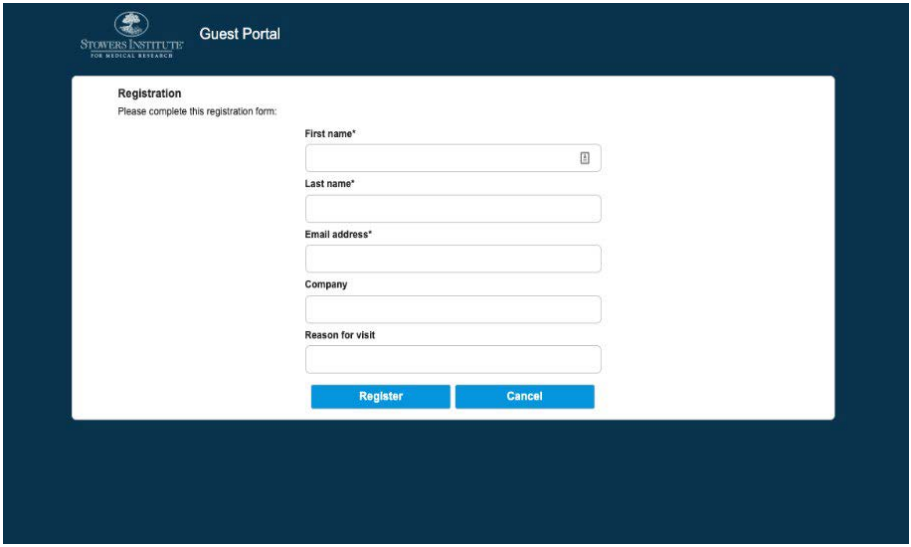
Mother's Room: At the base of the stairs on the B1 level, located next to the all gender restrooms . An entry key will be provided by security to guests that request to use it.

Tobacco Free Campus: Tobacco of any kind is prohibited in all the Institute facilities and on the Institute grounds. The Institute does not offer any designated smoking areas.

Wireless Access: Stowers Guest may connect to the **stowers_guest SSID**
When connected, you will see a captive portal. Click on the link at the top of the page to register for guest access.

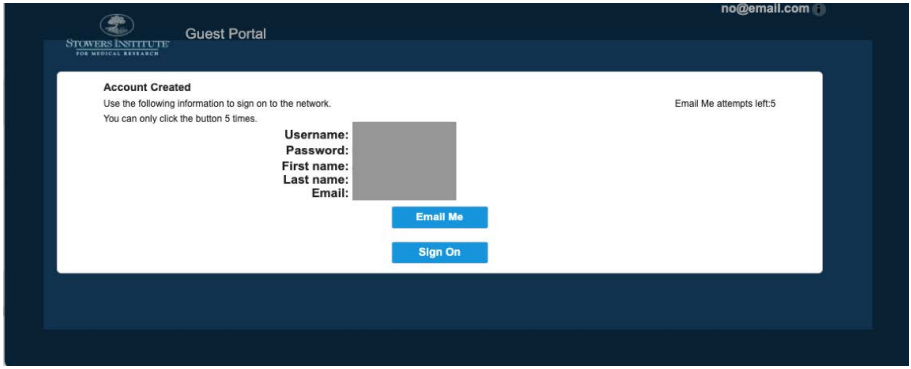


You will be prompted to enter their information. Name and email are the only required fields.
After entering the required information click Register



You will see a page like this with sign on information. You can use the userid and password to connect other devices without going through the registration, by entering the userid and password in the first page of the captive portal.

Click the "Sign On" button



After clicking sign on, you will be prompted to accept the "Acceptable use Policy". After clicking accept you should now have internet access.

If you have any questions, please contact the Stowers Help Desk at 816-926-4150.

Kansas City Fun: The Kansas City area offers a wealth of cultural, educational, and entertaining opportunities to explore, many of which are inexpensive or free of charge. The following is a brief listing of local attractions, event calendars, and resources.

Useful Resources
Information about Kansas City and the surrounding area can be found at www.kansascity.com, www.kcmo.gov or at www.visitkc.com. Two publications to help you discover things to do in the area are: "Insider's Guide to Kansas City" by Katie van Luchene and "Day Trips from Kansas City" by Shifra Stein. Both can be found in local bookstores.

Nelson Atkins Museum of Art
4525 Oak Street, 816-751-1278
Opened in 1933, the Nelson-Atkins Museum has more than 50 galleries and several period rooms. The museum's outstanding feature is its collection of Asian art. The collection ofChinese landscape paintings is one of the finest in the West, and the museum's holdings of Chinese ceramics and decorative arts are also noteworthy. Besides European paintings from the Renaissance on, the museum also has notable collections of ancient Egyptian sculpture, Japanese porcelains and lacquer, and English pottery. The E.F. Pierson Sculpture Garden was dedicated in 1972, and the Henry Moore Sculpture Garden opened in 1989. Admission is free. nelson-atkins.org

Kemper Museum of Contemporary Art
4420 Warwick, 816-753-5784
Founded in 1994, the Kemper Museum of Contemporary Art presents modern and contemporary art of the highest quality and significance. It collects, preserves, documents, interprets, and exhibits a growing permanent collection; develops and presents special exhibitions; and offers a variety of educational programs. Admission is always free, and the Museum serves a diverse and inclusive public population. kemperart.org

Loose Park
Intersection: Wornall Road and 51st Street, 816-784-5300
Loose Park is one of Kansas City's most beautiful parks. The park is home to a lake, a walking path, a shelter house, Civil War markers, tennis courts, a wading pool, picnic areas and the famous Rose Garden. The Rose Garden is popular for all types of outdoor special events including theatrical performances and wedding ceremonies.

Westport
Westport is one of Kansas City's premier destinations for dining, shopping, site seeing and is the heart of the city's nightlife. Located in the midtown, Westport is just north of the Country Club Plaza and a few miles south of downtown Kansas City. Historically, Westport was built along the Santa Fe Trail as an outfitting center for wagon trains heading west. Today the area is filled with renovated and new buildings housing trendy shops, restaurants, and nightspots.

Union Station
30 West Pershing Road, 816-460-2020
This fully restored 1914 landmark is Kansas City's most prominent destination for entertainment and cultural activities. The Station is home to a permanent rail exhibit with vintage rail cars, an interactive science center, a vibrant Theater District featuring giant screen movies and live theater, fine restaurants, unique shops, spaces for meetings and events and much more. Of course, you can still catch the train at Union Station, once again among Amtrak's busiest stops.
unionstation.org

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