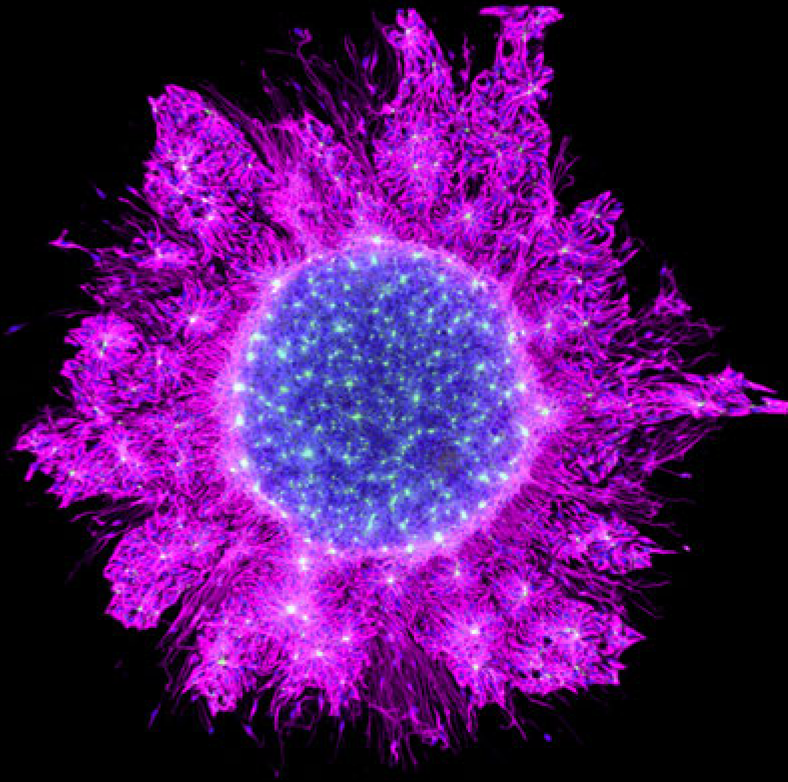
NYSTEM 2017



MAY 11 & 12

THE ROCKEFELLER UNIVERSITY

1230 York Avenue, New York, New York

Department of Health | Wadsworth Center | NYSTEM

NYSTEM 2017

May 11 & 12, 2017

Carson Family Auditorium
The Rockefeller University

PROGRAM COMMITTEE

Erika Bach, Ph.D., New York University Medical Center

Nicole Dubois, Ph.D., Icahn School of Medicine at Mount Sinai

Richard Gronostajski, Ph.D., University at Buffalo

Alexander Nikitin, M.D., Ph.D., Cornell University

Shahin Rafii, M.D., Weill Cornell Medical College

Viviane Tabar, M.D., Memorial Sloan-Kettering Cancer Center

ORGANIZING COMMITTEE

Kathy Chou, Ph.D., Scientific Officer, NYSTEM

Matthew Kohn, Ph.D., Scientific Officer, NYSTEM

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Gist Croft, Albert Ruzo, Szilvia Galgoski and Ali Brivanlou, the Rockefeller University

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GENERAL INFORMATION

Sessions
All sessions will take place in the Carson Family Auditorium.

Meals
Thursday's reception will take place in the lobby outside the Carson Family Auditorium. Thursday lunch and Friday breakfast and lunch will be served in the lobby outside the Carson Family Auditorium.

Posters
Odd numbered posters should be displayed throughout the day Thursday, presented during the evening reception, and taken down immediately following the reception. Even numbered posters should be displayed throughout the day Friday and presented at the Friday Poster Session, 11:10 AM – 12:30 PM. Posters must be removed at the end of the meeting.

Please refer to the Index of Poster Abstracts section of the program book for poster assignments.

The Program Book, including a final Participant List, will be posted on the NYSTEM website within several weeks of the meeting.

For more information about NYSTEM visit http://stemcell.ny.gov

PROGRAM SCHEDULE

THURSDAY, MAY 11, 2017

8:00 – 9:00 **REGISTRATION** and **POSTER I SETUP**

9:00 – 9:15 **OPENING REMARKS**

9:15 – 10:30 **PLENARY I**
Chair: Alex Nikitin, Cornell University

9:15 – 9:30 George Techiryan, University at Buffalo
Allogeneic Cardiosphere-Derived Cells Improve Infarct Remodeling and Left Ventricular Function When Given Via Global Intracoronary Infusion Early After Reperfusion in Swine with Myocardial Infarction

9:30 – 10:00 Alice Huang, Icahn School of Medicine at Mount Sinai
Identifying Mechanisms of Tendon Healing

10:00 – 10:30 Kateri Moore, Icahn School of Medicine at Mount Sinai
Elucidation of the Mechanisms of Hematopoietic Reprogramming

10:30 Poster Teaser #53: Shiwei Zheng, New York Genome Center
Unbiased Reconstruction of Early Fate Decisions in Human Hematopoiesis

BREAK

11:00 – 12:30 **PANEL DISCUSSION: REPRODUCIBILITY IN SCIENTIFIC RESEARCH**
Moderator: Erika Bach, New York University Medical Center

11:00 – 11:20 Daniel Klimmeck, *The EMBO Journal*

11:20 – 11:40 Dustin Graham, *Lab Animal* at Nature Research

11:40 – 12:00 Adam Marcus, *Retraction Watch* and *Gastroenterology & Endoscopy News*

12:00 – 12:30 Discussion

LUNCH and **POSTER VIEWING** in the atrium

1:30 – 3:30	**PLENARY II** Chair: Nicole Dubois, Icahn School of Medicine at Mount Sinai
1:30 – 1:45	Jianlong Wang, Icahn School of Medicine at Mount Sinai *RNA-Dependent Chromatin Targeting of TET2 for Endogenous Retroviral Control in Mammalian Cells*
1:45 – 2:00	Diogo Teles, Columbia University *Hypertrophy in a Human Tissue Chip*
2:00 – 2:30	Boris Reizis, New York University Medical Center *The Role of Hematopoietic Stem Cells in Steady-State Hematopoiesis*
2:30 – 3:00	Shahin Rafii, Weill Cornell Medical College *Conversion of Adult Endothelium into Long-Term Engraftable Immunocompetent Hematopoietic Stem Cells*
3:00 – 3:30	CONSORTIUM UPDATE Lorenz Studer & Viviane Tabar, Memorial Sloan Kettering Cancer Center *Developing a Human ES Cell Derived Dopamine Neuron Source for Cell Therapy in Parkinson's Disease*
3:30	Poster Teaser #39: Albert Ruzo, the Rockefeller University *Isogenic Huntington's Disease Embryonic Stem Cell Lines Reveal Novel Developmental Phenotypes*
	BREAK
4:00 – 5:30	**PLENARY III** Chair: Viviane Tabar, Memorial Sloan Kettering Cancer Center
4:00 – 4:30	Hans Snoeck, Columbia University Medical Center *Modeling Human Lung Development and Disease Using Pluripotent Stem Cells*
4:30 – 4:45	Dimitris Placantonakis, New York University School of Medicine *Low-Grade Astrocytoma Core Mutations in IDH1, p53 and ATRX Cooperate to Block Differentiation of Human Neural Stem Cells Via Epigenetic Repression of SOX2*
4:45 – 5:00	Holly Colognato, Stony Brook University *Ependymal Cell Dystroglycan Regulates Neural Stem Cell and Progenitor Function in Spatially Distinct Domains of the Subventricular Zone*

5:00 – 5:30 CONSORTIUM UPDATE
Steven Goldman, University of Rochester
Oligodendrocyte Progenitor Cell Delivery for Restoration of Function in Multiple Sclerosis: Development of a New York Consortium for the Treatment of Myelin Disease

5:30 Poster Teaser #1: Judith Agudo, Icahn School of Medicine at Mount Sinai
Jedi T Cells Reveal Quiescent Tissue Stem Cells Evade Immune Surveillance

– 7:00 **POSTER SESSION I** and **RECEPTION**

FRIDAY, MAY 12, 2017

8:30 AM **CONTINENTAL BREAKFAST** and **POSTER II SET-UP**

9:00 – 11:00 **PLENARY IV**
Chair: Richard Gronostajski, University at Buffalo

9:00 – 9:15 Qing Li, Memorial Sloan Kettering Cancer Center
Dissecting Human Endoderm Development Through a Genome-Wide CRISPR Screen in Human Embryonic Stem Cells

9:15 – 9:30 Shuangyu Ma, Columbia University
Restoration of Stem Cell Derived Beta-Cell Function from Permanent Neonatal Diabetes Patient by CRISPR/Cas9

9:30 – 10:00 Fraser Sim, University at Buffalo
M_3R Signaling Impairs Human and Rodent Progenitor-Based Myelin Repair

10:00 – 10:30 Iannis Aifantis, New York University Medical Center
3D Chromosomal Topology and Regulation of Adult Stem Cell Function

10:30 – 11:00 CONSORTIUM UPDATE
Sally Temple, Neural Stem Cell Institute
Retinal Stem Cell Consortium

11:00 – 11:10 Poster Teaser #22: Wenfei Kang, Albert Einstein College of Medicine
FGF Signaling Directs Neural Stem Cells in the Subventricular Zone Toward Oligodendrocyte Lineage and Improves Cell Regeneration After Demyelination

Poster Teaser #32: Thierry N'tumba-byn, Weill Cornell Medicine
Tyrosine Kinase Receptor EphB2 Loss-of-Function Alters Spermatogonial Stem Cell Activity

Poster Teaser #54: Jordana Lovett, Hunter College, CUNY
Compact Gene Regulatory Cassettes Support Key Aspects of T-Cell Receptor (TCR)-α Gene Locus Control Region (LCR) Activity

LUNCH and **POSTER SESSION II** in the atrium

12:30 – 1:30 **PLENARY V**
Chair: Shahin Rafii, Weill Cornell Medical College

12:30 – 1:00 Emmanuelle Passegué, Columbia University Medical Center
Hematopoietic Stem Cells in Regeneration, Disease and Aging

1:00 – 1:15 Daniel McIntyre, New York University Medical Center
Primordial Germ Cell Survival Depends on Contact with the Somatic Gonad in the C. elegans *Embryo*

1:15 – 1:30 Sophie M. Morgani, Memorial Sloan Kettering Cancer Center
Establishing a Pluripotent Stem Cell Culture System to Model Gastrulation

1:30 – 2:30 **KEYNOTE ADDRESS – AUSTIN SMITH**
Principles of Pluripotency

2:30 – 2:45 **CLOSING REMARKS** and **POSTER AWARDS**

2:45 **ADJOURN**

ALLOGENEIC CARDIOSPHERE-DERIVED CELLS IMPROVE INFARCT REMODELING AND LEFT VENTRICULAR FUNCTION WHEN GIVEN VIA GLOBAL INTRACORONARY INFUSION EARLY AFTER REPERFUSION IN SWINE WITH MYOCARDIAL INFARCTION

George Techiryan[1], Brian R. Weil[1], Gen Suzuki[1], Umesh C. Sharma[1], James A. Fallavollita[1,2], and John M. Canty, Jr[1,2].

[1]Division of Cardiovascular Medicine, University at Buffalo and the [2]VA WNY Healthcare System, Buffalo, NY

Background: Intracoronary administration of cardiosphere-derived cells (CDCs) is a novel strategy to protect the heart from reperfusion injury associated with acute myocardial infarction (MI). However, time-dependent changes in post-MI left ventricular (LV) remodeling following CDC treatment are poorly understood. We therefore utilized computed tomography (CT) to test the hypothesis that allogeneic CDCs favorably influence chronic LV remodeling when administered via global intracoronary infusion early after reperfusion in a porcine model of MI.

Methods: Immunosuppressed swine (cyclosporine 100mg/day, n=15) underwent a 60-minute LAD occlusion to produce MI. After 30-minutes of reperfusion, animals were randomized to receive vehicle or 20x10^6 allogeneic CDCs via global intracoronary infusion in a blinded fashion. Parameters of infarct remodeling and LV function were assessed via CT 2-days and 1-month post-MI. Post-mortem histopathological assessment of myocyte morphometry, proliferation (Ki67), and apoptosis (TUNEL) was also performed.

Results: Despite a similar reduction in infarct mass from 2-days to 1-month post-MI, a markedly different pattern of infarct remodeling was observed in each group. Vehicle-treated animals exhibited detrimental changes in infarct circumferential extent and transmurality, while CDC treatment tended to reduce both parameters. Histopathological analysis revealed increased myocyte proliferation and reduced myocyte apoptosis in viable myocardium of CDC-treated animals, which resulted in preservation of myocyte number in this region. This favorable pattern of infarct remodeling with CDC treatment was associated with enhanced regional wall thickening and a significant improvement in LV ejection fraction.

Conclusion: Global intracoronary delivery of allogeneic CDCs early after reperfusion promotes favorable changes in infarct remodeling during the month following MI without affecting total infarct mass. This effect is associated with an increased number of myocytes in viable myocardium and improved LV contractile function, supporting the use of allogeneic CDCs as a therapeutic tool to effectively ameliorate adverse LV remodeling after MI.

	Vehicle (n=7)			Allogeneic CDCs (n=8)		
	2 days post-MI	1 month post-MI	Δ	2 days post-MI	1 month post-MI	Δ
Infarct Mass (g)	13.1 ± 2.0	10.6 ± 1.8	-2.5 ± 0.7	10.6 ± 2.1	8.2 ± 1.6	-2.4 ± 1.5
Infarct Circumferential Extent (%)	33.7 ± 4.5	37.1 ± 4.2	3.4 ± 1.6	32.2 ± 4.1	27.6 ± 4.8	-4.5 ± 2.2†
Infarct Transmurality (%)	56.8 ± 8.4	57.1 ± 7.8	0.3 ± 2.8	54.8 ± 7.6	44.5 ± 8.1	-10.3 ± 5.0#
Infarct Segmental Wall Thickening (%)	4.4 ± 6.4	15.6 ± 8.1	11.1 ± 5.1	9.1 ± 6.5	30.5 ± 9.2*	21.4 ± 4.9
Remote Segmental Wall Thickening (%)	38.2 ± 2.2	38.6 ± 3.7	0.4 ± 3.4	36.1 ± 4.6	47.9 ± 4.2*	11.8 ± 3.4†
LV Ejection Fraction (%)	53.0 ± 2.2	50.7 ± 4.1	-2.3 ± 3.1	53.2 ± 3.0	57.7 ± 2.7*	4.5 ± 1.6†

Values are mean ± SEM; *p<0.05 vs. 2 days post-MI; †p<0.05 vs. Vehicle-Treated; #p=0.08 vs. Vehicle-Treated

IDENTIFYING MECHANISMS OF TENDON HEALING

Kristen Howell, Chun Chien, and Alice H. Huang

Department of Orthopaedics, Icahn School of Medicine at Mount Sinai

Adult tendon heals via fibrosis (scarring), and this failure to re-establish native tendon structure is likely the leading cause of injury recurrence. To date, most models of tendon injury are models of poor, fibrotic healing, which limits the ability to identify regenerative mechanisms. To establish a model of regenerative mammalian tendon healing, we therefore turned to the neonatal mouse, which is able to regenerate normally non-regenerative tissues when injured within a narrow window of time after birth (P0-P7). Using a simple and reproducible model of Achilles tendon transection, we show that functional properties are fully restored during neonatal tendon healing (gait and tensile mechanical properties) at 56 days after injury, in contrast to adult tendon function, which remains significantly impaired. Analysis of the ScxGFP tendon reporter showed early proliferation of ScxGFP+ tenocytes near the cut ends at day 3, followed by formation of a ScxGFP+ neo-tendon structure bridging the two tendon ends by day 14. Real-time qPCR analysis revealed tendon-specific differentiation from day 14 onward, with no aberrant expression of chondrogenic, osteogenic, or adipogenic markers. In contrast, adult healing is mediated by formation of ScxGFP-negative scar tissue, absence of tendon differentiation markers, enhanced deposition of non-tenogenic, scar-associated molecules, and aberrant differentiation toward cartilage and bone. Lineage tracing with an inducible Scx-CreERT2 to label tenocytes prior to injury showed that neonatal regeneration is mediated by activation and recruitment of Scx-lineage tenocyes while adult healing is driven by extrinsic cells. To test the molecular regulation of tendon recruitment during healing, we targeted key molecules in the TGFbeta and BMP signaling pathways, which have been implicated in tendon induction and repression during development, respectively. Deletion of TGFbR2 (the key receptor for TGFbeta signaling) using ScxCreERT2 prior to injury revealed loss of tenocyte recruitment during neonatal healing, while deletion of Smad4 resulted in strong recruitment of tenocytes during adult healing. Surprisingly, aberrant differentiation toward bone was still observed in Smad4/ScxCreERT2 mutants, which further suggests that the cellular origin of ectopic cartilage/bone formation may be extrinsic to tendon. Collectively, these studies establish the neonatal mouse as an exciting model of tendon regeneration, which can identify current limitations in adult healing and inform the development of therapeutic strategies to treat tendon injuries in adults.

ELUCIDATION OF THE MECHANISMS OF HEMATOPOIETIC REPROGRAMMING

Kateri Moore

Icahn School of Medicine at Mount Sinai

Hematopoietic stem cell (HSC) transplantation is widely used to treat a variety of disorders. Despite advances in the use of umbilical cord blood and mobilized stem cells, donor material remains limited. This is due to insufficient numbers of stem cells in cord blood, poor mobilization, and the lack of ethnic diversity to provide sufficient genetically matched material. Despite intensive efforts there has been limited success in generating transplantable HSCs from pluripotent stem cells (PSCs). Clearly, alternative approaches are necessary. Directly programmed hematopoietic stem/progenitor cells would provide an unlimited patient-specific source for cell replacement and genetic correction therapies as well as a platform for the future generation of patient specific therapeutics and blood products. In 2013 we demonstrated direct reprogramming of mouse fibroblasts into clonogenic hematopoietic progenitors with just four transcription factors (TFs), Gata2, Gfi1b, cFos and Etv6 (Pereira et al Cell Stem Cell). These four TFs induce a dynamic, multi-stage hemogenic process that progresses through an endothelial-like intermediate. As such, it appears to recapitulate definitive developmental hematopoiesis in vitro. We now have strong evidence that a similar hemogenic process can be optimally induced in human fibroblasts with Gata2, Gfi1b, and cFos. These reprogrammed cells are able to multi-lineage repopulate NSG mice. Therefore, we maintain that in vitro reprogramming provides a tractable system to address the underlying molecular mechanisms of hemogenesis not possible in primary cells. We have now studied how the TFs bind DNA and initiate a molecular program that changes the epigenetic landscape to allow a change in cell fate. The studies to date have revealed cooperative binding of two of the factors to the binding motif of the other causing simultaneous silencing of fibroblast genes and activation of endothelial and hematopoietic genes.

PANEL DISCUSSION: REPRODUCIBILITY IN SCIENTIFIC RESEARCH

Moderated by Erika Bach, New York University Medical Center

- **Dustin Graham, Chief Editor, *Lab Animal* at Nature Research**

Dustin Graham received his PhD in Neuroscience from Brown University, studying phototransduction in melanopsin ganglion cells in David Berson's lab. He then moved to University of Virginia and into *in vivo* research, studying the circuitry and population dynamics of gustatory cortical neurons in awake-behaving mice. Dustin joined Nature Research in 2015, taking over Lab Animal and managing its relaunch on Nature.com.

- **Daniel Klimmeck, Editor, *The EMBO Journal***

Daniel received his PhD in 2008 with work on ion channel signaling in sensory neurons in the laboratory of Stephan Frings at the University of Heidelberg. As a postdoc, he focused on the molecular characterization of cancer and hematopoietic stem cells with Andreas Trumpp at the DKFZ and Jeroen Krijgsveld at EMBL. Daniel joined The EMBO Journal in 2015.

The peer reviewed research paper remains the main conduit for the exchange of research discoveries. With the diversification and growth of global research, publishing in selected journals and citation metrics are increasingly employed as surrogates for quality in research assessment. Consequently, the pressure to publish in a handful of journals has increased, putting the peer review system under strain.

I will summarize EMBO’s organizational aim and activities and provide an overview of the four journals published by EMBO as an academic organization (*The EMBO Journal*/*EMBOreports*/*MSB*/*EMBO Mol Medicine*). Importantly, the Editorial Process as run at *The EMBO Journal* will be explained, thus outlining function and daily routines of scientific editors. Finally, I will present a number of policies that EMBO has implemented under its 'transparent peer review' scheme and that ensure a fast, fair and informed editorial process.

- **Adam Marcus, co-founder of *Retraction Watch*, Managing Editor of** *Gastroenterology & Endoscopy News*

Adam Marcus is co-founder of Retraction Watch and The Center for Scientific Integrity, and managing editor of *Gastroenterology & Endoscopy News*. Marcus, along with colleague Ivan Oransky, founded The Center for Scientific Integrity and Retraction Watch to promote transparency and integrity in science and scientific publishing. His articles have appeared in *Science*, *The New York Times, The Wall Street Journal, The Economist* and many other publications. He is a graduate of the University of Michigan and Johns Hopkins University, where he teaches science writing.

RNA-DEPENDENT CHROMATIN TARGETING OF TET2 FOR ENDOGENOUS RETROVIRUS CONTROL IN MAMMALIAN CELLS

Diana Guallar[1,2], Xianju Bi[3], Xin Huang[1,2], Carmen Saenz-Ausejo[1,2], Junjun Ding[1,2], Francesco Faiola[1,2], Dan Li[1,2,4], Carlos Sanchez-Priego[1,2,4], Arven Saunders[1,2,4], Feng Pan[5], Kevin Kelley[2], Mingjiang Xu[5], Miguel Fidalgo[1,2], Xiaohua Shen[3,*] and Jianlong Wang[1,2,4]

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[2]Department of Cell, Developmental and Regenerative Biology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA.
[3]Tsinghua-Peking Center for Life Sciences, School of Medicine, Tsinghua University, Beijing 100084, China.
[4]The Graduate School of Biomedical Sciences, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA.
[5]Sylvester Comprehensive Cancer Center, Department of Biochemistry and Molecular Biology, University of Miami, Miami, FL 33136, USA.

Ten-eleven translocation (TET) proteins maintain appropriate patterns of gene expression through epigenetic mechanisms that are relevant both in early embryonic development and in diseases, including cancer and neurodegenerative disorders. All TET family members play key roles in the regulation of the methylation status of DNA through oxidizing methyl groups in cytosines (5mC), generating intermediates such as 5-hydroxymethylcytosines (5hmC) that can both serve as stable epigenetic marks and participate in active demethylation. Given the substantial contribution of TET proteins to mammalian gene regulation and chromatin dynamics, understanding the molecular mechanisms mediating their functions is important for dissecting the complex processes involved in development and disease. Unlike the other TET-family members, TET2 does not contain a DNA-binding domain, and it remains unclear how it is recruited to chromatin. Here we show that TET2 is recruited by the RNA-binding protein Paraspeckle component 1 (PSPC1) through transcriptionally active endogenous retroviruses (ERVs). ERVs are remnants of ancestral viral infections that have been co-opted by mammalian genomes as stage- and tissue-specific transcriptional regulatory modules. We find that PSPC1 and TET2 contribute to ERV and ERV-associated gene regulation by both transcriptional repression via histone deacetylases and post-transcriptional destabilization of ERV RNAs through 5hmC modification. Our findings reveal a critical role for RNA in effecting TET2 function at the chromatin level, and provide evidence for a functional role of transcriptionally active ERVs as specific docking sites for RNA epigenetic modulation and gene regulation. Our study provides a new paradigm for post-transcriptional silencing of retroviral RNAs via 5hmC modification by RNA-binding protein mediated TET2 recruitment. Since ERV reactivation has been widely related to aging, cancer, and autoimmune diseases, our findings should also open new avenues for exploring ERV control by PSPC1 and TET2 in health and disease.

[Supported by NYSTEM contracts C028103 and C028121].

MODELING OF CARDIAC HYPERTROPHY IN A HUMAN TISSUE CHIP

Diogo Teles[1,2,3], Kacey Ronaldson[1], Alan Chramiec[1], Luke Hao[1], Jorge Correia-Pinto[2,3] and Gordana Vunjak-Novakovic[1,4]

[1]Laboratory for Stem Cells and Tissue Engineering, Department of Biomedical Engineering, Columbia University, New York, NY [2]Life and Health Sciences Research Institute, School of Medicine, University of Minho, Braga, Portugal [3]ICVS/3B's, PT Government Associate Laboratory, Braga/Guimarães, Portugal [4]Department of Medicine, Columbia University, New York, NY

The development of functional in-vitro tissue chips, combining biological fidelity with high-throughput experimentation for physiologically relevant predictions, are now enabling to model the "human body in a dish". Recent advances have the potential to revolutionize drug development towards the next era of precision based medicine. The environmental influences are of particular interest as they contribute to the functionality of engineered tissues, in health and throughout the initiation and progression of disease.

We developed a model of cardiac hypertrophy using engineered human cardiac tissues derived from patient-specific induced pluripotent stem cells, a disease acquired upon exposure to specific environmental conditions. We were able to demonstrate hypertrophy associated reversal of the positive force-frequency relationship and calcium-induced calcium response.

In order to faithfully recapitulate the disease phenotype, we investigated the differences between hypertrophic tissues derived from male and female cells, a component largely ignored in current tissue chip models. We demonstrate the importance of incorporating sex based hormones into tissue chips by showing the increased contractile duration and increased cardiotoxicity of Cisapride in female versus male cardiac tissues. Finally, we investigated how the male vs female derived cells and the presence of their respective sex hormones influence the behavior of engineered cardiac and bone tissues *in vitro*.

The development of sexualized tissue chip systems tissue chip systems for modeling disease that incorporate sex based hormones enables physiologically relevant manipulation and can provide valuable insight into the physiology of disease and organ-organ interactions.

[Supported by NYSTEM (contract C028119), NIH (grants UH3EB17103 and 3UH3EB017103-05S1) and FCT (PD/BD/105819/2014)].

THE ROLE OF HEMATOPOIETIC STEM CELLS IN STEADY-STATE HEMATOPOIESIS

Samik Upadhaya, Catherine M. Sawai and Boris Reizis

Dept. of Pathology, New York University School of Medicine, New York, NY 10032

Hematopoietic stem cells (HSCs) sustain long-term multilineage reconstitution of hematopoiesis when transplanted into myeloablated recipients. However, the role of HSCs in the endogenous steady-state hematopoiesis remains unclear. In particular, recent studies suggested that HSCs provide a relatively minor contribution to immune cell development in adults. To address this question, we undertook tamoxifen-inducible genetic labeling of HSCs in otherwise unperturbed recipients, and performed longitudinal studies of the contribution of labeled cells to hematopoiesis. The labeled HSCs (~30% of the HSC population conventionally defined by phenotypic markers) had the most immature phenotype and increased label-retaining capacity and localized in the perivascular space. Importantly, these labeled cells gave rise to other phenotypic HSCs, confirming their top position in the differentiation hierarchy and yielding >80% labeling of the total HSC population after ~6 months. The labeled HSCs rapidly contributed to committed progenitors of all lineages and to mature myeloid cells and lymphocytes, but not to embryo-derived cells such as tissue macrophages. By 6-9 month, the labeled HSCs gave rise to more than two-thirds of all myeloid cells and platelets, approaching but never equaling the fraction of labeled HSC. The contribution of HSCs could be accelerated by an induced interferon response, which also resulted in a full equilibration of HSC and peripheral cell labeling. Collectively, our data suggest that classically defined HSCs serve as a major sustained source of hematopoiesis in adult animals. Additional recent data that further confirm this notion and delineate the earliest steps of HSC differentiation will be presented.

CONVERSION OF ADULT ENDOTHELIUM INTO LONG-TERM ENGRAFTABLE IMMUNOCOMPETENT HEMATOPOIETIC STEM CELLS

Raphael Lis, Charles C. Karrasch, Michael G. Poulos, Balvir Kunar, David Redmond, Jose G. Barcia Duran, Chaitanya Badwe, Michael Ginsberg, Jenny Xiang, Koji Shido, Olivier Elemento, Jason M. Butler, Joseph Scandura, and Shahin Rafii.

Weill Cornell Medicine, New York, NY 10065, USA

The microenvironmental cues that choreograph the transition of endothelial cells (ECs) into true engraftable haematopoietic stem cells (HSCs) and progenitor cells (HSPCs) remain undefined. This is due to lack of models to recreate the ephemeral transition from endothelial to haemogenic cells and subsequently to HSCs. Here, we have developed a pluripotent-independent sequential tractable *in vitro* model in which by conditional on-off expression of transcription factors: *FosB*, *Gfi1*, *Runx1*, and *Spi1 (FGRS)*; and stimulation with angiocrine signals from the vascular niche, we were able to reprogram adult mouse ECs into true HSCs (rEC-HSCs) over a 28-day period. From day 0-8 (induction phase), *FGRS* are conditionally expressed in adult non-lymphatic ECs isolated from *Runx1*-IRES-GFP reporter mice and co-cultured with vascular niche. At specification phase (day 8-20), *FGRS*-transduced $VEcad^{+}Runx1^{-}CD45^{-}$ ECs switch on the endogenous expression of *Runx1,* initiating haematopoietic program and silencing EC fate in $VEcad^{+}Runx1^{+}CD45^{+}$ cells, setting the stage for full commitment to $VEcad^{-}Runx1^{+}CD45^{+}$ haematopoietic stem and progenitor cells (rEC-HSPCs). During expansion phase (day 20-28), vascular niche-derived angiocrine signals promote propagation and self-renewal of a large number of haematopoietic cells, at which time expression of exogenous *FGRS* is turned off. Subsets of rEC-HSPCs are endowed with distinctive features of engraftable rEC-HSCs, including single cell clonal and limiting-dilution multi-lineage reconstituting potential, ability to serially re-establish long-term primary and secondary marrow multi-lineage HSPC compartments, reconstitute adaptive immune function in $Rag1^{-/-}$ mice, and long-term self-renewal capacity. Molecular profiling shows that rEC-HSCs adopt the signature of adult HSCs, with complete erasure of the vascular genes and activation of the haematopoietic program. Employing *Runx1*-IRES-GFP reporter mouse enabled deconvolution of stage-specific pathways involved in generation of engraftable rEC-HSCs. Inhibition of Tgfβ signaling along with activation of Bmp and Cxcl12 pathways reinforced the induction phase. Cxcr4 deficient ECs failed to undergo conversion to rEC-HSPCs, indicating that Cxcl12 signaling is essential for specification and expansion phases. The conversion process is highly efficient with 70,000 $Runx1^{+}$ *FGRS*-transduced ECs, capable of giving birth to ~1500±610 (1500/70,000, 2.1%) authentic rEC-HSCs. This stepwise conversion method provides a tractable platform to decipher pathways involved in the transition of ECs into haematopoietic cells. Our approach also facilitates devising strategies to reprogram adult ECs into abundant autologous HSCs amenable to genetic modification for treatment of haematological disorders.

Lis R et al, *Nature*, In Press, 2017

CONSORTIUM UPDATE: DEVELOPING A HUMAN ES CELL DERIVED DOPAMINE NEURON SOURCE FOR CELL THERAPY IN PARKINSON'S DISEASE

Lorenz Studer & Viviane Tabar

Memorial Sloan Kettering Cancer Center

Parkinson’s disease (PD) is the second most common neurodegenerative disorder and is estimated to affect more than four million patients worldwide – a number predicted to more than double by 2030. A fundamental characteristic of PD is progressive, severe, and irreversible loss of specific dopamine-producing neurons (DA neurons) in the midbrain that ultimately may result in disabling motor dysfunction. Multiple therapies have been developed for PD, but none can replace the lost cells. Cell transplantation has been considered a promising therapy, but in spite of extensive efforts to develop it in laboratories across the world, this approach has faced multiple challenges, including the absence of an appropriate cell source that can match the lost cells in function and safety.

In 2011, our team made a major discovery that enables the derivation of nearly unlimited numbers of authentic, engraftable midbrain DA neurons from human embryonic stem cells (hESCs). We have since demonstrated that these cells can survive in three independent PD models and can reverse motor deficits of the disease. In addition, the cells have an excellent safety profile with no evidence of tumor or excessive growth in any of the animals tested.

We anticipate that by the end of the project period in 2017, our team will be ready to submit an Investigational New Drug (IND) application to the US Food and Drug Administration for a clinical trial in Parkinson’s patients. The team consists of scientists, neurologists, surgeons, industry leaders, ethicists, trial experts, and patient advocates who are dedicated to the achievement of this goal. The project further harnesses the expertise and strength present within Memorial Sloan Kettering at the Center for Cell Engineering (CCE) and the Center for Stem Cell Biology (CSCB) to deliver a first-in-man embryonic stem cell therapy for PD.

MODELING HUMAN LUNG DEVELOPMENT AND DISEASE USING PLURIPOTENT STEM CELLS

Hans Snoeck

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Recapitulation of lung development from human pluripotent stem cells (hPSCs) in three dimensions (3D) would allow deeper insight into human development, as well as the development of innovative strategies for disease modeling, drug discovery and regenerative medicine. We report here the generation from hPSCs of lung bud organoids (LBOs) that contain mesoderm and pulmonary endoderm and develop into branching airway and early alveolar structures after xenotransplantation and in Matrigel 3D culture. Expression analysis and structural features indicated that the branching structures reached the second trimester of human gestation. Infection *in vitro* with respiratory syncytial virus, which causes small airway obstruction and bronchiolitis in infants, led to swelling, detachment and shedding of infected cells into the organoid lumens, similar to what has been observed in human lungs. Introduction of mutation in HPS1, which causes an early-onset form of intractable pulmonary fibrosis, led to accumulation of extracellular matrix and mesenchymal cells, suggesting the potential use of this model to recapitulate fibrotic lung disease *in vitro*. LBOs therefore recapitulate lung development and may provide a useful tool to model lung disease.

pLOW-GRADE ASTROCYTOMA CORE MUTATIONS IN IDH1, P53 AND ATRX COOPERATE TO BLOCK DIFFERENTIATION OF HUMAN NEURAL STEM CELLS VIA EPIGENETIC REPRESSION OF SOX2

Aram S. Modrek[1], Danielle Golub[1], Themasap Khan[1], Jod Prado[1], Christopher Bowman[2], Jingjing Deng[3], Guoan Zhang[3], Pedro P. Rocha[2], Ramya Raviram[2], Harris C. Lazaris[2,4], James Stafford[5], Gary LeRoy[5], Michael Kader[1], Joravar Dhaliwal[1], N. Sumru Bayin[1], Joshua Frenster[1,6], Jonathan Serrano[2], Luis Chiriboga[2], Rabaa Baitalmal[2], Gouri Nanjangud[7], Andrew S. Chi[8,9,10], John G. Golfinos[1,9,10], Jing Wang[11], Matthias Karajannis[12,13], Richard A. Bonneau[14,15,16], Danny Reinberg[5,17], Aristotelis Tsirigos[2,4], David Zagzag[1,2,9,10], Matija Snuderl[2,8,10], Jane A. Skok[2], Thomas Neubert[3], and Dimitris G. Placantonakis[1,6,9,10].

Departments of [1]Neurosurgery, [2]Pathology, [3]Cell Biology, [5]Biochemistry and Molecular Pharmacology, [8]Neurology, [11]Department of Anesthesiology, [12]Pediatrics, [13]Otolaryngology, NYU School of Medicine, New York, NY 10016, USA [4]Applied Bioinformatics Center, NYU School of Medicine, New York, NY 10016, USA. [6]Kimmel Center for Stem Cell Biology, NYU School of Medicine, New York, NY 10016, USA [7]Molecular Cytogenetics Core Facility, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA [9]Laura and Isaac Perlmutter Cancer Center, NYU School of Medicine, New York, NY 10016, USA. [10]Brain Tumor Center, NYU School of Medicine, New York, NY 10003, USA. [16]Simons Center for Data Analysis, New York, New York, 10010, USA. [17]Howard Hughes Medical Institute, Chevy Chase, MD 20815, USA

To model low-grade astrocytoma (LGA) formation, we introduced R132H IDH1, P53 shRNA and ATRX shRNA in human neural stem cells (NSCs). These oncogenic hits blocked NSC differentiation, increased invasiveness *in vivo* and led to an epigenetic and transcriptional profile resembling IDH1-mutant human LGAs. The differentiation block was caused by transcriptional silencing of transcription factor *SOX2*. Ectopic expression of SOX2 was sufficient to rescue the differentiation block. We found that *SOX2* down-regulation was secondary to disassociation of its promoter from a putative long-range enhancer ~700kb downstream of *SOX2*. This occurred due to reduced binding of the chromatin organizer CTCF to its methylated DNA motifs and disrupted chromatin looping. Our human model of IDH-mutant LGA implicates impaired NSC differentiation due to epigenetic repression of *SOX2* as an early driver of gliomagenesis.

Supported by NIH/NINDS R03NS087349

EPENDYMAL CELL DYSTROGLYCAN REGULATES NEURAL STEM CELL AND PROGENITOR FUNCTION IN SPATIALLY DISTINCT DOMAINS OF THE SUBVENTRICULAR ZONE

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The subventricular zone (SVZ) neural stem cell niche is important to brain development and homeostasis but many of the mechanisms that regulate SVZ development remain unclear. In particular, the role of extracellular matrix (ECM)-mediated signals in shaping the development and function of the postnatal SVZ remain poorly understood despite the recognized role for ECM in adult stem cell niches. Loss of function of the ECM receptor dystroglycan is responsible for a class of muscular dystrophies termed "dystroglycanopathies", which also have profound deficits in brain structure and function in which the cell and molecular etiology remains unclear. However, we recently reported that dystroglycan is a key regulator of neural stem cell function in the perinatal SVZ, where it suppresses notch signaling in neural stem cells, niche supporting ependymal cells, and oligodendrocyte progenitor cells. Additionally, SVZ dystroglycan reorganizes ECM into laminin-rich "hubs" at the interface between neural stem cells and ependymal cells at the ventricular surface during niche construction. It remained unclear, however, whether niche stem- and ependymal cell dystroglycan had distinct roles in regulating SVZ form and function. We now report that ependymal cell dystroglycan has a role in regulating ventricular zone ECM architecture, and that the loss of dystroglycan in ependymal cells engenders non-cell autonomous alterations in SVZ proliferation in spatially distinct domains, alterations in neural stem cell morphology, and alterations in ependymal cell development. We also find that dystroglycan may play distinct and, at times, opposing roles in neural stem cells and ependymal cells in order to promote orderly development of the SVZ and maintain SVZ neural stem cell homeostasis in the postnatal period. Our findings shed new light on mechanisms of ECM remodeling in the SVZ and roles that ependymal cell-ECM interactions play in regulating stem cell function, as well as provide insight into potential neural stem cell dysfunction in dystroglycanopathies.

CONSORTIUM UPDATE: OLIGODENDROCYTE PROGENITOR CELL DELIVERY FOR RESTORATION OF FUNCTION IN MULTIPLE SCLEROSIS: DEVELOPMENT OF A NEW YORK CONSORTIUM FOR THE TREATMENT OF MYELIN DISEASE

Steven Goldman

University of Rochester

Myelin is the insulation around nerves that is damaged in the nervous system of Multiple Sclerosis (MS) patients. Early in the disease there are recurrent attacks of neurological dysfunction followed by recovery, referred to as relapsing-remitting MS (RRMS). There are medicines to treat RRMS. However, they only decrease disease activity by 30 to 67%. Thus, a large number of patients continue to worsen. In mid to late stages of the disease slow progression occurs, referred to as secondary progressive MS (SPMS). SPMS is thought to occur because the nerve (axon) has lost its myelin and begins to die. There is no specific therapy for SPMS. It is also known that the cells that make myelin, the oligodendroglia, are destroyed by inflammation during acute attacks. Our consortium aims to test the clinical hypothesis that immature oligodendroglia, called human oligodendroglial progenitor cells (hOPCs), will improve neurologic functions after brain transplantation.

Our consortium is focused on achieving all steps needed to initiate clinical trials of human oligodendrocyte progenitor cell grafts for the treatment of SPMS. Thus, the development of a therapy for this type of MS will have a major impact on the daily lives of these patients.

DISSECTING HUMAN ENDODERM DEVELOPMENT THROUGH A GENOME-WIDE CRISPR SCREEN IN HUMAN EMBRYONIC STEM CELLS

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Forward genetic screens have been instrumental for understanding lineage decisions during embryonic development. However, the screening approach used in model organisms cannot be directly extended to humans. Human embryonic stem (ES) cells are uniquely suitable for interrogating human development and birth defects with high-throughput genetic manipulation. Combining human ES cell differentiation with the CRISPR/Cas technology, we have conducted a genome-wide knockout screen for genes that cell-autonomously regulate the formation of human definitive endoderm (DE), which gives rise to most of the cells in respiratory and gastrointestinal organs including the lung, pancreas and liver. Our screen identified previously known as well as unknown regulators of endoderm differentiation. We further report that the MEKK1-MKK4/7-JNK1-JUN signaling axis acts as a previously unrecognized inhibitory pathway that constrains endoderm formation. JNK inhibitor treatment significantly improves the efficiency of ES cell differentiation to the endoderm lineage, which may be harnessed for creating therapeutically relevant endoderm-derived cells.

[Supported by NYSTEM, contract # pending]

RESTORATION OF STEM CELL DERIVED BETA-CELL FUNCTION FROM PERMANENT NEONATAL DIABETES PATIENTS BY CRISPR/CAS9

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Permanent neonatal diabetes mellitus (PNDM) can be caused by monogenic mutations in KCNJ11, ABCC8 or insulin. Here we described a novel mutation in the translation start site of the insulin gene in a patient with PNDM resulting in undetectable serum insulin at birth. Induced pluripotent stem cells (iPSCs) were generated by reprogramming somatic cells from a skin biopsy of the patient. Using differentiation to stem cell derived beta cells (sc-beta cells), we confirmed the absence of insulin production in sc-beta cells expressing MAFA, Nkx6.1, and synaptophysin. We corrected the mutation in patient iPSCs with CRISPR/Cas9 mediated ssDNA replacement. Upon differentiation to sc-beta cells, we found that insulin production and stimulated secretion was restored to levels comparable to wildtype cells. The functional testing of gene-corrected sc-beta cells in mice is ongoing. Therefore, our study provides proof of principle for the generation of genetically corrected sc-beta cells for patients with PNDM. Because of the lack of an auto-immunity, insulin dependent diabetes caused by single gene mutations should be amenable to cell therapy.

M_3R SIGNALING IMPAIRS HUMAN AND RODENT PROGENITOR-BASED MYELIN REPAIR

Fraser Sim

University at Buffalo

Muscarinic receptor antagonists act as potent inducers of oligodendrocyte differentiation and accelerate remyelination. However, the use of muscarinic antagonists in the clinic is limited by poor understanding of the operant receptor subtype, and questions regarding possible species differences between rodents and humans. Based on high selective expression in human oligodendrocyte progenitor cells (OPCs), we hypothesized that M_3R is the functionally relevant receptor. Lentiviral M_3R knock-down in human primary CD140a/PDGFaR$^+$ OPCs resulted in enhanced differentiation *in vitro* and substantially reduced the calcium response following muscarinic agonist treatment. Importantly, following transplantation in hypomyelinating *shiverer/rag2* mice, M_3R knock-down improved myelin repair by human OPCs. Furthermore, conditional M_3R ablation in adult NG2-expressing OPCs increased oligodendrocyte differentiation and led to improved spontaneous remyelination in mice. Together, these results demonstrate that M_3R receptor mediates muscarinic signaling in human and rodent OPCs that act to delay differentiation and myelin repair and suggest that M_3 receptors represent viable targets for human demyelinating disease.

[Supported by National Multiple Sclerosis Society and NYSTEM contract C028108].

3D CHROMOSOMAL TOPOLOGY AND REGULATION OF ADULT STEM CELL FUNCTION

Iannis Aifantis

New York University Medical Center

CONSORTIUM UPDATE: RETINAL STEM CELL CONSORTIUM

Sally Temple

Neural Stem Cell Institute

We previously showed that the adult human retina contains a rare population of stem cells capable of regenerating the retinal pigment epithelium (RPE), an essential support cell for the photoreceptors of the neural retina. These RPESCs can be extracted from cadaver eyes donated to eye banks and efficiently expanded into over a billion cells per donor, currently being done under GMP conditions in preparation for a clinical trial. We have demonstrated that RPESC-derived RPE cells are effective at preventing vision loss in the Royal College of Surgeons rat. Importantly, a particular stage of RPESC is most efficacious to preserve vision: neither very early proliferating RPESCs cells nor mature RPE cells are as effective as an intermediate RPESC-RPE cell stage. In this regard, RPESC-RPE are similar to other stem cell products applied to degenerative neural conditions in exhibiting stage-dependent efficacy. We have identified a novel long non-coding RNA, the expression of which tracks with potency of the product. RPESC-RPE have potential as a transplantable therapeutic cell to counter RPE loss due to degenerative conditions such as age-related macular degeneration (AMD). However, as this unique cell is present within the human retina throughout adulthood, even in nonagenarians, and in patients with AMD, an exciting possibility is to activate the RPESC in vivo to promote endogenous repair for degenerative eye conditions.

HEMATOPOIETIC STEM CELLS IN REGENERATION, DISEASE AND AGING

Emmanuelle Passegué

Columbia University Medical Center

PRIMORDIAL GERM CELL SURVIVAL DEPENDS ON CONTACT WITH THE SOMATIC GONAD IN THE C ELEGANS EMBRYO

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We are studying the formation of the germline stem cell niche in the *C. elegans* gonad, and are investigating how niche cell contact promotes stem cell survival. Niche-derived signals often control stem cell quiescence, proliferation and differentiation. As such, formation of the niche is a critical event in development. In *C. elegans*, the gonad primordium is a simple, easily observed organ consisting of just four cells – two primordial germ cells (PGCs), and two somatic niche cells (SGPs). The primordium forms via a fascinating morphogenetic process in which the two SGPs migrate posteriorly, recognize the PGCs, and surround the PGC cell body. Using genetics, fluorescent imaging and embryological manipulations we are testing the hypothesis that either signaling or adhesion between SGPs and PGCs is needed for germ cells to survive. Previously, it was reported that ablation of the SGPs resulted in a loss of the primordial germ cells. However, no mechanism has been reported for this phenomenon. We began by repeating this experiment using fluorescent reporters to follow cells in the primordium. We too found the PGCs are frequently lost in these animals. Surprisingly, we also observed that unprotected PGCs were engulfed by neighboring endodermal cells. We next repeated these ablations in mutants lacking endoderm. PGCs survived in these mutants. Previously, our lab showed that PGCs produce lobes that are pinched off and digested by endodermal cells. This process requires the coordinated activity of CED-10, LST-4 and DYN-1. We have shown that this pathway is also needed for death of PGCs following SGP ablation, which suggests SGP contact protects the PGC cell body, ensuring only the lobe is eaten by the endoderm. We are testing if SGP wrapping protects the PGCs by blocking the cytoskeletal re-arrangements needed for wrapping in the SGPs. Additionally, we are testing if niche cells ability to protect germ cells requires E-Cadherin and if so whether it acts through an adhesion or signaling based mechanism.

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ESTABLISHING A PLURIPOTENT STEM CELL CULTURE SYSTEM TO MODEL GASTRULATION

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Gastrulation is a paradigm for coordinated cell fate specification whereby the germ layers are specified and patterned providing a blueprint for the adult body. However, research on human embryonic material at this stage is not possible and the implanted mouse embryo is relatively inaccessible for long-term experiments. Hence, little is known about the dynamics of these events. Recently, an *in vitro* micropatterning system (MPS) using human embryonic stem cells (ESCs) demonstrated both differentiation and spatial patterning of germ layers, similar to events occurring *in vivo* during gastrulation. To determine whether this system accurately recapitulates mammalian embryonic development, I have established a comparable system using mouse pluripotent stem cells. To determine whether the identity and time of emergence of differentiated populations *in vitro* is comparable to those *in vivo*, I have compared this MPS to gastrulating mouse embryos at discrete stages. I have started to characterize the role and temporal requirements of individual cytokines for differentiation and patterning and will take advantage of fluorescent reporter lines and genetic tools to analyze these events in real time at a single cell level. This system offers a high-throughput, quantitative and easily manipulated tool to model gastrulation-like events *in vitro*.

Keynote Address by Austin Smith, Ph.D.

Medical Research Council Professor
Wellcome Trust/MRC Stem Cell Institute
University of Cambridge

Principles of Pluripotency

As an undergraduate in Oxford, Professor Smith became captivated by pluripotency. He graduated from the University of Oxford with a degree in Biochemistry in 1982 and then pursued Ph.D. studies in Developmental Genetics in Edinburgh. After a postdoctoral period back in Oxford, he returned to Edinburgh in 1990 as a Group Leader at the Centre for Genome Research. Professor Smith became Centre Director in 1995 and created the Institute for Stem Cell Research. In 2006, he moved to Cambridge and was founding Director of the Cambridge Stem Cell Institute. He is also a Medical Research Council Professor, an EMBO Member, and a Fellow of the Royal Societies of Edinburgh and of London. In 2010 he was awarded the Louis Jeantet Prize and in 2016 he received the ISSCR McEwen award for Innovation.

His research interest is stem cell biology and in particular pluripotent embryonic stem cells. He aims to elucidate generic principles of pluripotency establishment and progression in the mammalian embryo and derivative stem cells. His aspirations are firstly to advance fundamental understanding, and secondly to translate this knowledge into a transformative platform for biomedical applications. Professor Smith has published 186 research papers and has an h-index of 45 (Scopus).

The main objective of his research group is to characterize the cellular and molecular mechanisms governing the self-renewal and differentiation of multipotent embryonic stem cells of mouse, rat and human origin. Stem cells are defined by the ability both to produce identical daughter cells (self-renewal), and to produce progeny with more restricted fates (commitment and differentiation). This property of stem cells underpins growth and diversification during development and sustains homeostasis and repair processes throughout adult life. An understanding of molecular mechanisms that govern stem cell fate is therefore of fundamental significance in cell and developmental biology, and the capabilities arising from such knowledge have major biomedical applications. Embryonic stem (ES) cells, which are derived directly from the pluripotent cells of the early mouse embryo, can be propagated and manipulated in vitro whilst retaining their full potential for multi-lineage development. Our strategy is to exploit these prototypic stem cell cultures for the identification and characterization of key regulatory molecules, to determine the significance of these molecules *in vitro* and *in vivo*, and thence to develop improved methods of stem cell propagation and manipulation.

POSTER ASSIGNMENTS

(1) **AGUDO, J. –** JEDI T CELLS REVEAL QUIESCENT TISSUE STEM CELLS EVADE IMMUNE SURVEILLANCE

(2) **AKKIRAJU, H. –** NUCLEOTIDE LIGAND BINDING AND EXCHANGE IN RECOMBINANT KRAS USING NATIVE MASS SPECTROMETRY

(3) **ANDRES-MARTIN, L. –** BIPHASIC SELF-RENEWAL RESPONSE OF SPERMATOGONIAL STEM CELLS TO FGF SIGNALING

(4) **ARANDA-ORIGILLES, B. –** THE MEDIATOR OF ENHANCER ACTIVITY IN HEMATOPOIETIC STEM CELLS

(5) **BADWE, C. –** FLI1 IS ESSENTIAL FOR MAINTENANCE OF HEMATOPOIETIC STEM CELL FUNCTION

(6) **BARIC-PARKER, J. –** EXPANDING THE 14-DAY RULE: ETHICAL AND POLITICAL CONSIDERATIONS

(7) **BROWN, L. –** REMODELING OF AORTIC VALVE TISSUE IN PATIENTS WITH AND WITHOUT LEFT-VENTRICULAR ASSIST DEVICE (LVAD) IMPLANTATION MONITORED BY PROTEOMIC PROFILING

(8) **CHEN, X.** – A HISTAMINE-DEPENDENT CIRCUIT REGULATES MYELOID-BIASED STEM CELLS AND PROGENITORS

(9) **CHRISTIN, J. –** REGULATION OF STEM CELL FUNCTION IN THE MOUSE MAMMARY GLAND BY SOX9

(10) **CHUANG, Y.-C. –** THE IMPACT OF NANOPARTICLES ON DENTAL PULP STEM CELL DIFFERENTIATION

(11) **CROFT, G. –** ISOGENIC HUNTINGTON'S DISEASE HUMAN EMBRYONIC STEM CELL DERIVED NEURONS REVEAL NEUROGENESIS PHASE CHROMOSOMAL INSTABILITY DOMINANT NEGATIVE LOSS OF FUNCTION PHENOTYPE FOR MUTANT HTT

(12) **FRENSTER, J. –** GPR133, AN ORPHAN ADHESION G PROTEIN-COUPLED RECEPTOR, PROMOTES HYPOXIA-DRIVEN TUMOR PROGRESSION IN GLIOBLASTOMA

(13) **FUNATO, K. –** HUMAN ESC-BASED MODELING OF PEDIATRIC GLIOBLASTOMA BY HISTONE MUTATIONS

(14) **GE, Y. –** AT THE CROSSROADS OF WOUND-REPAIR AND CANCER

(15) **GOMEZ-SALINERO, J. –** EVALUATION OF THE SCALE UP PRODUCTION OF HUMAN UMBILICAL ENDOTHELIAL CELLS FOR THE EXPANSION OF HUMAN CD34+ HEMATOPOIETIC STEM PROGENITOR CELLS

(16) **GOSWAMI, S. –** METASTATIC NICHE IN BREAST CANCER INDUCES BREAST CANCER STEM CELL FORMATION VIA THE ACTIVATION OF WNT PATHWAY

(17) **GRONSKA, M. –** ADULT HIPPOCAMPAL NEUROGENESIS AND ITS REGULATION BY COMPONENTS OF FGF SIGNALING

(18) **GUENTHART, B. –** POROUS HYDROGEL PATCH SEEDED WITH STEM CELLS FOR TREATING ALVEOLAR AIR LEAKS

- **(19) GUO, P. –** ENDOTHELIAL JAGGED2 SUSTAINS HEMATOPOIETIC STEM AND PROGENITOR CELL RECONSTITUTION AFTER MYELOABLATIVE INJURY
- **(20) HARLAN, B. –** STEM CELLS OF THE TUBAL EPITHELIUM AND THEIR ROLE IN OVARIAN CANCER
- **(21) HUANG, X. –** THE PLURIPOTENCY FACTOR ZFP281 COORDINATES TRANSCRIPTIONAL AND EPIGENETIC CONTROL OF EPIBLAST MATURATION
- **(22) KANG, W. –** FGF SIGNALING DIRECTS NEURAL STEM CELLS IN THE SUBVENTRICULAR ZONE TOWARD OLIGODENDROCYTE LINEAGE AND IMPROVES CELL REGENERATION AFTER DEMYELINATION
- **(23) KYLE, J. –** PHARMACOLOGICAL ABLATION OF MICROGLIA MODULATES ADULT NEUROGENESIS AFTER ISCHEMIC STROKE
- **(24) LI, D. –** CONTEXT-DEPENDENT FUNCTIONS OF NANOG PHOSPHORYLATION IN PLURIPOTENCY AND REPROGRAMMING
- **(25) LIS, R. –** CONVERTING ADULT ENDOTHELIUM TO BONA FIDE IMMUNOCOMPETENT HEMATOPOIETIC STEM CELLS
- **(26) LIU, S. –** IMPAIRED EPIDERMAL TO DENDRITIC T CELL SIGNALING SLOWS WOUND REPAIR IN AGED SKIN
- **(27) MA, S. –** SPATIOTEMPORAL CONTROL OF HUMAN CARDIAC TISSUE USING AN OPTOGENETIC PLATFORM
- **(29) MAYOURIAN, J. –** HUMAN MESENCHYMAL STEM CELLS ENHANCE CARDIAC CONTRACTILITY THROUGH EXOSOMAL PARACRINE SIGNALING
- **(30) MOMENI, A. –** CELL SURFACE GLYCOENGINEERING IMPROVES SELECTIN-MEDIATED ADHESION OF MESENCHYMAL STEM CELLS (MSCS) AND CARDIOSPHERE-DERIVED CELLS (CDCS): PILOT VALIDATION IN PORCINE ISCHEMIA-REPERFUSION MODEL
- **(31) MORGANI, S. –** A SPROUTY4 REPORTER TO MONITOR FGF/MAPK/ERK SIGNALING ACTIVITY IN THE MOUSE
- **(32) N'TUMBA-BYN, T. –** TYROSINE KINASE RECEPTOR EPHB2 LOSS-OF-FUNCTION ALTERS SPERMATOGONIAL STEM CELL ACTIVITY
- **(33) ONWUMERE, O. –** CHEMOTACTIC DYNAMICS OF PHOTORECEPTOR PRECURSOR CELLS AND RETINAL PROGENITOR CELLS IN MICROFLUIDIC VEGF AND OSTEOPONTIN GRADIENTS
- **(34) ORDEK, G. –** IMAGING THE FUNCTIONAL INTEGRATION OF ADULT-BORN HIPPOCAMPAL GRANULE CELLS INTO THE DENTATE GYRUS NETWORK
- **(35) PAPA, L. –** EXPANSION OF HSCS FROM CORD BLOOD $CD34^{+}$ CELLS BY VALPROIC ACID REQUIRES BOTH MITOCHONDRIAL REMODELING AND P53
- **(36) PINKAS-SARAFOVA, A. –** THE INTERACTION OF DENTAL PULP STEM CELLS WITH 3-D SCAFFOLDS-MOLDED VS. PRINTED – CAN WE TELL THE DIFFERENCE?
- **(37) RAGHUPATHY, G. –** A CHROMATIN BARRIER INSULATOR IN THE T-CELL RECEPTOR α (TCRα) GENE LOCUS CONTROL REGION (LCR)

(38) **RONG, N. –** NANOG RESTORES MECHANICAL PROPERTIES AND EXTRACELLULAR MATRIX DEPOSITION BY SENESCENT STEM CELLS

(39) **RUZO, A. –** ISOGENIC HUNTINGTON'S DISEASE EMBRYONIC STEM CELL LINES REVEAL NOVEL DEVELOPMENTAL PHENOTYPES

(40) **SHAHINI, A. –** NANOG EXPRESSION RESTORES THE REGENERATIVE CAPACITY OF SENESCENT MYOBLAST

(41) **SIMON, M. –** INDUCING ODONTOGENIC DIFFERENTIATION OF HUMAN DENTAL PULP STEM CELLS VIA ENZYMATIC CROSS LINKING OF HYDROGELS

(42) **SMITH, R. –** MODELING PEDIATRIC GLIOMAS OF THE THALAMUS BEARING HISTONE MUTATIONS

(43) **TSEROPOULOS, G. –** THE ROLE OF FGF IN REPROGRAMMING OF EPIDERMAL KERTINOCYTES TOWARD NEURAL CREST FATE

(44) **VADAKKE-MADATHIL, S. –** CDX2-DERIVED CELLS FROM PLACENTA: A NOVEL MULTIPOTENT CELL SOURCE FOR CARDIAC DIFFERENTIATION

(45) **VERMA, N. –** MZF1 AND GABP ACT AS KEY TRANSCRIPTIONAL ACTIVATORS OF YAP1 IN OSTEOPROGENITOR LINEAGE

(46) **VILA, O. –** AN OPTOGENETIC 3D MODEL OF HUMAN NEUROMUSCULAR JUNCTIONS

(47) **VILLASANTE, A. –** ENGINEERING TUMOR MICROENVIRONMENTS FOR STUDIES OF OSTEOLYSIS

(48) **WANG, J. –** CELL-CELL INTERACTION MICROCHIP FOR ANALYSIS OF STEM CELL COOPERATIVITY

(49) **WHITE, E. –** OPTOGENETIC STIMULATION FOR THE MATURATION OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

(50) **XU, P.-X. –** IDENTIFICATION OF MOUSE COCHLEAR PROGENITORS THAT DEVELOP HAIR AND SUPPORTING CELLS IN THE ORGAN OF CORTI

(51) **YANG, Y. –** DEVELOPMENT OF NOVEL MENIN INHIBITORS FOR TREATMENT OF GLIOMAS

(52) **ZHANG, L. –** GRAPHENE LOADED POLY(4-VINYLPYRIDINE) FIBROUS SCAFFOLDS SUPPORTS DENTAL PULP STEM CELL PROLIFERATION AND DIFFERENTIATION

(53) **ZHENG, S. –** UNBIASED RECONSTRUCTION OF EARLY FATE DECISIONS IN HUMAN HEMATOPOIESIS

(54) **LOVETT, J. –** COMPACT GENE REGULATORY CASSETTES SUPPORT KEY ASPECTS OF T-CELL RECEPTOR (TCR)-A GENE LOCUS CONTROL REGION (LCR) ACTIVITY

(55) **COLOGNATO, H. –** EPENDYMAL CELL DYSTROGLYCAN REGULATES NEURAL STEM CELL AND PROGENITOR FUNCTION IN SPATIALLY DISTINCT DOMAINS OF THE SUBVENTRICULAR ZONE

FACILITIES POSTERS

(F1) **ARDUINI, B. –** THE RENSSELAER CENTER FOR STEM CELL RESEARCH: BIOENGINEERING AND TISSUE SCIENCE

(F2) **FU, D.-J. –** THE STEM CELL PATHOLOGY UNIT AT CORNELL UNIVERSITY

(F3) **GOETA, S. –** SHARED RESOURCE: LARGE-SCALE BIOCHEMICAL PROFILING FOR STEM CELL RESEARCH IN NEW YORK

(F4) **HARRINGTON, K. –** A CLINICAL-SCALE, cGMP-COMPLIANT PRODUCTION PROCESS FOR TISSUE-DERIVED RETINAL PIGMENT EPITHELIAL CELLS: MANUFACTURE AND CHARACTERIZATION OF MASTER CELL BANKS AND FINAL CELL PRODUCT FOR PRE-CLINICAL EFFICACY STUDIES AND GLP SAFETY-TOXICOLOGY STUDIES

(F5) **PAVLOVA, I. –** OPTIMIZATION OF WHOLE-BRAIN IMMUNOLABELING TECHNIQUES FOR INDELIBLY-LABELED MEMORY TRACES

(F6) **CHANG, C.-C., –** THERMOMECHANICAL & IMAGING NANOSCALE CHARACTERIZATION (ThINC)

(1) JEDI T CELLS REVEAL QUIESCENT TISSUE STEM CELLS EVADE IMMUNE SURVEILLANCE

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The surface of all nucleated cells contains MHC class I that presents peptides from endogenously processed proteins. T cells scan the surface of a cell, and engage only cells in which their T cell receptor (TCR) has affinity for a specific MHC-peptide complex. Once recognition occurs, killing of the target cells is dependent on the cell type, the co-receptors they express, and the presence of immunomodulatory signals in the microenvironment. While we know the outcome of T cell interaction with some cell types, the outcome of the interaction with many populations have not been studied. To address this issue, we recently generated a new antigen-specific TCR mouse, called the Jedi, whose CD8 T cells recognize green florescent protein (GFP). By using GFP as the cognate antigen, we can take advantage of hundreds of GFP-reporter mice to study T cell interactions with virtually any cell type (Agudo, NBT 2015).

There is a long-standing interest in understanding the immunogenicity of stem cells. They have a unique capacity to re-grow replacement tissues for transplantation, which could be subject to immune rejection. Moreover, their self-renewing capacity means they are long-lived, and can accumulate mutations over time, which would result in neo-antigens. These antigens make stem cells potential targets of T cells. However, whether they are subject to immune surveillance is unknown.

Here, we utilized the Jedi, along with mice in which GFP is expressed exclusively in specific stem cell populations, to interrogate the immunogenicity of adult tissue stem cells in their niche. We provide conclusive demonstration that immune privilege is not a general property of adult stem cells. Instead, our studies reveal that cycling stem cells, such as those in the gut, ovary, and mammary gland, are subject to immune clearance, but that quiescent stem cells, specifically hair follicle stem cells and satellite cells, escape immune detection. This is an intrinsic property of the resting stem cells resulting from downregulation of MHC class I, and other key genes involved in the antigen presentation machinery, and protects the stem cells from immune clearance. These studies establish that quiescent tissue stem cells hide from immune surveillance and protect their integrity. This helps explain why mutations in long-lived stem cells do not lead to immune editing, and suggests how cancer-initiating cells may evade immune surveillance.

(2) NUCLEOTIDE LIGAND BINDING AND EXCHANGE IN RECOMBINANT KRAS USING NATIVE MASS SPECTROMETRY

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It is well known that KRAS mutations can activate cancer stem cells, contributing to tumorigenesis and metastasis. Studies of RAS proteins and their effectors have potential to reveal biophysical mechanisms of protein function and assist in creation of chemical probes and therapeutic agents. To support these efforts, we utilized native mode and denaturing mass spectrometry to validate binding of recombinant $KRAS^{G12D}$ exchanged with ligands GDP, GTP or GppNHp (a GTP analog). In general, these nucleotides are tightly bound to KRAS. We demonstrated variably efficient competition of nucleotides for $KRAS^{G12D}$ samples exchanged with GTP, GppNHp or GDP, depending on the particular nucleotide used. Assessment of the extent of binding and exchange of guanine nucleotides is an essential prerequisite for evaluation of small molecule RAS ligands, and MS-based analysis of bound nucleotide identity is feasible using these methods.

Intact mass analysis of the recombinant $KRAS^{G12D}$ exchanged with GDP, GTP or GppNHp was performed using direct infusion on a Q Exactive HF (Orbitrap) mass spectrometer (Thermo Fisher)

In native mode with ammonium acetate, the endogenous protein contained a mixture of GDP and GTP ligands. However, exchange was 100% effective for both GDP and GTP. No contaminating GTP was detected in the GDP-exchanged samples, and no contaminating GDP was detected in the GTP-exchanged samples. In contrast, the GppNHp exchange with $KRAS^{G12D}$ resulted in a mixture that contained either the GppNHp or the GDP ligands. GTP, which started out as less abundant in the endogenous samples, was completely displaced by GppNHp. We demonstrate that exchanged preparations of $KRAS^{G12D}$-GTP and $KRAS^{G12D}$-GDP were pure with the expected nucleotide present. In contrast, exchange with the lower-affinity GppNHp resulted in the protein binding a mixture of GDP and GppNHp ligands. In contrast, in all of the methanol (denaturing condition) experiments, the apoprotein ($KRAS^{G12D}$) was the predominant species.

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(3) BIPHASIC SELF-RENEWAL RESPONSE OF SPERMATOGONIAL STEM CELLS TO FGF SIGNALING

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Sperm-forming (spermatogonial) stem cells (SSCs) in the mammalian testis require a specific set of tightly regulated growth factors, including GDNF and FGF. During aging, pathogenic mutations accumulate in the testes, paralleling an increase in genetic disorders among children of older fathers, referred to as the paternal age effect (PAE). Intriguingly, most PAE-related mutations consist of weakly acting, gain-of-function

mutations in the FGFR/RAS/MAPK pathway. We previously demonstrated that SSCs expressing the activating mutation in FGFR2 associated with Apert syndrome (AS SSCs), exhibit enhanced competitiveness, contingent upon growth factor dose. AS SSCs exhibited increased sensitivity to FGF2 manifested by enhanced MAPK signaling and proliferated in suboptimal GDNF and FGF2 conditions, suggesting that slightly elevated FGF signaling preserves stem cell activity when growth factors are scarce, through activation of the MAPK pathway. Paradoxically, we found recently that excessive FGF2 impairs colonization activity of wild type SSCs and affects their ability to respond to retinoic acid, which is known to drive SSC differentiation. Consistent with this, low/moderate doses of FGF2, unlike high FGF2, induced the strongest and most persistent MAPK activation detectable at least 2 hr after stimulation. We have since uncovered the FGF-dependent negative feedback mechanism that controls MAPK signaling and comprises elevated Spry4 among other genes. Spry4 depletion in SSCs via shRNA induced a differentiation phenotype and decreased stem cell activity measured by transplantation. We propose that FGF dose-dependent MAPK signaling is a fundamental molecular switch controlling SSC fate and that mutations affecting the MAPK pathway have profound effects, determining whether SSCs are positively or negatively selected. Our data shed light on how pathogenic mutations accumulate and may explain the limited spectrum of such mutations that are compatible with positive selection in the testis.

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(4) THE MEDIATOR OF ENHANCER ACTIVITY IN HEMATOPOIETIC STEM CELLS

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Hematopoietic stem cells (HSCs) rely on tightly regulated networks of transcription factors to self-renew, differentiate or transform in response to oncogenic triggers. In this processes, cofactors necessarily assist transcription factors located at distal enhancers to communicate with the general transcription machinery at promoters. One such crucial cofactors is the Mediator complex (Mediator), a huge macromolecular complex composed of four submodules (head, middle, tail and kinase). Mediator acts as a “molecular bridge” to facilitate promoter-enhancer communication. Tail-subunits interact with transcription factors at enhancers and connect through the middle module to the head, which then binds RNA Polymerase II at promoters. Mediator is able to

adapt its subunit composition to integrate signaling cues and, thereby, activate specific transcriptional programs to achieve functional biological responses. To date, little is know about the role of Mediator in adult stem cell self-renewal and differentiation. Here, we demonstrate that MED12, a member of the kinase mediator module, is an essential regulator of HSC homeostasis as *in vivo* deletion of *Med12* causes rapid bone marrow aplasia and leads to acute lethality. Strikingly, deletion of other members of the Mediator kinase module does not affect HSC function suggesting kinase-independent functions for MED12 during early hematopoiesis. We reveal that MED12 is located at key HSC-enhancer, where its hematopoietic-specific deletion destabilizes P300 chromatin association. Consequently, P300 loss prompts the deactivation of enhancers of crucial HSC genes, which are often perturbed in leukemia (c-KIT, RUNX1). The subsequent disruption of essential gene expression signatures compromises the survival of HSCs. The growing amount of reports identifying MED12 mutations in multiple blood malignancies make these findings particularly relevant, as they shed light on the molecular mechanisms underlying Med12-dependent enhancer regulation in the control of physiological and, potentially, malignant hematopoiesis. Future efforts will determine the contribution of human MED12 mutations to leukemia initiation and/or progression.

(5) FLI1 IS ESSENTIAL FOR MAINTENANCE OF HEMATOPOIETIC STEM CELL FUNCTION

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The ETS family of transcription factors is known to play an essential role in hematopoietic development. One such factor that is widely expressed in almost all hematopoietic lineages, extending from long term hematopoietic stem cells to terminally differentiated hematopoietic cells, is Fli1. Global deletion of Fli1 leads to embryonic lethality at E12.5 due to dramatic hemorrhaging caused by poor vascular integrity and platelet dysfunction. The role of Fli1 in adults has been explored in several different cells types ranging from megakaryocytes to B and T cells and has also been implicated in transcriptional regulation of hematopoietic stem and progenitor cells through combinatorial analysis, however its exact contribution to stem cell maintenance and function remains unclear. In this study, we identify Fli1 as one of the critical regulators of adult hematopoietic stem cell function. Using the cre-lox system and various transplantation strategies we conclude that Fli1 is required for maintenance of hematopoietic homeostasis and is required for hematopoietic reconstitution post radiation. Additionally, Fli1 deletion abolishes the ability of stem cells to engraft in the bone marrow and contribute to peripheral blood lineages. This study offers an insight into one of the few transcription factors that plays a role in HSC function during development as well as in adults.

(6) EXPANDING THE 14-DAY RULE: ETHICAL AND POLITICAL CONSIDERATIONS

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ISCCR guidelines limit the length of time that human embryos may be gestated *in vitro* to 14 days. This is a respected scientific standard used by researchers throughout the world. The limit of 14 days is based upon the emergence of the embryo's primitive streak, which indicates the beginning development of the central nervous system. This is also the identified point at which no further division (twinning) or fusing of embryos may occur, thereby firmly establishing the existence of a unique human being. This policy seemed to broker a solution between some scientists and the broader community, who were divided on whether nascent human embryos should be sacrificed to advance knowledge and develop clinical treatments. Until recently, it was not possible to sustain *in vitro* human embryos for longer than 13 days. However, with recent advances, viability will likely exceed the 14-day threshold. As a result, some are seeking to extend the rule to 21 or 28 days.

In this complex matter, ethical and political factors must be carefully weighed against potential scientific advances. Ethical arguments against extending the deadline include making what many believe to be a morally objectionable act even more grievous, since the 28-day-old embryo would have a beating heart, limb buds, and neural tube – the precursor to the central nervous system. Further, concerns of the 'slippery slope' would be validated, with good reason to believe the 'goal post' would advance repeatedly, to suit scientific appetites. Lastly, one must ask if weakening embryonic restrictions, coupled with other novel developments such as artificial uteri, may open the door to unconscionable experimentation at a later point. Political considerations for maintaining the current rule include preservation of public confidence in scientific integrity and transparency, public funding considerations, and assurance that scientists can operate within consensual guidelines.

(7) REMODELING OF AORTIC VALVE TISSUE IN PATIENTS WITH AND WITHOUT LEFT-VENTRICULAR ASSIST DEVICE (LVAD) IMPLANTATION MONITORED BY PROTEOMIC PROFILING

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Remodeling of heart valve tissue is a process dependent on stem cells recruited from the circulation. Such changes in valve tissue result in a substantial proportion of Left-Ventricular Assist Device (LVAD) patients developing "leaky" aortic valves (AV) over time. This results in worsening heart failure. However, the mechanism underlying aortic regurgitation is poorly understood. Based on our knowledge of valve biology and mechanics, we hypothesized that the altered mechanical environment in LVAD patients leads to altered protein composition of aortic valves, and their altered structural and mechanical properties. We used large-scale protein profiling to assess the proteome of aortic valves in LVAD patients undergoing heart transplant and compared them to

control patients also undergoing heart transplant. Knowledge of proteome changes during LVAD support may lead to interventions to prevent this detrimental complication of valve regurgitation.

Aortic valves (AV) were collected from 16 LVAD and 6 non-LVAD patients at the time of heart transplant. Tissue was frozen in liquid nitrogen, ground, extracted with SDS in Tris-buffered saline and digested with trypsin. Analyses were performed on an Ultimate 3000 RSLCNano chromatograph coupled to a Q Exactive HF (Orbitrap) mass spectrometer (Thermo Fisher Scientific). Searches were done with Mascot v. 2.5.1 (Matrix Science Ltd.) against a Swiss-Prot human protein database followed by post-processing with Elucidator software (Ver. 4.0.0.2.29).

Twenty-two LC/MS/MS chromatograms from LVAD and non-LVAD heart valves revealed a total of 774 proteins with 422 proteins identified with high certainty, supported by two or more detected peptides. High abundance proteins in both LVAD and non-LVAD tissues included collagens, vimentin, biglycan, blood proteins, serum amyloid P-component, fibrillin-1, prolargin, vitronectin, lumican, elastin and many others. Preliminary results indicate that there is a subset of proteins relating to fibrosis and scar tissue development accumulated in LVAD patients, suggesting an impact on mechanical properties of the valves.

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(8) A HISTAMINE-DEPENDENT CIRCUIT REGULATES MYELOID-BIASED STEM CELLS AND PROGENITORS

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Myeloid-biased hematopoietic stem cells (MB-HSCs) play critical roles in transplantation, inflammation, and recovery from radiation injury. But little is known regarding their regulation in the native bone marrow niche. Here, we show that histidine decarboxylase (Hdc) marks quiescent MB-HSCs and myeloid lineage-restricted progenitors, and contributes to an auto/paracrine physiologic circuit. Histamine,

predominantly produced by lineal descendants in close anatomical proximity to MB-HSCs and progenitors, feeds back through the histamine H_2 receptor (H_2R) on these biased stem cells and progenitors to promote their quiescence and self-renewal upon provocation. Removal of histaminergic tone enforces cell cycle entry, loss of serial transplant capacity and sensitizes animals to chemotherapeutic injury. Myeloid demand induced by lipopolysaccharide (LPS) specifically recruits MB-HSCs and progenitors, but not lymphoid-biased HSCs (LB-HSCs), into the cell cycle. In the absence of histamine feedback, cycling MB-HSCs fail to revert into quiescence, leading to exhaustion. Injection of an H_2 agonist in vivo protects these stem cells from exhaustion after sepsis. Thus, intrinsically primed MB-HSCs and myeloid lineage-restricted progenitors are recruited to meet lineage-specific physiological demands, and restored to homeostasis by the lineage autocrine/paracrine factor, histamine, which is produced by their myeloid lineal descendants, thereby establishing a feedback loop.

(9) REGULATION OF STEM CELL FUNCTION IN THE MOUSE MAMMARY GLAND BY SOX9

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Sox9, a transcription factor, has been demonstrated by our lab to be a useful marker for two novel stem populations in the mammary gland epithelium: one that contributes to the Estrogen Receptor Negative (ER-) luminal epithelia and a second to the basal epithelia. Importantly, this pattern of expression is replicated in human mammary gland. However, whether Sox9 is required in these stem cells pools, is unclear. By crossing $Sox9^{Flox/Flox}$ with a novel mammary gland specific Cre, that allows for ECFP lineage tracing of recombined cells. *In vivo* the only gross morphological defect observed is an incompletely penetrant, defect of early alveologenesis which is resolved by the time pups are born. By using the lineage tracing Cre though, we have been able to determine that this is likely the result of incomplete recombination as the ER- population responsible for alveologenesis is not entirely recombined. However, we have been able to demonstrate a marked deficiency in the *ex vivo* acini forming ability in recombined ER- luminal cells along with the loss of expression of CD61 (a progenitor cell marker). Furthermore, upon examining ECFP+ basal cells, we discovered that despite the presence of homozygous Sox9-Flox, only 50% of the alleles are recombined, suggesting a strict requirement for Sox9 in the basal lineage. These results suggest that Sox9 is functionally indispensable for both ER– luminal and basal stem/progenitor cells.

These findings are further supported by our lab's organoid and acini culture system wherein shRNA mediated knockdown leads to a complete ablation of both basal and luminal stem cell activity. With this loss of stem cell activity in mind, we have now crossed our Sox9 condition knockout animals to a basal-like breast cancer model to determine Sox9's role in basal-like mammary tumorigenesis.

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(10) THE IMPACT OF NANOPARTICLES ON DENTAL PULP STEM CELL DIFFERENTIATION

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Stem cells isolated from the dental pulp (dental pulp stem cells (DPSCs)) when grown in specific inducing media can differentiate and express markers of odontoblasts, osteoblasts, adipocytes or neuronal cells. The use of materials whose biochemical and mechanical cues can regulate or direct stem cell proliferation and differentiation offers exciting applications. We have previously shown using spun cast polybutadiene (PB) as substrate, that DPSCs respond to surface mechanics, such that by day 4 on thin (20nm), hard PB surfaces, cell modulus increases, and by day 28 cells biomineralize without dexamethasone. In contrast, on the thicker (200nm) softer PB surfaces, cell modulus was lower and biomineralization required dexamethasone. It has been shown that nanoparticles (NPs) also offer a means of regulating cell function. In the current work, we asked whether NPs might alter the response of DPSC to substrate mechanics. TiO_2 NPs (0.1 mg/mL) were added at either day 1 or day 4 post-plating onto thin PB. Cells were then grown in medium without dexamethasone. At week 1, cell moduli were measured using shear modulation force microscopy. At week 4 biomineralization was examined using SEM/EDS. We found that the addition of TiO_2 on day 1 post-plating inhibited biomineralization. However, the addition of TiO_2 on day 4 was without effect and cells biomineralized as did the TiO_2-free control. This result suggests an early response that once complete cannot be reversed by TiO_2 NPs.

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(11) ISOGENIC HUNTINGTON'S DISEASE HUMAN EMBRYONIC STEM CELL DERIVED NEURONS REVEAL NEUROGENESIS PHASE CHROMOSOMAL INSTABILITY DOMINANT NEGATIVE LOSS OF FUNCTION PHENOTYPE FOR MUTANT HTT

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disease caused by an expansion of CAG repeats in the Huntingtin gene *(HTT).* Despite decades of scrutiny neither the pathogenic mechanism of mutant-Htt in HD, nor the normal function of Htt are known. Here we used an isogenic series of gene-edited, human embryonic stem cell (hESCs) lines representing a graded series of disease severity to identify an early neurodevelopmental phenotype. During in vitro forebrain differentiation, a fraction of HD neural progenitors, proportional to CAG-expansion length, exhibit significantly expanded cell soma. These cells also show aneuploidy, multiple centrioles, DNA damage including nuclear strands, lagging DNA, and failed cytokinesis. This novel human neurodevelopmental cellular phenotype complements

existing mouse-model HD literature and suggests region specific DNA damage and mutant cells compromise early striatal neurodevelopment in HD. Next, using heterozygous and homozygous Htt knockout lines (Het, KO) we demonstrate this same phenotype in KO, but not Het lines. This finding suggests a dominant-negative loss of function phenotype for mutant Htt in HD, and suggests an important evolutionary function for Htt role in regulating neural progenitor cell division in the human forebrain. Together these findings illuminate a novel function of *HTT* and argue for the requirement for understanding the developmental effects of mutant *HTT* for therapeutics development.

(12) GPR133, AN ORPHAN ADHESION G PROTEIN-COUPLED RECEPTOR, PROMOTES HYPOXIA-DRIVEN TUMOR PROGRESSION IN GLIOBLASTOMA

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Glioblastoma (GBM) is a deadly primary brain malignancy. GBM stem cells (GSC) drive tumor growth, as well as resistance to current therapies. Hypoxia, a hallmark of GBM, promotes the GSC phenotype and leads to tumor progression via Hypoxia Inducible Factors (HIFs). However, therapeutically HIFs has so far been unsuccessful. In the effort to identify other targetable mediators of the hypoxic response in GBM, we recently discovered that GPR133 (ADGRD1), an orphan adhesion G protein-coupled receptor (GPCR), is enriched in GSCs. Staining of biospecimens specifically detects GPR133 in the hypoxic areas of pseudopalisading necrosis, where it colocalizes with CD133, HIF1α, and CA9, but not in normal brain. *In vitro*, patient-derived GBM cell cultures upregulate *GPR133* transcript in hypoxia, while HIF1α knockdown decreases the level of *GPR133* transcript ($P<0.05$). We found direct binding of HIF1α to a Hypoxia Response Element (HRE) upstream of the *GPR133* transcriptional start site using ChIP-qPCR ($P<0.001$). These findings support the idea that GPR133 is upregulated in hypoxia via direct transactivation by HIF1α. Functionally, GPR133 knockdown decreases tumor cell proliferation *in vitro* under both normoxic and hypoxic conditions ($P<0.05$). GPR133 knockdown also impairs sphere formation in extreme limiting dilution assays ($P<0.001$) and lowers the CD133+ GSC population, suggesting an effect on "stem" properties. *In vivo*, knockdown cells are unable to initiate tumor xenografts ($P<0.002$), while control cells lead to death of mouse hosts (n=4 mice/group; $P<0.01$), indicating a robust role for GPR133 in tumor initiation. TCGA data from 160 GBM patients revealed that higher *GPR133* mRNA levels correlate with worse survival

(P=0.0062). RNA-sequencing followed by gene ontology analysis demonstrated that GPR133 knockdown affects cell cycle regulation, stem-related pathways, telomere maintenance and DNA damage response. We believe that GPR133 plays an important pro-tumorigenic role in hypoxia-driven GBM progression and represents a promising novel therapeutic target.

(13) HUMAN ESC-BASED MODELING OF PEDIATRIC GLIOBLASTOMA BY HISTONE MUTATIONS

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Recent findings reveal recurrent mutations in histone H3 genes in pediatric glioblastoma, including $H3^{K27M}$ mutation in DIPGs and $H3.3^{G34R/V}$ mutation in supratentorial glioblastomas. Our lab has previously successfully modeled the $H3.3^{K27M}$ mutation in human embryonic stem cell (hESC) progeny. However, recent efforts by our team and others to model $H3.3^{G34R/V}$ tumor by introducing the histone mutation into neural precursors have failed and the oncogenic mechanisms of this “oncohistone” remain unknown. We therefore hypothesized that the $H3.3^{G34R/V}$ and its co-mutations must be highly context-dependent and require a specific cell of origin.

Computational analysis of gene expression profiles of $H3.3^{G34R/V}$ tumors reveals a significant enrichment of interneuron signature, as demonstrated by specific expression of DLX1, DLX5 and SP8. We therefore optimized an interneuron differentiation protocol and specified hESC differentiation into interneuron progenitors. We next evaluated the effect of each mutation by a stepwise transduction of $H3.3^{G34R}$, shp53, shATRX and constitutively active mutant (D842V) of PDGFRA. Our data demonstrate increased proliferation in the presence of shp53 but rapid depletion of the cells by knockdown of ATRX due to increased DNA damage (4.5-fold increase in γH2AX-index and 5.4-fold increase in apoptotic sub-G1 fraction). We also found that cell death induced by ATRX knockdown is partially rescued by the $H3.3^{G34R}$, although the extent of DNA damage is not changed (γH2AX-index of 22.5 ± 4.0 % in $H3.3^{G34R}$ group *vs* 24.4 ± 3.2 % in $H3.3^{WT}$ group), suggesting that $H3.3^{G34R}$ contributes to the resistance to DNA damage. Furthermore, we have analyzed the impact of these oncogenes, alone or in combination, on neuronal and astrocytic differentiation. Our data show that $H3.3^{G34R}$ could suppress the differentiation to both neuronal and astrocytic lineages, only if combined with shATRX and shp53. Taken together, these preliminary data suggest potential mechanisms of $H3.3^{G34R}$-mediated oncogenesis and further highlight the cell-lineage-specific roles of oncohistones.

(14) AT THE CROSSROADS OF WOUND-REPAIR AND CANCER

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Human adult tissues harbor resident stem cells responsible for homeostasis and wound-repair. Tumors often hijack these normal cellular programs and exploit them for malignancy. The parallel between wound and tumor, initially postulated by Rudolf Virchow in the mid nineteenth century, has been coined by many researchers and clinicians over the decades. It has long been recognized that human patients suffering from chronic wounds have increased susceptibility to cancers. Additionally, mice with gene mutations that enhance stem cell activity heal their wounds faster, but they also exhibit enhanced susceptibility carcinomas. By contrast, mice who possesses mutations that impede stem cell activation display reduced efficiency in wound closure, but also an increased resistance to cancers. Intimate connections between wounds and tumors have also been drawn at the molecular level. Following serum stimulation, cultured fibroblasts elicit a robust wound repair signature resembling that of certain human carcinomas and predictive of poor patient prognosis. Gene profiling studies in various wounded and tumorigenic whole tissues have further highlighted a concordant gene signature. Although intriguing, it remains unclear which of the normal stem cell remodeling pathways are exploited by tumor stem cells and how cancers rewire pre-installed regulatory networks to support malignancy. Here using mouse skin, an excellent genetically tractable model system to tackle these issues, we exploit transcriptional, epigenetic and CRISPR functional genomic approaches to revisit this hypothesis, and dive into the molecular mechanisms underlying the convergence and divergence of wounds and cancer. Our study further illuminates clinical paths where molecular tools can be tailored to selectively repair wounds and target malignancy.

(15) EVALUATION OF THE SCALE UP PRODUCTION OF HUMAN UMBILICAL ENDOTHELIAL CELLS FOR THE EXPANSION OF HUMAN CD34+ HEMATOPOIETIC STEM PROGENITOR CELLS

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The endothelium is the layer of cells that covers the blood vessels. Endothelial cells are important in the maintenance of organ homeostasis providing with them with angiogenic factors. Importantly, endothelial cells generate a niche for several stem cell populations *in vivo*, such as hematopoietic or brain stem cells among others. Our laboratory has previously developed a technology to expand *in vitro* human CD34+ hematopoietic stem progenitor cells (hHSPCs) using endothelial cells as a niche. Our current focus is the scale up of this protocol to a clinical grade protocol in order to treat humans. The growth an expansion of Human Umbilical Endothelial Cells (HUVECs) has been challenging for many years. Our laboratory has previously described that the

overexpression of the protein E4-ORF1 from Adenovirus Class 5 allows the long-term culture and expansion of HUVEC cells in vitro, maintaining their angiogenic properties. Our results have shown that we can achieve 0.7 to 1.8 billion HUVECs in less than 1 month using classical 2D culture expansion or the use of a Quantum bioreactor. Importantly, after the expansion, these cells do not loose their capacity to expand engraftable hHSPCs. In conclusion, we have set up a scale up protocol to expand HUVECs transduce with E4-ORF1 that can be used to expand hHSPCs in the clinic.

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(16) METASTATIC NICHE IN BREAST CANCER INDUCES BREAST CANCER STEM CELL FORMATION VIA THE ACTIVATION OF WNT PATHWAY

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Biomarkers CD44, CD24, CD133 and ALDH1 have been used to identify breast cancer stem cells (BCSC). BCSC are chemotherapy resistant bearing high tumorigenesis capability. Recent studies suggest that juxtacrine signaling from monocytes and macrophages support BCSC niche. Macrophage is a component of the micro-anatomical sites of breast cancer hematogenous dissemination called Tumor Micro Environment of Metastasis (TMEM). We hypothesized that TMEM rich tumor microenvironments support BCSCs.

We used flowcytometry to quantify $CD44^{high}/CD24^{low}$ cells, as well as mRNA fluorescent in *situ* hybridization (FISH) and qRT-PCR to quantify CD133 and ALDH1 expression in breast cancer cells from 50 invasive ductal carcinomas obtained from patients' cancer excisions by fine needle aspiration (FNA) before fixation. Formalin-fixed paraffin embedded tissue section from each sample was also analyzed for TMEM score using triple immunohistochemistry and the stem cell marker expression was compared to TMEM scores for each corresponding sample.

We observed very strong correlation between percentage of $CD44^{high}/CD24^{low}$ cells and TMEM scores (r=0.91), as well as the percentage of CD133 and ALDH1 expressing cells with TMEM scores (r=0.88 and 0.86 respectively). FISH results were validated using qRT-PCR and showed very strong correlation with TMEM scores (r=0.76 and 0.73 for CD133 and ALDH1 respectively).

Wnt/β-catenin pathway is involved in stem cell generation and in pathogenesis of various types of cancer. We hypothesized that interactions between cancer cells, macrophages, and endothelial cells induce cancer stemness via activation of Wnt/-β catenin pathway. Indeed, co-culture of macrophages with cancer cells, as well as endothelial cells with cancer cells, significantly increase the expressing stem cells markers, while salinomycin, a selective inhibitor of Wnt receptor activation, significantly reduced macrophage or endothelial cell induced increase in BCSCs. Using a specific antibody to block the Wnt receptor we found significant reduction in stemness. Blocking the Wnt/β-catenin pathway can have therapeutic potential in breast cancer.

(17) ADULT HIPPOCAMPAL NEUROGENESIS AND ITS REGULATION BY COMPONENTS OF FGF SIGNALING

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Understanding adult neurogenesis is crucial to advancements in regenerative medicine as adult neural stem cells (ANSCs) can generate neurons that integrate into existing hippocampal circuits. Thus, identification of the molecular pathways that control the generation, maturation, and integration of newborn neurons has the potential to pinpoint therapeutic targets for certain brain disorders, such as age-related memory decline. We previously showed that loss of Fibroblast Growth Factor Receptors (FGFRs)1-3 in ANSCs decreases proliferation and dendritic elaboration. However, the identities of the ligands and signal transducers for these FGFR-dependent processes are unknown. To address this gap, we are first examining proliferation and dendritic elaboration in the dentate gyrus (DG) of FGFR conditional mutant mice that lack binding sites for the downstream mediators Phospholipase-C gamma (PLCγ) and Fgf Receptor Substrate (FRS) proteins. Surprisingly, our data thus far suggest that not only FRS, which was previously implicated in FGFR-mediated proliferation in other contexts, but also PLCγ, which was not previously implicated in FGFR-mediated cell proliferation, are together non-redundantly required to transmit FGFR activity in promoting ANSC expansion. The potential roles of FRS and PLCγ in dendrite elaboration are currently being addressed using AAV-Cre and CreER transgene drivers that target newborn neurons and bypass their requirements fro FGFRs in stem and progenitor cells. Effects on learning and memory in hippocampus-related tasks will be examined. Using multiple Cre and CreER drivers, we are also determining whether L1CAM, a non-canonical FGFR ligand implicated in dendritogenesis, acts in a cell-type autonomous or non-autonomous manner to promote dendritogenesis in the DG. Determining which intra- and extracellular pathways differentially affect adult stem cell expansion and the generation and integration of new neurons will provide a better understanding of potential therapeutic targets for reversing deficiencies which lead to age-related memory decline.

(18) POROUS HYDROGEL PATCH SEEDED WITH STEM CELLS FOR TREATING ALVEOLAR AIR LEAKS

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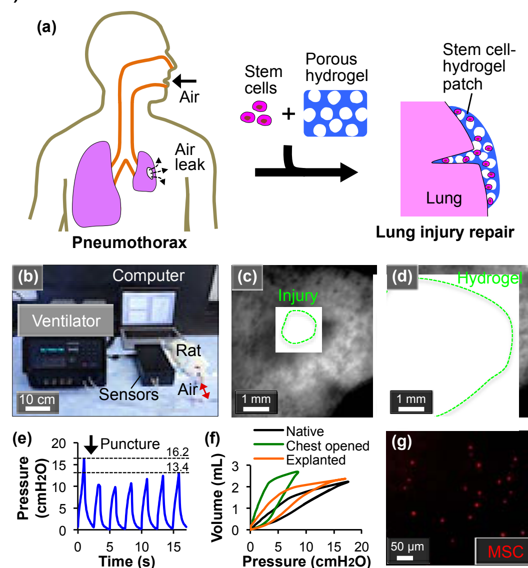
Statement of Purpose: Prolonged alveolar air leaks (AAL) following pulmonary resection have many devastating consequences to the patient including prolonged hospitalization, increased risk of infection, and the need for additional invasive procedures. Although pleural sealants such as fibrin glue, collagen patches, and synthetic hydrogels have been employed to decrease AAL, they are typically expensive

and ineffective [1]. Here, we present a novel approach to repair damaged lung pleura using a lung extracellular matrix-derived hydrogel mixed with stem cells. Our highly deformable stem cell-hydrogel system effectively seals the injured site while promoting wound healing. The structural similarity of our material to the native lung, achieved by the incorporation of air micro-bubbles, helps to increase the mechanical compliance of the material during ventilation.

Methods: In this study, we used lung-derived hydrogel loaded with mesenchymal stem cells (MSC) (**Fig. 1a**). Lung hydrogel (MatriTek) was derived from porcine lungs [2]. MSCs (2×10^5 cells) labeled with quantum dots were introduced, while a multi-channel microfluidic device introduced air pockets. Approximately 50 µL of the MSC-hydrogel mixture was applied to injured lung. During the experiment, the lungs were ventilated using a mechanical ventilator, while pressure and volume of the lung were measured using pressure and flow rate sensors (**Fig. 1b**).

Results: Lungs were punctured using a needle, and air leak was visually assessed (**Fig. 1c**). By applying the MSC-hydrogel mixture, the hole was sealed and leakage was completely prevented. While the hydrogel patch adhered firmly to the lung surface, the porous structure of the patch allowed the lung to expand and contract normally without any noticeable structural resistance (**Fig. 1d**). We demonstrated visualization of quantum dot-labeled MSCs within the patch using fluorescent microscopy (**Fig. 1g**).



Conclusions: We present an approach to treat air leak in the lung using deformable lung matrix-derived hydrogel seeded with therapeutic stem cells. The porous patch was derived from the native lung pleura and processed to mimic the lung architecture and biomechanical properties of native lung for maximal biocompatibility and integration. The patch induced rapid and complete sealing of the injured lung leaking air, and supported healing and sustained ventilation function. Our ongoing studies are focused on investigating the long-term patch durability and the mechanisms of cell engraftment and therapeutic benefit.

References: [1] P.D. Leyn et al., J Thorac, Cardiovasc Surg, 141(4), 881-887 (2011); [2] J.D. O'Neill et al., Ann Thorac Surg, 96(3), 1046-1055 (2013).

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Figure 1. Treatment of lung air leak using a porous lung-derived hydrogel seeded with stem cells. (a) Treatment overview. (b) Experimental setup. (c) Rat lung injured and (d) repaired. (e) Pressure and (f) pressure-volume loop obtained during treatment. (g) MSCs in the hydrogel patch on the lung.

(19) ENDOTHELIAL JAGGED2 SUSTAINS HEMATOPOIETIC STEM AND PROGENITOR CELL RECONSTITUTION AFTER MYELOABLATIVE INJURY

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Adult hematopoietic stem and progenitor cells (HSPCs) reside within the perivascular niche. We interrogated the function of endothelial cells (ECs) in HSPC homeostasis and reconstitution via conditional deletion of the Notch ligand Jagged2 from endothelium. We demonstrate that Jagged2 is differentially expressed in tissue-specific vascular beds at steady state, and its expression is dynamically regulated post myelosuppresive injury. Endothelial Jagged2 is not required to maintain HSPC repopulating capacity under steady state conditions, but contributes to recovery of HSPCs after myelosuppressive conditions. Following myeloablation, the engraftment and/or expansion of HSPCs were dependent on endothelial Jagged2. Jagged2 expressed in bone marrow ECs increased the survival and reconstitution of HSPCs via induction of Notch target Hey1 in HSPCs. Jagged2 serves as one activating module in Notch signaling to promote hematopoietic recovery. Modulating Jagged2 also affects the mobilizing potential of HSPCs and their lineage outcomes.

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(20) STEM CELLS OF THE TUBAL EPITHELIUM AND THEIR ROLE IN OVARIAN CANCER

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Ovarian cancer is the 5th leading cause of cancer deaths among women in the US. However, its cell of origin is still up for debate. There is evidence that high-grade serous carcinomas (HGSC), the most common and aggressive type of ovarian cancer, are originating from the ovarian surface epithelium (OSE) and the tubal epithelium (TE) of the fallopian tube. Our lab identified a stem cell pool in the OSE that was shown to form HGSC upon inactivation of *Trp53* and *Rb1*, tumor suppressor genes commonly altered in this malignancy. No studies to date have definitively identified TE stem cells (TE-SCs); furthermore, the susceptibility of TE-SCs to the malignant transformation remains unclear. Sphere assays and label retaining cell assays suggest that the TE-SCs reside mainly in the distal region of the uterine tube (aka Fallopian tube or oviduct), near the ovary. We isolated putative TE-SCs by using CD49f^{+}/CD24^{med} cell surface markers and identified keratin 5 (KRT5) as a potential marker of stem/progenitor cells in the TE. Using lineage tracing techniques, we followed the fate of KRT5 expressing cells. These experiments revealed that KRT5 expressing cells reside in the distal region of the uterine tube, a potential location of SCs, and give rise to both secretory and ciliated cells, the two major cell lineages in the TE. In addition, the KRT5 stem/progenitor cell pool was able to contribute to regenerated tissue after mechanical wounding of the TE. Ongoing research will elucidate whether KRT5 stem/progenitor cells are susceptible to

malignant transformation by conditional inactivation of *Trp53* and *Rb1*. This study will shed light on the origin of HGSCs and can inform preventative measures and future treatments for ovarian cancer.

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(21) THE PLURIPOTENCY FACTOR ZFP281 COORDINATES TRANSCRIPTIONAL AND EPIGENETIC CONTROL OF EPIBLAST MATURATION

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Pluripotency is defined by a cell's potential to differentiate into any somatic cell type. How pluripotency factors regulate lineage specification events resulting in the establishment of the basic body plan is poorly understood. Here we report how the transcription factor Zfp281 functions in the naive-to-primed pluripotency transition occurring during pre- to post-implantation embryonic development. By characterizing mouse mutants and identifying gene targets and protein partners of Zfp281 during the transition of pluripotency, we establish critical roles for Zfp281 in activating Nodal signaling components and DNA methylation modifying enzymes within pluripotent epiblast cells. *Zfp281* mutant embryos reach the blastocyst stage with indistinguishable gross morphology, but exhibit defects in epiblast maturation resulting in a failure to establish an anterior-posterior (A-P) axis at post-implantation stage. Transcriptomic profiling of E6.5 embryos reveals a failure in activating Nodal signaling in the *Zfp281* mutant embryos. Whole mount mRNA in situ hybridization and immunofluorescence analysis confirm the impairment of Nodal signaling in *Zfp281* mutant embryos dramatically reduces expressions of A-P specification markers Cer1, Hex, Lefty1, Hesx1, and Dkk1, leading to defects in distal and anterior visceral endoderm (DVE/AVE) specification and migration. Global chromatin-binding study of Zfp281 in naive mouse embryonic stem cells (ESCs) and primed epiblast stem cells (EpiSCs) reveals dynamic binding pattern and rearrangement of Zfp281 at target loci in transition between these two pluripotent states. Mechanistically, we demonstrate that Zfp281 is required for coordinating transcription factors (Oct4 and Otx2) and epigenetic regulators, including histone acetyltransferases Tip60-Ep400 complex and p300, and polycomb repressive complex PRC2 to modulate expressions of lineage-specific genes (Otx2, T, Fgf5, and Fgf8), Nodal signaling components (Nodal, Lefty2) and DNA methyltransferases (Dnmt3a/3b/3l) during development. Our results provide mechanistic insights into the functions of a pluripotency factor in activating lineage-specific genes and reprogramming DNA methylation for transcriptional and epigenetic control of epiblast maturation.

(22) FGF SIGNALING DIRECTS NEURAL STEM CELLS IN THE SUBVENTRICULAR ZONE TOWARD OLIGODENDROCYTE LINEAGE AND IMPROVES CELL REGENERATION AFTER DEMYELINATION

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FGF signaling plays a critical context-dependent role in both dorsal-ventral cell fate specification and neurogenesis during brain development. But its role in the regulation of adult stem/progenitor cells in the subventricular zone (SVZ) in vivo is not clear. Here in this study, a conditional mouse genetic approach was used to modulate FGF signaling specifically in adult neural stem/progenitor cells in the SVZ, and the effects of both loss and activation of FGF signaling on stem/progenitor cell fate specification was examined. The results showed that although FGF signaling is not essential for the neurogenesis in the SVZ, its increased activity leads to a greatly increased generation of Olig2+ transit amplifying progenitor (type C) cells that are committed to oligodendrocyte lineage at the expense of neurogenic progenitor cells. Importantly, upon demyelination, the increased generation of oligodendrocyte lineage progenitor cells in the SVZ results in an improved cell regeneration and replacement in the corpus callosum, whereas the neurogenesis from the SVZ is temporarily decreased, likely due to the re-directed differentiation of stem/progenitor cells toward oligodendrocyte lineage. Whether the oligodendrocyte fate specification by FGF signaling can lead to improved remyelination and functional recovery after demyelination will be further examined. The finding that the fate of neural stem cells in the SVZ can be respecified toward oligodendrocyte fate by FGF signaling has important implications for the potential use of endogenous stem/progenitor cells in demyelinating diseases.

(23) PHARMACOLOGICAL ABLATION OF MICROGLIA MODULATES ADULT NEUROGENESIS AFTER ISCHEMIC STROKE

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According to the American Stroke Association, Every year 795,000 people in the United States suffer a stroke. Stroke results in long-term disability as it causes loss of neurons. Treatment options for stroke are limited, necessitating research for additional options to repair the evident neural damage. Mammals have an endogenous mechanism, called neurogenesis, to generate new neurons to repair the damage caused by ischemia. Such endogenous repair processes are of therapeutic interest. However, the pro-inflammatory environment caused by stroke hinders neurogenesis. Microglia, the resident macrophages of the central nervous system, are a primary contributor to this environment as they respond to the damage and release locally pro-inflammatory cytokines. Microglia also have been shown to modulate neurogenesis at homeostasis. Additionally, there is extra complexity as microglia continuously shift between anti-inflammatory, resting, or pro-inflammatory states in response to their environment. Anti-inflammatory microglia are thought to promote neurogenesis, while pro-inflammatory hinder neurogenesis. Using a specific inhibitor of microglia, the PLX5622 compound, microglia can be depleted non-invasively from the CNS. We have started studies

examining the relationship between microglia and neurogenesis at homeostasis and after endothelin-1 (ET1) induced ischemia. Our preliminary results suggest that microglia depletion at homeostasis for 1 week causes no changes of the neural stem cell population in the lateral subventrical zone. Additionally, microglia ablation for 2 weeks after ET1 induced ischemia results in reduced infarct volumes compared to control, as well as the presence of more neural stem cells in the infarct and its penumbra.

(24) CONTEXT-DEPENDENT FUNCTIONS OF NANOG PHOSPHORYLATION IN PLURIPOTENCY AND REPROGRAMMING

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Nanog is a core pluripotency transcription factor that is critical for embryonic stem cell (ESC) self-renewal and the faithful reprogramming of somatic cells to induced pluripotent stem cells (iPSCs). Although Nanog is phosphorylated at multiple residues, the role of Nanog phosphorylation in ESC self-renewal is incompletely understood, and no information exists regarding its functions during reprogramming. Here we report our findings that Nanog phosphorylation is beneficial, although nonessential, for ESC self-renewal, and that loss of phosphorylation enhances Nanog activity in reprogramming. Mutation of serine 65 in Nanog to alanine (S65A) alone had the most significant impact on increasing Nanog reprogramming capacity. Mechanistically, we find that pluripotency regulators (Esrrb, Oct4, Sall4, Dax1, and Tet1) are transcriptionally primed and preferentially associated with Nanog S65A at the protein level due to presumed structural alterations in the N-terminal domain of Nanog. These results demonstrate that a single phosphorylation site serves as a critical interface for controlling context-dependent Nanog functions in pluripotency and reprogramming.

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(25) CONVERTING ADULT ENDOTHELIUM TO BONA FIDE IMMUNOCOMPETENT HEMATOPOIETIC STEM CELLS

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Developmental pathways choreographing the ephemeral transition of endothelial cells (ECs) into haematopoietic stem cells (HSCs) remain undefined. Here, we developed a tractable approach for converting adult murine ECs to HSCs (rEC-HSCs) by transiently expressing transcription factors *FosB*, *Gfi1*, *Runx1*, and *Spi1 (FGRS)* and angiocrine stimulation by vascular-niche. Induction phase (day 0-8) of conversion is initiated by expressing *FGRS* in mature ECs resulting in endogenous *Runx1* expression. During specification phase (day 8-20), endogenous *Runx1*$^{+}$ *FGRS*-transduced ECs commit to a haematopoietic fate and no longer require *FGRS* expression. The vascular-niche drives robust self-renewal and expansion of rEC-HSCs (day 21-28). rEC-HSCs have a transcriptome and long-term self-renewal capacity similar to adult HSCs, are competent for clonal engraftment and multi-lineage reconstituting potential, including antigen-dependent adaptive immune function. Inhibition of TGF-β and Cxcr7 or activation of Bmp and Cxcr4 signaling enhanced rEC-HSCs generation. Conversion of ECs into autologous authentic HSCs promises treatment of haematological disorders.

(26) IMPAIRED EPIDERMAL TO DENDRITIC T CELL SIGNALING SLOWS WOUND REPAIR IN AGED SKIN

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Aged skin heals wounds poorly, increasing susceptibility to infections. Restoring homeostasis after wounding requires the coordinated actions of epidermal and immune cells. We found that aged epidermal keratinocytes display significantly reduced proliferation and migration at the wound edge. In addition, the aged wound edge failed to properly activate and maintain dendritic epidermal T cells (DETCs), a skin resident γδT cell that is important for wound healing. To further understand the mechanism, we find that aged keratinocytes near the wound edge don't efficiently upregulate Skints (selection and upkeep of intraepithelial T cells) for proper DETC maintenance and activation. We then identified Stat3 as the transcription factor that regulates Skint

upregulation at the wound edge and mediates proper DETC function. Notably, when epidermal Stat3, Skints, or DETCs are silenced in young skin, re-epithelialization following wounding is perturbed. Administration of IL-6, a STAT3 signaling activator, accelerates keratinocyte migration in aged skin explants in vitro. In sum, these findings underscore epithelial-immune crosstalk perturbations in general, and Stat3 and Skints in particular, as critical mediators in the age-related decline in wound-repair. Importantly, our study offers novel insights into the treatment of poor wound-repair associated with aging.

(27) SPATIOTEMPORAL CONTROL OF HUMAN CARDIAC TISSUE USING AN OPTOGENETIC PLATFORM

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The connection between the functional geometry of the heart and its dynamic behavior has informed a number of recent therapeutic advances, such as targeted catheter ablation for refractory arrhythmias. Progress in arrhythmia management requires better characterization of cardiac behavior as a function of geometry. Such characterization, however, has been impeded by limited methods for carrying out these studies. Better tools are required for the development of novel spatiotemporal control schemes in biological systems.

In pursuit of these goals, ChR2- and eNpHR-expressing hiPSCs were created through lentiviral transduction. These cell lines were differentiated into cardiomyocytes and subsequently formed into cardiac constructs. In parallel, we also developed an optical system that integrates a projector with an optical mapping system, providing for simultaneous illumination and imaging. The behavior of the tissue constructs in response to patterned illumination was investigated by analyzing the resulting videos with custom Matlab code.

Localized conduction blocks of arbitrary spatiotemporal parameters were created through patterned illumination of halorhodopsin-expressing constructs. Similarly, patterned illumination of constructs expressing channelrhodopsin was performed to overwrite existing behavior.

To demonstrate the relevance of our system, we studied spatially heterogeneous monolayers, which were used as a proxy for the peri-infarct zone following a myocardial infarction. Light was used to overwrite the disorganized waveforms in these substrates, restoring the normal propagation of traveling waves.

The flexibility of this platform enables systematic study of tissue arrhythmogenicity as a function of tissue geometry, and high-throughput testing of novel spatiotemporal control algorithms for arrhythmia control. Furthermore, use of hiPSCs allows for the exploration of these topics in disease- and patient-specific contexts.

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(29) HUMAN MESENCHYMAL STEM CELLS ENHANCE CARDIAC CONTRACTILITY THROUGH EXOSOMAL PARACRINE SIGNALING

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Introduction: An emerging therapy for non-ischemic cardiomyopathy involves the delivery of human mesenchymal stem cells (hMSCs). Clinical trials document modest benefits on cardiac contractility, underscoring a need to better understand and exploit the underlying mechanisms governing hMSC-cardiomyocyte interactome. Our recent work has demonstrated that hMSC paracrine signaling favorably counteracts heterocellular coupling effects on cardiac contractility. However, little is known about the key components of the hMSC secretome for enhancing cardiac function. We hypothesize that bilayer membrane bound exosomes play a key role in hMSC paracrine mediated enhancement of cardiac contractility.

Methods: We utilize three-dimensional human engineered cardiac tissues (hECTs) as an in vitro model to investigate the role of hMSC exosomes in the enhancement of cardiac function. Following baseline hECT contractile function testing on day 5, hECTs cultured in serum-free defined media (SFDM) were replaced with the following treatments: 1) SFDM (Control); 2) hMSC conditioned media (hMSC CdM); 3) SFDM supplemented with hMSC exosomes (hMSC exo); or 4) hMSC exosome-depleted conditioned media (hMSC exo-depl). hECTs were cultured an additional 5 days, and then developed force (DF) was measured again.

Results: The hMSC CdM and hMSC exo treatments led to statistically significant increases in DF ($p<0.05$), whereas the control and hMSC exo-depl groups were unchanged relative to pre-treatment (n=4-7). hECTs were then snap-frozen for prospective real time quantitative polymerase chain reaction of cardiac-specific, apoptosis, and calcium handling genes; mRNA levels of SERCA2a and LTCC significantly increased for hECTs treated with hMSC CdM and hMSC exo ($p<0.01$), while the BAX/BCL2 ratio, an established apoptosis marker, significantly decreased ($p<0.01$).

Summary/Conclusion: These findings demonstrate at functional and molecular levels that hMSC exosomes play a central role in hMSC paracrine mediated enhancement of hECT function, motivating further investigation of the key exosomal cargo responsible for these cardioactive effects.

(30) CELL SURFACE GLYCOENGINEERING IMPROVES SELECTIN-MEDIATED ADHESION OF MESENCHYMAL STEM CELLS (MSCS) AND CARDIOSPHERE-DERIVED CELLS (CDCS): PILOT VALIDATION IN PORCINE ISCHEMIA-REPERFUSION MODEL

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Stem cell therapy has been shown to have promising results in cardiovascular applications and there are many ongoing clinical trials. One of the shortcomings of such clinical trials is the poor homing and engraftment ability of the natural stem cells as they lack the ability to target the site(s) of injury. In this paper, we show that glycoengineering of stem cells enables them to mimic neutrophils and address this limitation. Here two modifications have been implemented on CDCs and MSCs to facilitate their interaction with selectin molecules expressed on the vascular endothelium: i. a P-selectin glycoprotein ligand-1 (PSGL-1) mimetic 19Fc[FUT7$^+$] was non-covalently coupled to the surface of the stem cells via lipid-protein G, and ii. α(1,3) fucosyltransferase FUT7 was overexpressed in these cells. Functionalized cells with 19Fc[FUT7$^+$] showed enhanced binding to P-selectin, while cells overexpressing FUT7 had elevated interaction with E-selectin. In an *ex vivo* setting, these surface modifications aided the capture and rolling of mesenchymal and cardiosphere-derived stem cells, under physiological shear, on substrates bearing either IL-1β stimulated endothelial cells or immobilized selectin proteins. A pilot study using a swine ischemia-reperfusion injury model confirmed the safety of the implemented modification technique, and the efficacy of the functionalized cells to target and home to sites of ischemia. Further studies are ongoing, to improve the efficiency of this cell capture process, and to enhance engraftment. Overall, the glycoengineering of stem cells may enable the targeting of stem cells, thus enhancing the success rate of current and future clinical trials.

(31) A SPROUTY4 REPORTER TO MONITOR FGF/MAPK/ERK SIGNALING ACTIVITY IN THE MOUSE

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Fibroblast growth factors (FGF) are a family of cytokines that bind to and activate receptor tyrosine kinase (RTK) FGF receptors (FGFRs). FGF ligands are the main triggers of MAPK/ERK signaling, which plays a fundamental role in many basic cellular processes including proliferation, metabolism, migration, cell survival and differentiation. For this reason, a reporter of MAPK/ERK signaling has long been sought. So far, the distribution of MAPK/ERK activity has been analyzed at static snapshots in time but to understand how the role of this complex pathway changes dynamically, tools are required that allow quantitative single cell resolution monitoring of signaling activity over time in fixed and live sample preparations. Some of the earliest target genes activated in response to MAPK/ERK signaling are negative regulators of this pathway such as *Sprouty* (*Spry*) genes. We have generated embryonic stem (ES) cells and mice with a knock-in fluorescent reporter allele for *Spry4*. We observe that the *Spry4* reporter (*Spry4$^{H2B\text{-}Venus}$*) is expressed in regions of the embryo with known FGF signaling activity such as the somites, tail bud, isthmus, pharyngeal arches and limb buds. *Spry4$^{H2B\text{-}Venus}$* exhibits highly localized expression within adult organs potentially giving novel insights into the mechanisms of FGF regulation in tissue homeostasis.

Furthermore, the reporter responds to manipulations of MAPK/ERK signaling in ES cells and early embryos.

(32) TYROSINE KINASE RECEPTOR EPHB2 LOSS-OF-FUNCTION ALTERS SPERMATOGONIAL STEM CELL ACTIVITY

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Spermatogonial stem cells (SSCs) represent the proximal origin for sperm production in mammals. SSCs can be extracted from the adult mouse testis and maintained long-term in vitro; these are referred to as adult germline stem cell (GSC) lines. Recently, members of the Eph receptor family of tyrosine kinase have been detected in murine spermatogonia. EphB2 is expressed on progenitor cells in the intestinal tract and is critical for normal cell proliferation. We detected EphB2 at the level of mRNA and protein in GSC lines. Based on these findings, we hypothesized that EphB2 plays a role in the maintenance of SSCs. To interrogate EphB2 genetically, we employed the CRISPR-Cas9 method and generated mutant GSCs with a complete loss of EphB2 (EphB2-KO), which we validated by flow cytometry. We then assessed the characteristics of EphB2-KO cells using various phenotypic and functional assays. First, we observed that, *in vitro*, EphB2-KO GSCs exhibit a reduced proliferation rate compared to matched, untargeted wild type (WT) GSCs, while the apoptosis rate remains unchanged. Therefore, we examined whether loss of EphB2 correlates with the activity of canonical signaling pathways involved in SSCs self-renewal and proliferation. EphB2-KO GSCs exhibited reduced activity of the MAPK/Erk signaling. To assess whether loss of EphB2 impairs SSC function, *in vivo* colonization assays were performed. Fluorophore-expressing WT or EphB2-KO GSCs were transplanted into the seminiferous tubules of busulfan-treated C57Bl6 mice. Animals were sacrificed after 6-8 weeks. We found that EphB2-KO GSCs formed smaller and fewer colonies than WT GSCs, indicating a role for EphB2 in the stem cell activity of GSCs. Finally, the challenging aspect of studying Eph receptor signaling pathways resides in promiscuous ligand binding and the concept of bidirectional signaling. Hence, our current investigations focus on identifying the partners specific to the EphB2-related stem cell activity in SSCs.

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(33) CHEMOTACTIC DYNAMICS OF PHOTORECEPTOR PRECURSOR CELLS AND RETINAL PROGENITOR CELLS IN MICROFLUIDIC VEGF AND OSTEOPONTIN GRADIENTS

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The biochemical interactions influencing migration of retinal progenitor cells (RPCs) and photoreceptor precursor cells (PPCs) following retinal transplantation have not been well defined. Chemotaxis is an intrinsic mechanism of homing and integration of these cells to appropriate retinal lamina. A number of potential chemotactic signaling molecules are localized to the retinal microenvironment in the adult mouse and receptors to the surface of RPCs and PPCs. Bioinformatic analysis of the VEGF and Osteopontin ligand and tissue-specific interaction pathway predicted a chemotactic function for mouse retina (Fig. 1), which is tested at 500ng concentrations of the ligand using Ibidi microfluidic devices (Fig. 2). Single cell RPC migration studies in a microfluidic chamber, which ensures a steady-state gradients of VEGF and Osteopontin, supports a chemotactic of RPCs and PPCs (Fig. 3). The receptors for VEGF and Osteopontin, KDR and ITGA, respectively are present in both RPCs and PPCs by qPCR (Fig. 4) and immunocytochemical analysis (Fig. 5). The results indicate that VEGF or Osteopontin function as a chemoattractant for RPC and PPCs in this *in vitro* model system, to a degree that is both concentration and gradient dependent. Further analysis will elucidate the downstream signaling mechanisms active during RPC and PPC migratory responses to VEGF or Osteopontin in vitro. Data derived from this work may improve the efficiency of neural progenitor transplantation to restore damaged retinal and other CNS tissue.

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(34) IMAGING THE FUNCTIONAL INTEGRATION OF ADULT-BORN HIPPOCAMPAL GRANULE CELLS INTO THE DENTATE GYRUS NETWORK

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The ability to discriminate among similar experiences is an important feature of episodic memory. The dentate gyrus of the hippocampus and its ability to incorporate newborn granule cells throughout life is crucial for the neural computation that generates spatial and contextual discrimination behaviors. In particular, preferential activation of 1-2-month old adult born granule cells (abGCs) within the predominantly silent dentate network, is hypothesized to allow abGCs to actively participate in these behaviors. Our lab has recently shown that abGCs exhibit higher firing rate and reduced spatial tuning with similar degree of spatial remapping between contexts compared to the mature GCs (mGCs) *in vivo* (Danielson NB. et al, 2016), suggesting that abGCs indirectly modulate the activity of the mGCs by recruiting local inhibitory networks (Drew LJ. et al, 2015). Nevertheless, circuit interactions of abGCs that contribute to information processing within the dentate gyrus during discrimination behaviors are poorly understood. To this end, we will use two-photon calcium imaging in awake behaving mice to monitor the activity of abGCs and mGCs over the 4-week period during which abGCs functionally integrate into the dentate gyrus circuits. Then, rate of the activity in DG cells will be extracted with z-score matrices and used as a functional feature to identify cell subsets:

abGCs and mGCs. Lastly, spatial tuning properties of DG cells will be investigated with place field/cell analysis to address whether there are any contextual and sequential encoding preferences in abGCs and mGCs. This preparation will highlight the emergence of spatiotemporal activity of abGCs during encoding of contextual information and will allow us to test how manipulating their activity affects the circuit dynamics of mGCs.

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(35) EXPANSION OF HSCS FROM CORD BLOOD CD34$^+$ CELLS BY VALPROIC ACID REQUIRES BOTH MITOCHONDRIAL REMODELING AND P53

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The use of umbilical cord blood (UCB) as a graft for adult transplant recipients has been limited due to the small numbers of hematopoietic stem cells (HSC). We have reported that UCB CD34$^+$ cell numbers can be expanded by *ex vivo* treatment with a cytokine combination and the histone deacetylase inhibitor, valproic acid (VPA). We show that VPA triggers two distinct phases of HSC behavior. The first phase is characterized by a transient expression of pluripotency genes and a limited increase in the numbers of HSCs. The second phase is characterized by a significant increase of HSC numbers due to greater numbers of cell divisions and loss of quiescence. VPA-treated HSCs maintain a mitochondrial profile characterized by low mitochondrial potential, ROS levels and mass. Remarkably, the mitochondrial network is comprised of primitive and cristae-poor mitochondria. Removal of VPA from the culture suppressed HSC expansion and augmented mitochondrial ROS, mass and potential. Thus, VPA triggers both the epigenetic modifications and remodeling of a primitive mitochondrial network to dedifferentiate and expand the pool of HSCs from UCB CD34$^+$ cells.

In addition to a restructured mitochondrial network, VPA transiently up-regulates p53 to suppresses excessive ROS. Mechanistically, we found that p53 triggered antioxidant defense mechanisms that rely on activation of MnSOD and Sestrin2. Notably, inhibition of the p53-MnSOD axis limited the ability of VPA to expand the pool of HSCs by increasing ROS generation but not the mitochondrial mass. Conversely, co-treatment with VPA and Nutlin, an inhibitor of p53 degradation enhanced the expansion of HSCs without altering the mitochondrial mass. Therefore, both mitochondrial remodeling and p53 activation are required for dedifferentiation and the functional fitness of the expanded HSCs by VPA.

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(36) THE INTERACTION OF DENTAL PULP STEM CELLS WITH 3-D SCAFFOLDS-MOLDED VS. PRINTED – CAN WE TELL THE DIFFERENCE?

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Additive manufacturing technologies are increasingly being used to replace standard extrusion or molding methods in engineering polymeric biomedical implants and devices. The principal advantage of this new technology is the ability to print directly from a scan and hence produce parts which are an ideal fit for an individual, which eliminates much of the sizing and fitting associated with standard manufacturing methods. 3-D Fused Deposition Modelling (FDM) can produce components which have nearly identical functionality and meet the same macroscopic manufacturing criteria as the equivalent molded components, and hence in most cases qualify for FDA Pre-Market 510(k) approval. Here we show that the situation is far more complex if the device is in contact with living tissue. Scanning electron microscopy shows that while molded of Poly Lactic Acid (PLA) components have microscopically flat surfaces, extrusion of PLA filaments through the printer head nozzle produces multi-scaled roughness. Microscaled roughness occurs due to incomplete filament fusion occurs at low speed and temperatures, while nanoscaled periodic roughness due to “sharkskin” (slip-stick phenomenon) occurs at high temperatures and high extrusion rates. Here we demonstrate that dental pulp stem cells cultured on PLA scaffolds are very sensitive to the manufacturing process. In contrast to DPSC cultured on molded substrates, where dexamethasone is required to induce differentiation, DPSC cultured on FDM 3-D printed scaffolds of equivalent dimensions, can be induced to differentiate without dexamethasone, along either odontogenic or osteogenic lineages depending on the specific details of the extrusion process, indicating that in addition to functionality, the manufacturing process should also be considered in obtaining FDA approval for devices.

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(37) A CHROMATIN BARRIER INSULATOR IN THE T-CELL RECEPTOR α (TCRα) GENE LOCUS CONTROL REGION (LCR)

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The efficacy of stem cell gene therapy is often hindered by lack of, or variability in, the expression of a therapeutic gene due to the influence of the chromatin environment at

its site of integration. Flanking a therapeutic gene with barrier insulators can counteract this problem to enable robust, long-term and site-of-integration independent expression. Barrier insulators are DNA regulatory elements that prevent cross-regulatory influences from surrounding chromatin. There are only a few promising barrier insulators that are currently available for application in gene therapy. The impact and risk involved with gene therapy demand the identification of new insulators that can be incorporated into gene delivery vectors. This study identifies a novel barrier insulator within the mouse TCRα LCR. LCRs are *cis* regulatory elements that, along with their ability to overcome site-of-integration dependence, impose specific spatiotemporal expression pattern on a linked gene. Various sub-regions of this LCR were tested for their insulation capacity in stably-transfected T cell lines. We find that a 4-kb region of this LCR is sufficient for barrier insulation, in that it prevents silencing of a linked reporter gene over a 90-day period. TCRα LCR insulators enable maintenance of histone acetylation at the reporter gene locus, which is generally associated with euchromatin. This activity is also confirmed by DNase I sensitivity assay that demonstrate the open chromatin form of the insulator-linked reporter gene. Thus, we show that the TCRα LCR contains elements that support typical features of well-characterized barrier insulators. Further understanding of this insulation capacity will enable sub-regions that possess barrier activity to be incorporated into gene therapy vectors, to help achieve stable and long-term therapeutic gene expression

(38) NANOG RESTORES MECHANICAL PROPERTIES AND EXTRACELLULAR MATRIX DEPOSITION BY SENESCENT STEM CELLS

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Extracellular matrix (ECM) is critical in regulating the mechanical properties of many tissues including arteries and skin. It is well established that aging is accompanied by significant loss of tissue strength and elasticity mainly due to loss of elastin fibers, increased collagen crosslinking and decreased collagen synthesis. Therefore, there is a need to develop strategies to restore the impaired deposition of ECM.

Here we report that ectopic expression of NANOG could restore the impaired ECM deposition by human senescent stem cells and mechanical properties of bioengineered tissues prepared from these cells. To this end, we employed two widely established models of aging - replicative senescence of human Mesenchymal Stem Cells (MSC), and myofibroblasts derived from Hutchinson's Guilford Progeria Syndrome (HGPS) patients.

Our results show that senescence reduced synthesis of both collagen 3 and elastin. Consequently, tissues engineered using senescent cells showed inferior mechanical properties. Notably, NANOG restored the capacity of senescent cells to express both ECM molecules and increased the mechanical properties of engineered tissues. NANOG restored the activity of the TGFβ pathway that was impaired in senescent stem cells and is critical for ECM synthesis and crosslinking. Chemical inhibition (SB431542) or shRNA knockdown of SMAD4 (shSMAD4) prevented reversal of the senescent phenotype, suggesting that TGFβ pathway activation mediated the effects of NANOG.

Further, knockdown and gain of function approaches indicated that both SMAD2 and SMAD3 were necessary to mediate the effects of NANOG but only SMAD3 was sufficient. In summary, our study provides a novel strategy to restore the impaired production of ECM by senescent stem cells and may have broad applications in stem cell therapies for tissue regeneration as well as anti-aging treatments.

(39) ISOGENIC HUNTINGTON'S DISEASE EMBRYONIC STEM CELL LINES REVEAL NOVEL DEVELOPMENTAL PHENOTYPES

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Huntington’s disease (HD) is a dominant autosomal neurodegenerative disease caused by an expansion of CAG repeats in the first exon of the *HTT* locus that translates into an increase of poly-glutamine (polyQ) repeats in the Huntingtin protein (HTT). Despite the fact that HTT was among the first disease-causing genes to be cloned over 20 years ago, the molecular mechanism of disease is still unknown, and more strikingly, even the wild-type function of HTT is still not clearly defined. Animal models of HD have been useful in understanding some aspects of cellular dysfunction, but unfortunately they do not fully reproduce the full symptomatology of human HD, and therefore better models systems are required. Here we describe the generation of a set of isogenic, CRISPR/Cas9 gene-edited, human embryonic stem cell (hESCs) lines with different CAG lengths, representing different levels of disease severity and onset. In order to understand the mechanisms of the disease, we also generated HTT+/- and HTT-/- isogenic hESC lines to determine whether HD phenotypes arise due to a gain- or a loss-of-function of HTT, a question still unresolved. We found that the simple alteration of the CAG length produced a constellation of early developmental alterations at multiple stages: We found that when cultured on micropatterned substrates and induced to differentiate with BMP4, our isogenic lines self-organize and generate distinct CAG length-dependent, radially symmetrical patterns of germ fates, thus providing the earliest reported phenotypic signature for human HD. When submitted to neural induction, all lines generated neural rosettes and neural progenitors, but self-organization was perturbed in the HD lines. Finally, examination of post-mitotic cortical neurons revealed the appearance of multinucleated giant cells. The frequency of these abnormal progenitors, which are caused by a failure in cytokinesis, increased proportionally to the CAG length, and were phenocopied in HTT-/- lines. Our results provide for the first time highly quantitative, human phenotypic signatures of HD, and strongly suggest that HD mutations cause abnormalities during embryonic development of the human brain, with the devastating consequences manifesting symptoms decades later.

(40) NANOG EXPRESSION RESTORES THE REGENERATIVE CAPACITY OF SENESCENT MYOBLAST

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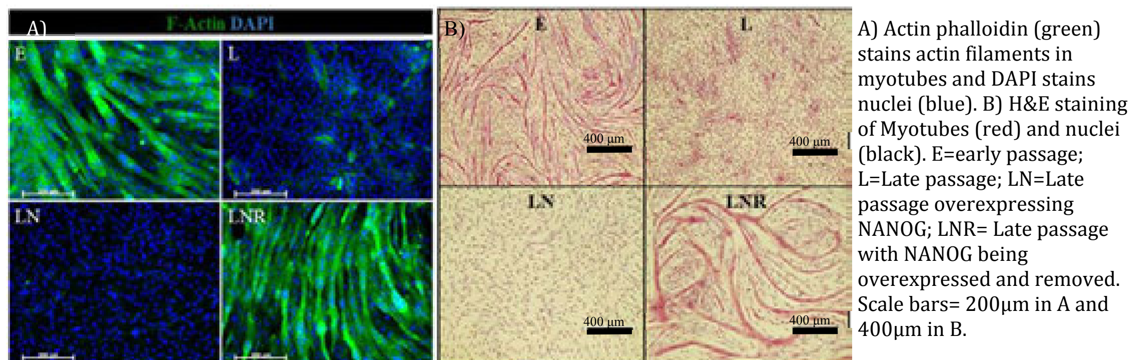
Skeletal Muscle constitutes 45-55% of the total body mass and carries important physiological functions such as skeletal movements, maintaining body posture, regulating body temperature, etc. However, the function of skeletal muscle declines rapidly with aging. Age-related loss in muscle mass, sarcopenia, is a major medical problem facing the elderly and correlates with disabilities, loss of metabolic function, morbidity, and mortality. Adult skeletal muscle regeneration relies on the activity of satellite cells resident in skeletal muscle niche. However, systemic and intrinsic factors decrease the myogenic differentiation potential of senescent satellite cells. Previous attempts for restoring the function of satellite cells and improving muscle regeneration targeted intracellular signaling pathways or extracellular factors. In a novel approach, our lab employed an embryonic transcription factor, NANOG, to reverse the effects of senescence. Previous works in our lab showed that ectopic expression of NANOG restored the impaired contractile function and myogenic differentiation potential of senescent mesenchymal stem cells (MSCs).

In this study, we also observed down-regulation in the myogenic differentiation potential of late passage myoblasts that was accompanied by significant changes in the expression of myogenic regulatory factors (Myf5, Myod, Myogenin, and MRF4) and members of myocyte enhancer factor 2 family (Mef2a-d). Interestingly, overexpression of NANOG in proliferating myoblasts restored their myogenic differentiation. A course of 20 days of NANOG expression was enough to rescue the myogenic differentiation capacity and this reversal was stable after removal of NANOG from the cell. Overexpression of NANOG also preserved the morphology and size of myoblasts over serial passaging.



A) Actin phalloidin (green) stains actin filaments in myotubes and DAPI stains nuclei (blue). B) H&E staining of Myotubes (red) and nuclei (black). E=early passage; L=Late passage; LN=Late passage overexpressing NANOG; LNR= Late passage with NANOG being overexpressed and removed. Scale bars= 200µm in A and 400µm in B.

In summary, these results shed light on the potential of NANOG to restore the myogenic differentiation potential of senescent myoblasts and to reverse the loss of muscle regeneration due to aging.

(41) INDUCING ODONTOGENIC DIFFERENTIATION OF HUMAN DENTAL PULP STEM CELLS VIA ENZYMATIC CROSS LINKING OF HYDROGELS

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Hydrogels are increasing in popularity as substrates for cell culture and cell delivery, since they simulate more closely the in-vivo environment. In particular, with the advent of bioprinting enzymatically cross linked gels are becoming a popular alternative to synthetic gels containing to the more common, but potentially damaging UV cross linking functionalities. In contrast to chemical cross linking, enzymatic cross linking is specific to well defined functional groups, and hence controls not only the stiffness or modulus of the gel, but also the supra-molecular structure of the protein component of the gel. But even though, as has been shown recently using cryo-volume SEM imaging, the supra-molecular structures generate multiple conformations with equivalent moduli, the functional domains expressed depend on the protein structure, and can vary greatly with the parameters of the enzymatic reactions. Apical regeneration is a well known phenomenon in children when a blood clot is produced near an injury affecting the root canal, while gelatin has been shown to induce massive biomineralization when plated with dental pulp stem cells. Here we try to understand these phenomena in greater depth by correlating the observed differentiation lineage (odontogenic vs osteogenic) of the dental pulp stem cells with the underlying structure of the hydrogel and the functionalities exposed during the enzymatic cross linking process.

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(42) MODELING PEDIATRIC GLIOMAS OF THE THALAMUS BEARING HISTONE MUTATIONS

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Pediatric high-grade gliomas are malignant brain tumors arising in children that are associated with very poor clinical outcomes. Recent efforts to profile the genetic alterations found in these tumors have revealed frequent mutations in histone H3 genes, including lysine-to-methionine "$H3^{K27M}$" mutations in 80% of diffuse intrinsic pontine gliomas (DIPGs) and other subcortical gliomas. Tumors with $H3^{K27M}$ mutations, including DIPGs, possess somatic alterations in a variety of growth factor signaling

pathways as well. Such co-alterations include activating mutations in the fibroblast growth factor (FGF) receptor *FGFR1* in tumors that arise preferentially in the thalamus. The roles of these mutations in tumorigenesis remain unclear; moreover, their potential values as therapeutic targets have yet to be fully explored. Here, we aim to establish a model of pediatric thalamic gliomas harboring *FGFR1* and $H3^{K27M}$ mutations. To date, our findings indicate that $H3^{K27M}$ reduces overall H3K27 trimethylation in neural precursor cells (NPCs) expressing thalamic progenitor-associated genes along with activated *FGFR1*. We have found that both of these mutations drive a variety of cancer-associated phenotypes in these NPCs *in vitro* and that NPCs bearing the mutations can be transplanted into the mouse brainstem to model the disease *in vivo*. We have also found that activated *FGFR1* and $H3^{K27M}$ increase expression of genes associated with early neuroectodermal cells but not highly expressed in NPCs, including *LIN28A* and *LIN28B*, and that multiple mechanisms may underlie their upregulation. Finally, we report that targeting FGFR activity with a small-molecule inhibitor depletes wild-type NPCs and NPCs bearing the mutant receptor at similar doses, thus indicating limited efficacy and potential toxicity of this therapeutic strategy. Further efforts are underway to thoroughly characterize this model *in vitro* and *in vivo* via cell grafting, with the goal of providing novel insights into the biology and potential treatment of these refractory pediatric brain tumors.

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(43) THE ROLE OF FGF IN REPROGRAMMING OF EPIDERMAL KERTINOCYTES TOWARD NEURAL CREST FATE

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Neural crest cells (NC) play a central role in the formation of the peripheral nervous system, the craniofacial skeleton and pigmentation of the skin during embryonic development. Due to their broad differentiation potential these cells have been a focal point of research for disease therapeutics via cell transplantation. However, an easily accessible autologous cells source for therapeutic cell transplantation remains one of the main challenges facing the field. Recently our lab discovered that human epidermal inter-follicular keratinocytes (KC) can turn into neural crest (NC) stem cells without

ectopic expression of transcription factors or reprogramming to the pluripotent state. Here we studied the role of FGF2 in this novel phenomenon.

We identified two pathways to be critical for reprogramming leading to expression of the NC-specific transcription factors including *SOX10*, *Pax3, FoxD3* as well as intermediate filament gene *Nestin*. Most notably, KC-NC could be coaxed to differentiate into peripheral neurons, Schwann cells, melanocytes and smooth muscle cells as shown by molecular as well as functional assays.

In conclusion, we provide mechanistic insight into the process of keratinocyte reprogramming to neural crest stem cells. This work represents a paradigm shift in stem cell biology as it demonstrates the unusual plasticity of human adult KC that can turn into many different cells types without genetic modification or reprograming to the pluripotent state. The dearth of cell sources for treatment of neurogenic disorders, combined with the accessibility and growth potential of human epidermal cells suggest that the proposed work could have tremendous implications for the use of cell therapy for treatment of many debilitating diseases.

(44) CDX2-DERIVED CELLS FROM PLACENTA: A NOVEL MULTIPOTENT CELL SOURCE FOR CARDIAC DIFFERENTIATION

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Placenta is an easily available and rich source of multipotent cells. We previously reported that fetal cells from placenta “home” to injured maternal heart and approximately 40% of the migrating cells expressed homeodomain protein Cdx2. This interesting observation led us to hypothesize that placental Cdx2 could be a novel cell target for cardiac differentiation. To understand this phenomenon, we employed a cre-lox strategy that labeled Cdx2 cells in placenta with e-GFP and induced myocardial infarction (MI) in pregnant mice at mid-gestation. The maternal heart was analyzed 4 weeks post-MI for the presence of Cdx2-eGFP-derived cardiomyocytes. Additionally, Cdx2-eGFP cells were isolated from late-gestation placenta and assayed for cardiac differentiation *in vitro* followed by live cell imaging. Phenotypic and whole cell proteomic analysis, clonal and vascular lineage differentiation and immune profiling was carried out subsequently. We observed that Cdx2 cells migrated to injured maternal hearts and differentiated into cardiomyocytes revealing the functional significance of fetal-maternal stem cell transfer. Additionally, isolated Cdx2-eGFP cells from the late placenta differentiated into spontaneously beating cardiomyocytes in culture and expressed structural proteins Cardiac Troponin T(cTnT), α-sarcomeric actinin and gap junction protein Cx43. These cells underwent clonal expansion and differentiated into endothelial and smooth muscle lineages in culture indicative of their multipotent nature. Low expression of MHC molecules and components of the immune-responses, in these cells suggested the capability to evade host immune surveillance. Furthermore, Cdx2 cells responded robustly to chemokine-mediated migration and the proteomic analysis

confirmed these enriched cell functions in Cdx2 cells compared to the embryonic stem cells. In summary, Cdx2 cells from placenta represent a novel mutipotent cell-type that possesses cardiomyogenic and vasculogenic potential with an inherent “homing” ability. Our findings thus clearly infer that Cdx2 cells may be a potential candidate in allogeneic cell therapy strategies for cardiac repair.

[Supported by NYSTEM contract C029565]

(45) MZF1 AND GABP ACT AS KEY TRANSCRIPTIONAL ACTIVATORS OF YAP1 IN OSTEOPROGENITOR LINEAGE

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The Hippo pathway discovered in Drosophila as a regulator of organ size, consists of a phosphorylation cascade that restrains the activity of the transcriptional coactivator, YAP1 and TAZ. Hippo signaling in mammals restrains cell proliferation and stemness. This pathway also has a significant tumor suppressive role, as promotes the phosphorylation and nuclear exclusion of YAP1, which can function as a potent oncogene. We have shown that the stem cell transcription factor SOX2 antagonizes the Hippo pathway and affects the lineage differentiation fates of mesenchymal stem cells (MSCs) through direct induction of YAP1 *(Seo et al, Cell Reports, 2013)*. SOX2 and YAP1 also maintain cancer stem cells in osteosarcomas, a cancer that arises from the MSC lineage. SOX2 antagonizes the Hippo pathway by directly inducing YAP1 and repressing Hippo upstream activators *(BasuRoy et al, Nature Comm, 2015*). We are now determining how SOX2 and other transcription factors regulate YAP1 expression in the osteogenic lineage by examining the importance of putative SOX2 binding sites in the YAP1 genomic regions. In particular, we are using nucleotide substitutions to delete putative functional elements in the 243 bp enhancer region of YAP1. Together with proteomic analysis we aim to determine the coactivators of YAP1 that are cooperating with SOX2 to regulate YAP1 by mutagenesis approach. We have generated luciferase reporter constructs driven by SOX2 bound genomic regions of YAP1, and identified the enhancers for YAP1 expression in mesenchymal stem cells (MSCs) and osteoprogenitor cells. We have identified additional transcription factor (TFs) Myeloid Zinc Finger 1 (MZF1) and GA Binding Protein (GABP) that play a role in regulation of YAP1 in cancer stem cells.

Our data revealed that Myeloid Zinc Finger domain (MZF1) and GA binding protein (GABP) are required to maintain basal activity of YAP1 in osteoproginator cells. Knocking down of MZF1 and GABP decreases the expression of YAP1 while, overexpression of MZF1 activates YAP1 expression. Interestingly, these and other related studies have led to the conclusion that YAP also regulates SOX2 expression creating self sustaining expression loop.

Elevated YAP1 activity due to mutations in Hippo pathway components or *YAP1* amplification is observed in several types of human cancers such as osteosarcoma and glioblastomas which also express high level of SOX2. Therefore disruption of YAP1 transcriptional activity could be a therapeutic strategy for YAP1 dependent tumours.

References:
Seo et al, Cell Reports, 2013 Jun 27;3(6):2075-87,
BasuRoy et al, Nature Comm, 2014 Apr 2;6:6411.

(46) AN OPTOGENETIC 3D MODEL OF HUMAN NEUROMUSCULAR JUNCTIONS

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Neuromuscular junctions (NMJ) are synapses formed between motor neurons and skeletal muscle fibers, and are targets of several motoneuron diseases. Since repeated visualization and manipulation of human NMJs is not feasible, it is necessary to develop *in vitro* models for the study of NMJ formation and evolution under physiological or pathological conditions.

Here we present a novel microfluidic system for compartmentalized 3D culture of human skeletal muscle cells and hiPSC-derived motoneurons. The muscle chamber features two compliant pillars for myotube attachment and force sensing, and connects to the motoneuron chamber via a channel for axon outgrowth.

For controlled testing of neuromuscular connectivity, hiPSCs were transduced with a lentivirus containing the channelrhodopsin-2 protein (Boyden et al., 2005), a cation channel that opens in response to blue light. Following myotube differentiation (Guo et al, 2014), motoneurons derived from the channelrhodopsin-expressing hiPSCs (Maury et al., 2015) are seeded in the adjacent chamber, and NMJ function is confirmed by muscle contraction in response to motoneuron activation with blue light.

We observe the first signs of NMJ formation as early as 5 days after motoneuron seeding, with improvement in NMJ functionality over the following 2 weeks. Furthermore, our system enables the study of NMJ maturation over time, as evidenced by increased muscle contractility and structural changes in the NMJ.

The platform described here can be easily used for high-throughput drug testing and disease modeling. In particular, the use of hiPSCs enables the use of patient-specific cell lines for the study of genetic diseases and personalized medicine.

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(47) ENGINEERING TUMOR MICROENVIRONMENTS FOR STUDIES OF OSTEOLYSIS

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The lack of ability to model with fidelity the complexity of the osteolytic process in bone cancer constitutes a barrier to understand the mechanisms underlying the progression of this disease and limits the evaluation of anti-osteolytic drugs. In response to these limitations, we have established a set of Tissue-Engineered (TE) models of Ewing's sarcoma (ES) that mimic crucial bone tumor microenviromental factors *in vitro*.

Firstly, we investigated the role of mechanical forces within bone tumors in regulating osteolytic-related proteins. We seeded ES cells onto 3D porous collagen 1- hyaluronic acid scaffolds cultured in a bioreactor with mechanical loading. Our data indicate that bone osteolytic-related proteins (i.e. RUNX2) expressed in ES tumors from patients, are down-regulated in monolayer cultures but re-expressed in ES tumors generated in a biomimetic system under mechanical forces. We also demonstrated that biomechanical stimuli modulated sensitivity to anti-cancer drugs, such as Sorafenib.

In addition, we established a TE model of ES within a bone niche to address the role of cancer cells in osteoclasts activation during osteolysis. The bone tissue was formed by co-culturing within a native decellularized bone matrix (*i*) ES cells, (*ii*) human mesenchymal stem cells differentiated into osteoblastic lineages and (*iii*) monocytes differentiated into osteoclasts. We observed decreases in bone volume density, bone connectivity and organic bone matrix deposition. Interestingly, the TE model recapitulated the effects of the therapeutic reagent zoledronic acid observed in patients.

(48) CELL-CELL INTERACTION MICROCHIP FOR ANALYSIS OF STEM CELL COOPERATIVITY

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Stem cell based neural therapies offer tremendous hope for treating neurodegenerative diseases as evident through numerous in vitro and in vivo studies in rat and non-human primate models. A barrier to CNS cell therapies is in choosing what cells or cell combinations will present optimal therapeutic potential for a given neurodegenerative disease such as traumatic brain injury (TBI). We start this study by analyzing cell-cell interactions and heterogeneity of embryonic stem cells (hESCs) which will be differentiated to neural stem cell rosettes and to pyramidal and GABAergic interneurons in our study. The cell-cell interaction microchip (CCIM) platform enables detection of ~20 proteins secreted by separate communicating cells from a heterogeneous population. In an analysis of intercellular communication in WA01 (H1) or WA09 (H9) hESCs, secreted factors related to WNT, IGF, FGF and BMP signaling were selected for detection on the barcode for the CCIM. Immunostaining revealed that cells secreting high levels of IGFBP2 and DKK3 remain associated with the colony periphery while BMP2+/FGF+ cells are regularly interspersed throughout the colony. Data obtained from the CCIM for ESCs revealed that the 2D growth of ESCs depends on extensive paracrine communication mediated by secretory factors and quantified changes to those signaling components. Thus ESCs in a colony spontaneously establish a

regulatory stem cell niche where cells on the periphery are partially differentiated and communicate with the more centrally positioned cells. This initial study provides direct evidence of heterogeneous signaling networks occurring between hESCs within the same colony and further defines how these networks may interact to control maintenance of self renewal and pluripotency. Our future work extends the NCCIM to evaluate neural rosettes, neurons, and mixed neuronal microenvironments as well as neuron-microglia interactions upon mechanical stress.

[NYSTEM award pending, DH01-STEM5-2016-00290]

(49) OPTOGENETIC STIMULATION FOR THE MATURATION OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

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Cardiovascular disease is the leading cause of patient mortality. Human induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs) are a promising tool for *in vitro* modeling of disease and drug studies. However, hiPS-CMs are far less mature than their native counterparts. To overcome this limitation, electrical stimulation has been used for maturation of cardiomyocytes over the past decade. Cardiomyocytes expressing light-responsive ion channels would allow for the use of optical pacing as an alternative means for maturation. Here we demonstrate transcriptional and functional maturation in 1) three-dimensional embryoid body (EB) constructs and 2) engineered tissues seeded into a custom fabricated bioreactor through optical pacing.

ChR2-expressing hiPSCs were created via lentiviral transduction, differentiated into cardiomyocytes, and subsequently seeded into EBs and engineered tissues. Custom image processing software was used to determine: 1) the spontaneous beating frequency and beat-by-beat deformation, 2) the maximum capture rate, in conjunction with an arduino-driven optical setup for reproducible ramped pacing, and 3) conduction velocity, in conjunction with a combined patterned illumination/optical mapping platform. Quantitative real time reverse transcription PCR (qRT-PCR) was used to quantify gene transcription and calculated as fold change over control.

Video analysis of EBs revealed entrainment to both higher and lower pacing frequencies. Optically paced tissue constructs showed functional improvements including an increased maximum capture rate and conduction velocity. Gene expression analysis showed upregulation of multiple genes associated with calcium handling, connexins, and sarcomeric structure. These findings demonstrate that optical pacing on transgenic hiPS-CMs leads to functional and genetic changes linked with cardiac maturity.

[Supported by NYSTEM contracts C028119 and C030291]

(50) IDENTIFICATION OF MOUSE COCHLEAR PROGENITORS THAT DEVELOP HAIR AND SUPPORTING CELLS IN THE ORGAN OF CORTI

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The adult mammalian cochlear sensory epithelium houses two major types of cells, mechanosensory hair cells and underlying supporting cells, and lacks regenerative capacity. Recent evidence indicates that a subset of supporting cells can spontaneously regenerate hair cells after ablation only within the first week post-parturition. Here, in vivo clonal analysis of mouse inner ear cells during development demonstrates clonal relationship between hair and supporting cells in sensory organs. We report the identification in mouse of a previously unknown population of multipotent stem/progenitor cells that are capable of not only contributing to the hair and supporting cells but also to other cell types, including glia, in cochlea undergoing development, maturation and repair in response to damage. These multipotent progenitors originate from Eya1-expressing otic progenitors. Our findings also provide evidence for detectable regenerative potential in the postnatal cochlea beyond one week of age.

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(51) DEVELOPMENT OF NOVEL MENIN INHIBITORS FOR TREATMENT OF GLIOMAS

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Diffuse intrinsic pontine gliomas (DIPGs) are rare, highly aggressive brainstem tumors primarily affecting children that lack effective treatment. Our lab has previously modeled histone mutant DIPG using a human ES cell platform. A drug screen identified a menin inhibitor (MI) as a potentially effective therapy. This was a novel finding as menin was not known to play a role in tumorigenesis or tumor maintenance in any gliomas, including DIPG. In addition, we found that menin is highly expressed in undifferentiated human ES cells and primitive neuroectoderm, but is rapidly downregulated as the cells progress through fate commitment.

We have also tested menin inhibitors on non DIPG glioblastomas and have found significant drug sensitivity in a range of low-passage patient-derived glioblastoma cell lines. In addition, high level expression of MEN1 (the gene encoding for menin) correlated with worse survival of glioma patients according to patient genome sequencing data from TCGA. (cBioportal, TCGA). These finding suggested an important oncogenic role for menin in gliomas.

Menin inhibitors are effective in MLL fusion leukemias whereby they inhibit the menin-MLL interaction resulting in cell death. We therefore performed quantitative proteomics,

using SILAC, on glioma lines treated with menin inhibitor or vehicle. Menin pull down uncovered a number of new menin interactors that may play a more relevant role than MLL in glioma. Our data suggest a key oncogenic role in gliomas for mechanisms involved in development and cell specification.

[Supported by RO1 CA208405]

(52) GRAPHENE LOADED POLY(4-VINYLPYRIDINE) FIBROUS SCAFFOLDS SUPPORTS DENTAL PULP STEM CELL PROLIFERATION AND DIFFERENTIATION

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Dental pulp stem cells (DPSCs) respond to multiple environmental cues. Their responses to topographical and mechanical cues and factors derived from other cell types has been well documented. In order to assess their responses in a 3-D environment we fabricated a 3-D nano-sized fibrous scaffold of poly(4-vinylpridine) (P4VP) with graphene nanoplatelets (GNPs) to mimic tissue's natural extracellular matrix and investigated DPSC behavior. GNP dispersion was verified by electron microscopy. DPSCs were cultured on these scaffolds for 28 days in medium devoid of the differentiation factor, dexamethasone. At day 7 post-plating, cell morphology and actin filaments were visualized by laser scanning confocal microscopy in cultures incubated with Alexa fluor® 488 Phalloidin. Cells were attached to the fibers with actin filaments oriented with the fibers. After 28 days of incubation, SEM was used to characterize the surface of the scaffolds and a rich deposition of mineralized matrix was revealed. By energy-dispersive X-ray spectroscopy (EDX) the elemental composition of the matrix was identified to be Ca, P, and O. The expression of the differentiation marker osteocalcin was also observed by confocal microscopy. Thus, utilization of 3D scaffolds should be considered for production of differentiated cells.

(53) UNBIASED RECONSTRUCTION OF EARLY FATE DECISIONS IN HUMAN HEMATOPOIESIS

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Hematopoietic stem cells (HSC) give rise to diverse cell types in the blood system, yet our molecular understanding of the earliest fate decisions taken towards lineage

commitment remains incomplete. Here we leverage Drop-seq, a massively parallel single cell sequencing approach, to individually profile more than 20,000 progenitor cells from human cord blood, without prior enrichment or depletion for individual lineages based on surface markers. Our data reveal a transcriptional 'atlas' of progenitor states in human cord blood, including a newly described progenitor of eosinophils, basophils, and mast cells. We leverage this dataset to reconstruct differentiation trajectories from HSC to four downstream lineages, identifying three transcriptomic 'branch points' that provide detailed molecular characterization of the earliest HSC fate decisions. In particular, we discover that distinct subsets of human myeloid cells are generated by two independent paths, falling on opposing sides of the initial HSC fate bifurcation. Intersecting our data with ATAC-seq measurements, we find that intermediate states exhibit transcriptomic and epigenetic multi-lineage priming, while cross-antagonistic mechanisms help to promote lineage commitment. Finally, we demonstrate that Drop-seq data can be utilized to identify new heterogeneous surface markers of cell state. Our findings shed significant new light on early fate decisions in human hematopoiesis, and highlight the exciting potential for unbiased single cell approaches to reconstruct complex developmental processes.

(54) COMPACT GENE REGULATORY CASSETTES SUPPORT KEY ASPECTS OF T-CELL RECEPTOR (TCR)-α GENE LOCUS CONTROL REGION (LCR) ACTIVITY

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Despite the promise of emerging stem cell transplant gene therapy strategies, there remains a gap in the ability to predictably generate robust, temporally controlled long-lasting, therapeutic gene expression in specific target cell lineages following stem cell differentiation. There exists a locus control region (LCR) in the mouse T-cell receptor (TCR)-α locus that confers an αβ TCR-like spatiotemporal expression pattern upon a linked transgene, regardless of its site of integration in the genome. These properties are well suited to direction of high-level, physiological expression of therapeutic antigen receptor genes to the T cell progeny of vector-transduced stem cells. The endogenous LCR spans over 13-kb of DNA. To accommodate LCR activity within the space constraints of gene delivery vectors, we aim to reduce the size of the LCR, while retaining its characteristics. Mini-LCRs have been constructed with varying combinations of previously characterized sub-elements of the TCRα LCR, ranging in size from 1.3- to 4.0-kb. Here, we present results of two mini-LCRs (TαLCR4.0 and TαLCR1.3) tested in an *in vitro* mouse embryonic stem (ES) cell differentiation system. In T cells derived from ES cells, reporter gene expression driven by TαLCR4.0 approximates that observed in the presence of the full length LCR, in that TCRα LCR-linked reporter gene expression levels are robust, mostly cell type-restricted and are upregulated during the expected stage transition of T cell development. Reporter gene expression driven by a smaller mini LCR, TαLCR1.3, while robust in all clones, displays early activation kinetics suitable for directing therapeutic gene expression to correct inherited immunodeficiency. Incorporating TCRα-derived mini LCRs into gene delivery vectors may provide the controlled and predictable therapeutic gene expression required to overcome some of the current hurdles to the use of genetically engineered stem cells as vehicles for delivering T cell gene therapy against a variety of diseases.

(55) EPENDYMAL CELL DYSTROGLYCAN REGULATES NEURAL STEM CELL AND PROGENITOR FUNCTION IN SPATIALLY DISTINCT DOMAINS OF THE SUBVENTRICULAR ZONE

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The subventricular zone (SVZ) neural stem cell niche is important to brain development and homeostasis but many of the mechanisms that regulate SVZ development remain unclear. In particular, the role of extracellular matrix (ECM)-mediated signals in shaping the development and function of the postnatal SVZ remain poorly understood despite the recognized role for ECM in adult stem cell niches. Loss of function of the ECM receptor dystroglycan is responsible for a class of muscular dystrophies termed "dystroglycanopathies", which also have profound deficits in brain structure and function in which the cell and molecular etiology remains unclear. However, we recently reported that dystroglycan is a key regulator of neural stem cell function in the perinatal SVZ, where it suppresses notch signaling in neural stem cells, niche supporting ependymal cells, and oligodendrocyte progenitor cells. Additionally, SVZ dystroglycan reorganizes ECM into laminin-rich "hubs" at the interface between neural stem cells and ependymal cells at the ventricular surface during niche construction. It remained unclear, however, whether niche stem- and ependymal cell dystroglycan had distinct roles in regulating SVZ form and function. We now report that ependymal cell dystroglycan has a role in regulating ventricular zone ECM architecture, and that the loss of dystroglycan in ependymal cells engenders non-cell autonomous alterations in SVZ proliferation in spatially distinct domains, alterations in neural stem cell morphology, and alterations in ependymal cell development. We also find that dystroglycan may play distinct and, at times, opposing roles in neural stem cells and ependymal cells in order to promote orderly development of the SVZ and maintain SVZ neural stem cell homeostasis in the postnatal period. Our findings shed new light on mechanisms of ECM remodeling in the SVZ and roles that ependymal cell-ECM interactions play in regulating stem cell function, as well as provide insight into potential neural stem cell dysfunction in dystroglycanopathies.

(F1) THE RENSSELAER CENTER FOR STEM CELL RESEARCH: BIOENGINEERING AND TISSUE SCIENCE

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The Rensselaer Center for Stem Cell Research (RCSCR) encompasses faculty from RPI's departments of Biomedical Engineering, Chemical and Biological Engineering, Chemistry and Chemical Biology, and Physics, as well as a state-of-the-art core facility. Interdisciplinary research teams pursue a variety of approaches to advance cell and tissue science, including design of bio-compatible and inductive materials, tissue fabrication, microscale technologies and novel screening platforms. The Stem Cell Research Core provides comprehensive assistance for fundamental stem cell research with tissue engineering and regenerative medicine applications. The multi-laboratory facility emphasizes support for analysis of three-dimensional materials, high content screening and hypoxia studies, as well as real-time imaging systems. Beginning in July 2017, the RCSCR will host a NYSTEM-funded immersive summer experience for pre-college teachers, including theoretical and practical aspects of stem cell research together with curriculum development.

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(F2) THE STEM CELL PATHOLOGY UNIT AT CORNELL UNIVERSITY

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Stem cells and their niches are crucial for normal life functions, and are frequently involved in various diseases, such as cancer. To support the rapidly growing fields of stem cell biology and regeneration medicine, the Stem Cell Pathology Unit offers help with project planning, animal phenotyping, and digital slide scanning and analysis. Both internal and external researchers can use these services. The unit is a part of Cornell's Stem Cell Modeling and Phenotyping Core, which assures a critical integration with other units, the Core Stem Cell Optical Imaging Unit and the Stem Cell and Transgenics Core Facility. Project planning assistance includes guidance about design or selection of animal models and strains, tissue collection and fixation, and consultations with other Core Units. Animal phenotyping services are available for a variety of animal species and performed by experienced board-certified veterinary anatomic pathologists. The Stem Cell Pathology Unit provides a wide spectrum of service from slide consultation to complete necropsy and histologic examination of experimental and control animals. Histological evaluation of stem cell niches, as a part of routine phenotyping, offers an extra value for all investigators, independent of their immediate involvement in stem cell research. Digital slide preparation for both bright field and fluorescent samples is performed with Leica/Aperio ScanScopes. Glass slides are scanned at giga-pixel resolutions, and the resulting digital slides allow for network sharing and highly efficient evaluation of histological and cytological materials without the use of a microscope.

Additional viewing software, image analysis algorithms and one month of free of charge image storage are offered for all users. The Stem Cell Pathology Unit continues to evolve to meet the needs of the stem cell research community both inside and outside the Cornell University.

[Supported by NYSTEM contract C024174]

(F3) SHARED RESOURCE: LARGE-SCALE BIOCHEMICAL PROFILING FOR STEM CELL RESEARCH IN NEW YORK

Shahar Goeta, Hemanth Akkiraju, Clark T. Hung, Lewis M. Brown, Columbia University, New York, NY

The ability to fully understand metabolic and protein modification changes that occur as a result of manipulations such as the induction of pluripotency or differentiation are critical goals of stem cell science. The large-scale study of the global sets of small molecules in cells is called metabolomics. Metabolomics is expected to have a transformative impact on the understanding of stem cell fate and physiology as well as patient care in regenerative medicine. Concentrations of metabolites cannot be deduced from genomic information. Dedicated, high-sensitivity, non-targeted screening of metabolites is enabled by technology utilizing mass spectrometry. Posttranslational modifications of proteins are also important drivers of development and physiology of stem cells. Analogous to the situation in studying the metabolome, the identities and concentrations of these modified proteins cannot be deduced from genomic information. To address both problems, we have established a NYSTEM-supported shared resource with state-of-the-art equipment such as the Thermo Scientific Orbitrap Q Exactive HF mass spectrometer and an Agilent gas chromatograph/mass spectrometer. The Q Exactive HF platform has proven itself to provide excellent results and has resulted in successful studies, some of which have resulted in high impact publications (e. g., Welsch et al., 2017, Cell 168, 878–889). The Agilent system has also proven to be a reliable addition to the shared laboratory. This facility should have an important impact on stem cell research, and will serve as an important addition to the statewide stem cell infrastructure.

[Supported by NYSTEM contract C029159]

(F4) A CLINICAL-SCALE, cGMP-COMPLIANT PRODUCTION PROCESS FOR TISSUE-DERIVED RETINAL PIGMENT EPITHELIAL CELLS: MANUFACTURE AND CHARACTERIZATION OF MASTER CELL BANKS AND FINAL CELL PRODUCT FOR PRE-CLINICAL EFFICACY STUDIES AND GLP SAFETY-TOXICOLOGY STUDIES

Karen Harrington[1], Megan Granger[1], Sharyl Zaccaglino[2], Carol Charniga[3], Richard Davis[3], Sally Temple[3], Jeffrey Stern[3], Timothy Blenkinsop[4], Nazia Alam[5], Glen Prusky[5], Claudia Müller[6], Silvia C. Finnemann[6], Michael Fiske[1]
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Age-related macular degeneration (AMD) is a degenerative disease of the retina, of which there is no treatment for the vast majority of patients. Stem cell research offers the opportunity to generate replacement cells for AMD patients. Efforts at The Neural Stem Cell Institute have led the discovery of a new stem cell in the adult human retina which can be isolated from cadaver-donated eyes, activated *in vitro* to divide and produce new retinal pigment epithelial cells (RPE), offering a novel cell source for replacement. The RPESC differs significantly from embryonic or induced pluripotent derived sources of RPE, currently being pursued in clinical studies, in being less proliferative and having a much narrower range of developmental potency.

This presentation describes the manufacture and product release testing and characterization of RPE Master Cell Banks (MCB) and Final Cell Product (FCP) using the Upstate Stem Cell cGMP Facility (USCGF) developed GMP-compliant, clinical-scale cell culture process.

To demonstrate the consistency of the manufacturing process and to provide data for setting final product specifications, a total of 3 Comparability MCBs and 6 Comparability FCP Batches were manufactured and fully characterized. MCB Batches are characterized for cell number, viability, purity, identity, potency, sterility, endotoxin, mycoplasma, adventitious viruses, karyotype and stability. FCP Batches are characterized for cell number, viability, purity, potency, sterility, endotoxin and mycoplasma.

From the Comparability MCB Batches an additional 35 FCP Batches were manufactured and fully characterized for evaluation in pre-clinical efficacy studies and 15 FCP Batches for GLP (Good Laboratory Practice) safety-toxicology studies.

The USCGF is a multi-use, contract cGMP facility with capabilities for: development of clinical-scale manufacturing processes; the production of materials for preclinical efficacy and safety-toxicology studies; development and validation of analytical test methods for clinical trial materials; and cGMP manufacturing of clinical-grade materials for early phase human studies.

[Supported by NYSTEM contracts C026713 and C028504]

(F5) OPTIMIZATION OF WHOLE-BRAIN IMMUNOLABELING TECHNIQUES FOR INDELIBLY-LABELED MEMORY TRACES

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Recent genetic tools have allowed researchers to visualize and manipulate memory traces in small brain regions. However, the ultimate goal is to visualize whole-brain memory traces, in order to better understand how memories are stored in neural networks and how multiple memories may coexist. Intact whole-tissue clearing and imaging is a new and rapidly growing area of focus that could accomplish this. Here,

we utilized the leading protocols for whole-brain clearing of ArcCreERT2 brain tissue. CLARITY and PACT greatly distorted the tissue, whereas iDISCO quenched enhanced yellow fluorescent protein (EYFP) fluorescence and hindered immunolabeling. Alternative clearing solutions, such as tert-Butanol, circumvented these harmful effects, but still did not permit whole-brain immunolabeling. Development of CUBIC with Reagent-1A* resulted in EYFP preservation and immunolabeling of the immediate early gene (IEG) Arc. In summary, CUBIC with Reagent-1A* is the ideal method for reproducible, whole-brain clearing and immunolabeling for the visualization of memory traces.

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(F6) THERMOMECHANICAL & IMAGING NANOSCALE CHARACTERIZATION (ThINC)

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Thermomechanical and Imaging Nanoscale Characterization (ThINC) laboratory is a new comprehensive core facility, dedicated to advancing interdisciplinary research at the interface of materials and the life sciences. The facility combines cutting-edge instrumentation, with full time scientists, who are available to teach and guide users in their use and finding the best approach to understanding their needs in nanotechnology. The facility works closely with multiple New York State programs aimed at economic development and fostering job creation in in the biotech and environmental areas by sponsoring closer university-industrial relations, providing assistance to small companies and start ups, and establishing a gateway for industrial access to national laboratory resources.

ThINC has unique instrumentation specifically designed for research on the impact of the chemical, mechanical, electronic, and morphological, properties of scaffolds on tissue engineering, stem cell development, drug delivery, and nanotoxicology. The location of the facility within the Advanced Energy Center (AEC) emphasizes the need for close collaboration between scientists involved in the development of new energy sources, nanotechnologies, and materials with those concerned with environmental impact, sustainability, and biomedical applications.

Some of the instrumentation specifically targeted at the life sciences are: (a) an UPRIGHT CONFOCAL MICROSCOPE Leica TCS SP8 X, specially designed for three dimensional imaging of cells plated on opaque structured scaffolds or embedded in hydrogels. While light pulsed laser optics and a heated gas perfused stage allows for in operando spectroscopic analysis and single molecular imaging of living cell cultures (b) SCANNING PROBE MICROSCOPE BRUKER DIMENSION ICON, with wet cell attachments for simultaneous imaging of topographical , mechanical, and electronic properties of scaffolds and live cell cultures; (c) LEICA EM UC7/ FC7 ultra cryo-microtome facility for the preparation of soft samples for transmission electron microscopy (d) JEOL JEM 1400 Transmission electron microscope, with low voltage imaging , electron diffraction and elemental analysis capabilities, and special cryo and liquid sample holders, specifically suited for life sciences applications (e) and our newest instrument; ZEISS CROSSBEAM 340 scanning electron microscope with FIB

and Cryo attachements specially designed for 3-D volume imaging of biological cryo-samples.

For further information and a complete list describing our thermomechanical processing and characterization instrumentation contact the instrument scientist and project director, Dr. Chung-Chue Chang (Simon) chung-chueh.chang@stonybrook.edu, phone 631-216-7412, or website http://www.stonybrook.edu/commcms/thinc.

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PROGRAM AT A GLANCE

THURSDAY, MAY 11, 2017

8:00 – 9:00	REGISTRATION & POSTER I SETUP
9:00 – 9:15	OPENING REMARKS
9:15 – 10:30	PLENARY I
10:30 – 11:00	BREAK
11:00 – 12:30	PANEL DISCUSSION: REPRODUCIBILITY IN SCIENTIFIC RESEARCH
12:30 – 1:30	LUNCH & POSTER VIEWING
1:30 – 3:30	PLENARY II
3:30 – 4:00	BREAK
4:00 – 5:30	PLENARY III
5:30 – 7:00	POSTER SESSION I & RECEPTION

FRIDAY, MAY 12, 2017

8:30 – 9:00	CONTINENTAL BREAKFAST & POSTER II SETUP
9:00 – 11:10	PLENARY IV
11:10 – 12:30	POSTER SESSION II & LUNCH
12:30 – 1:30	PLENARY V
1:30 – 2:30	KEYNOTE: AUSTIN SMITH
2:30 – 2:45	POSTER AWARDS & CLOSING REMARKS
2:45	ADJOURN