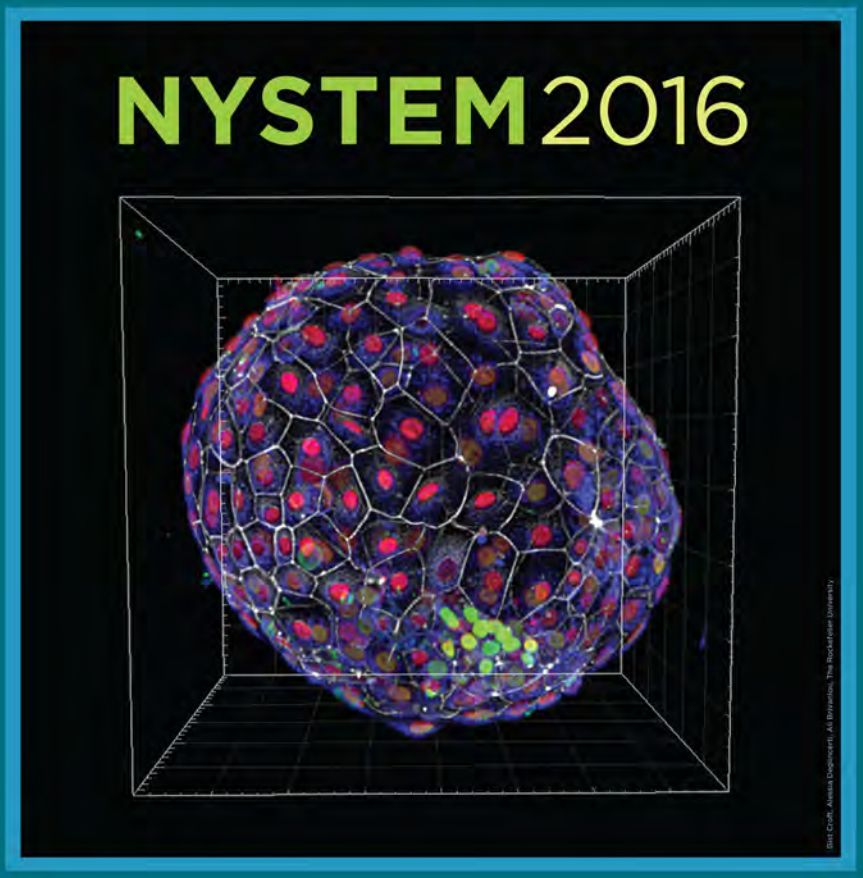
NYSTEM2016



MAY 10-11

THE ROCKEFELLER UNIVERSITY
1230 York Avenue, New York, New York

NEW YORK STATE | Department of Health | Wadsworth Center | NYSTEM

NYSTEM 2016

Carson Family Auditorium
The Rockefeller University

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Cover photo "Human blastocyst, day 6," courtesy of
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TABLE OF CONTENTS

GENERAL

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

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

Posters
Odd numbered posters should be displayed throughout the day Tuesday, presented during the evening reception, and taken down immediately following the reception. Even numbered posters should be displayed throughout the day Wednesday and presented at the Wednesday Poster Session, 11-12 PM. Posters must be removed at the adjournment of the meeting.

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

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

PROGRAM SCHEDULE

TUESDAY, MAY 10, 2016

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

9:00 – 9:15 **OPENING REMARKS**

9:15 – 10:15 **PLENARY I: CONTROLLING CELL FATE**
Chair: Viviane Tabar, *Memorial Sloan Kettering*

9:15 – 9:30 John O'Neill, *Columbia University*
Region-Specific Extracellular Matrix for Advanced Cell Culture and Modeling of Disease

9:30 – 9:45 Raphael Lis, *Weill Cornell Medicine*
Stepwise Direct Conversion of Adult Endothelial Cells into Immune-Competent *Bona Fide* Hematopoeitic Stem Cells

9:45 – 10:00 Hani Shayya, *University at Buffalo*
Acceleration of Oligodendrocyte Progenitor Cell Differentiation by Genetic Ablation and Pharmacological Inhibition of Extracellular 6-O Endosulfatases SULF1 and SULF2

10:00 – 10:15 Jian Feng, *University at Buffalo*
Direct Conversion of Human Fibroblasts to Induced Serotonergic Neurons

10:15 – 10:45 **BREAK**

10:45 – 12:15 **PLENARY II: BIOENGINEERING IN STEM CELL BIOLOGY**
Chair: Alex Nikitin, *Cornell University*

10:45 – 11:15 Lawrence Bonassar, *Cornell University*
Image-Based Generation of Tissue Engineered Cartilage Using Mesenchymal Stem Cells

11:15 – 11:30 Robert Schwartz, *Weill Cornell Medicine*
Engraftment and Function of Human Pluripotent Stem Cell-Derived Hepatocyte-Like Cells in Mice Via 3D Co-Aggregation and Encapsulation

11:30 – 11:45 Wei Liu, *Albert Einstein College of Medicine*
Generation of Three-Dimensional Retinal Tissues from Human Embryonic Stem Cells

11:45 – 12:15 Gordana Vunjak-Novakovic, *Columbia University*
Engineering Human Tissues for Regenerative Medicine and Study of Disease

12:15 – 1:15 **LUNCH** and **POSTER VIEWING** in the atrium

1:15 – 2:00 **PLENARY III: STEM CELL BIOLOGY**
Chair: Elaine Fuchs, *the Rockefeller University*

1:15 – 1:30 Kang Liu, *Columbia University*
Remapping Human Dendritic Cell Lineage Using Clonal Data Reveals Commitment upon Initial Differentiation

1:30 – 1:45 Marc Amoyel, *New York University School of Medicine*
Stem Cell Differentiation is an Active Selection Process Determined by PI3K/TOR Levels

1:45 – 2:00 Gist Croft, *the Rockefeller University*
Self-Organization of the *In Vitro* Attached Human Embryo

2:00 – 3:30 **IMMUNOTHERAPIES**
Chair: Sean Morrison, *Howard Hughes Medical Institute University of Texas Southwestern Medical Center*

2:00 – 2:30 Isabelle Riviére, *Memorial Sloan Kettering Cancer Center*
CAR T Cell Therapy: the CD19 Paradigm and Beyond

2:30 – 3:00 Michel Sadelain, *Memorial Sloan Kettering Cancer Center*
Alternative Cell Sources for Cancer Immunotherapy

3:00 – 3:30 Thinle Chodon, *Roswell Park Cancer Institute*
Combined Adoptive Transfer of Engineered Hematopoietic Stem Cell and T Cell Immunotherapy for Cancer

3:30 – 4:00 **BREAK**

4:00 – 5:30 **PLENARY IV: MODELING DISEASES**
Chair: Shahin Rafii*, Weill Cornell Medical College*

4:00 – 4:30 Eirini Papapetrou, *Icahn School of Medicine at Mount Sinai*
Disease Stage-Specific Human Induced Pluripotent Stem Cells (iPSCs) Delineate a Phenotypic Roadmap to Myeloid Transformation

4:30 – 4:45 Andrea Flesken-Nikitin, *Cornell University*
Defining Cancer-Prone Stem Cell Niche in the Human Tubal Epithelium

4:45 – 5:00 Zhong-Dong Shi, *Memorial Sloan Kettering Cancer Center*
Pluripotent Stem Cell Disease Modeling of Gata6 Haploinsufficiency in Human Pancreatic Development

5:00 – 5:15 Zongdong Li, *Stony Brook University*
Acquired Loss of the Glutamate Transporter SLC1A6 in BLVRB-Deficient Pluripotent Stem Cells Identifies a Novel Mechanism for Altering Cellular Glutamate Metabolism

5:15 – 5:30 Evelyne Tassone, *New York University School of Medicine*
KLF4 Regulates Human Prostate Stem Cell Differentiation and Malignant Transformation

5:30 – 7:00 **POSTER SESSION I** and **RECEPTION**

WEDNESDAY, MAY 11, 2016

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

9:15 – 11:00 **PLENARY V: MESODERMAL LINEAGES**
Nicole Dubois, *Icahn School of Medicine at Mount Sinai*

9:15 – 9:30 Ming Yang, *New York University School of Medicine*
Regulation of mRNA Binding Protein AUF1 Expression by CTCF Controls Muscle Stem Cell Differentiation

9:30 – 9:45 Claudio Peña, *University of San Martin de Porres, Lima, Peru*
In Vitro Osteogenic Differentiation of Human Periodontal Ligament Stem Cells (PDLSCs)

9:45 – 10:00 Kacey Ronaldson, *Columbia University*
Bio-Engineered Adult-Like Heart Tissue from Human iPS Derived Cardiomyocytes Enables Predictive Modeling of Toxicity and Disease

10:00 – 10:15 Janet Paluh, *SUNY Polytechnic Institute*
Developing Cardiomyocyte Contractility Bioinformatic Insights from Ethnically and Epigenetically Diverse iPSC Lines

10:15 – 10:30 Evan Bardot, *Icahn School of Medicine at Mount Sinai*
Foxa2 Marks a Ventricular-Specific Progenitor Population During Gastrulation

10:30 – 11:00 Robert Krauss, *Icahn School of Medicine at Mount Sinai*
Niche Regulation of Muscle Stem Cell Quiescence by Classical Cadherins

11:00 – 12:30 **LUNCH** and **POSTER SESSION II** in the atrium

12:30 – 1:30 **KEYNOTE ADDRESS – SEAN MORRISON**
Niches for Hematopoietic and Osteogenic Stem Cells

1:30 – 1:45 **CLOSING REMARKS and POSTER AWARDS**

1:45 **ADJOURN**

Keynote Address by Sean Morrison, Ph.D.
Director, Children's Research Institute
Mary McDermott Cook Chair in Pediatric Genetics
Investigator, Howard Hughes Medical Institute
University of Texas Southwestern Medical Center

Niches for Hematopoietic and Osteogenic Stem Cells

The Morrison laboratory studies the cellular and molecular mechanisms that regulate stem cell function in the nervous and hematopoietic systems and the role these mechanisms play in cancer. Dr. Morrison obtained his B.Sc. in biology and chemistry from Dalhousie University (1991), then completed a Ph.D. in immunology at Stanford University (1996), and a postdoctoral fellowship in neurobiology at Caltech (1999). Dr. Morrison is currently at the University of Texas Southwestern Medical Center where he is the founding Director of Children's Research Institute, which performs research at the interface of stem cell biology, cancer, and metabolism. Dr. Morrison's laboratory studies the mechanisms that regulate stem cell self-renewal and stem cell aging, as well as the role these mechanisms play in cancer. Dr. Morrison was a Searle Scholar (2000-2003), was named to Technology Review Magazine's list of 100 young innovators (2002), received the Presidential Early Career Award for Scientists and Engineers (2003), the International Society for Hematology and Stem Cell's McCulloch and Till Award (2007) the American Association of Anatomists Harland Mossman Award (2008), and a MERIT Award from the National Institute on Aging. Dr. Morrison has also been active in public policy issues surrounding stem cell research and is currently the President of the International Society for Stem Cell Research. He has twice testified before Congress and was a leader in the successful "Proposal 2" campaign to protect and regulate stem cell research in Michigan's state constitution.

REGION-SPECIFIC EXTRACELLULAR MATRIX FOR ADVANCED CELL CULTURE AND MODELING OF DISEASE

John D. O'Neill, M.S., Jennifer C. Xiong, Gordana Vunjak-Novakovic, Ph.D.

Department of Biomedical Engineering, Columbia University, New York, NY

The extracellular matrix (ECM) is a critical regulator of cell function *in vivo* and *in vitro*. The mechanical, biochemical, and ultrastructural properties of the ECM vary with the organ/tissue type (e.g., bone, heart, or liver), the region within the organ (e.g., kidney cortex or medulla), and the state of disease (e.g., healthy or fibrotic lung). Previously, we showed that ECM derived from native heart, lung, kidney, and bone provides highly instructive scaffolding for cell culture.

Here we describe optimized methods for producing ECM from 25 different organs/tissues – including bone, cartilage, heart, kidney, large intestine, liver, lung, spleen, and stomach – in four formats: acellular scaffolds, sponges, hydrogels, and soluble medium supplements. We also developed methods to produce region-specific ECM from kidney cortex, medulla, and papilla; lung airway cartilage, airway mucosa, and parenchyma; and stomach cardia, corpus, and fundus. When compared with conventional cell culture substrates (i.e., collagen I or Matrigel), tissue-specific ECM substrates consistently show advantages in cell culture applications, such as enhanced cell proliferation, higher expression of tissue-specific markers, more efficient cell differentiation, and better cell attachment and viability. We also show that disease-specific ECM (e.g., from fibrotic lung, neoplastic breast, failing heart) retains hallmark features of the diseased tissue. Such disease-specific ECM could be used to develop more realistic models of disease and drug screening platforms. Overall, we show multiple examples of how the native tissue-specific ECM can be used to markedly advance cell culture and tissue engineering in studies of development, regeneration, and disease.

STEPWISE DIRECT CONVERSION OF ADULT ENDOTHELIAL CELLS INTO IMMUNE-COMPETENT *BONA FIDE* HEMATOPOEITIC STEM CELLS.

Raphael Lis[1,2], Charles C. Karrasch[1,2], Michael G. Poulos[1], Jose G. Barcia Duran[1], Will Schachterle[1], Michael Ginsberg[3], Arash Rafii Tabrizi[4], Koji Shido[1], Nancy Speck[5], Jason M. Butler[1], Joseph Scandura[1], and Shahin Rafii[1].

[1]Ansary Stem Cell Institute, Department of Medicine, Weill Cornell Medicine, New York, NY 10065, USA. [2]Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine and Infertility, New York, NY 10065, USA. [3]Angiocrine Bioscience, New York, NY 10065, USA. [4]Stem Cell and Microenvironment Laboratory, Department of Genetic Medicine and Obstetrics and Gynecology, Weill Cornell Medical College in Qatar, Education City, Qatar Foundation, P.O. Box 24144, Doha, Qatar. [5]Abramson Family Cancer Research Institute, Institute for Regenerative Medicine and Department of Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA 19104, USA

Molecular pathways and micro-environmental cues that choreograph the conversion of endothelial cells (ECs) into hematopoietic stem cells (HSCs) remain undefined. This is due to lack of models to recreate the ephemeral transition from endothelial to hemogenic cells and to HSCs. Here, we developed a sequential *in vitro* model whereby conditional on-off expression of transcription factors: *FosB*, *Gfi1*, *Runx1*, *Spi1 (FGRS)*; and re-establishing proper inductive niche, high-efficiency reprogramming of adult mouse ECs into HSCs (rEC-HSCs) was achieved over a 28-day period. Induction phase (day 0-8): *FGRS* are conditionally expressed in adult non-lymphatic ECs isolated from *Runx1*-IRES-GFP reporter mice, co-cultured with vascular niche. Specification phase (day 8-20): *FGRS*-transduced VEcad^{+}Runx1^{-}CD45^{-} ECs switch on endogenous expression of *Runx1,* initiating hematopoietic program and silencing EC fate in VEcad^{+}Runx1^{+}CD45^{+}-cells, setting the stage for full commitment to VEcad^{-}Runx1^{+}CD45^{+} hematopoietic stem/progenitor cells (rEC-HSPCs). Expansion phase (day 20-28): niche cells promote HSPC large-scale expansion, at which time expression of exogenous *FGRS* is turned off. Transplantation of rEC-HSPCs (*FGRS-off*, CD45.2^{+}) into lethally irradiated recipient mice (CD45.1^{+}) reconstituted both short-term and long-term hematopoiesis, with secondary engraftment potential (rEC-HSCs), giving rise to functional myeloid and lymphoid cells with full compliment of polarized T cell. rEC-HSC-derived immune cells undergo T-cell receptor (TCR) rearrangement and restore adaptive immune function in *Rag1*$^{-/-}$ mice. Employing *Runx1*-IRES-GFP reporter mice enabled deconvolution of stage-specific pathways involved in generation of engraftable rEC-HSCs. TGFβ inhibition along with BMP and CXCL12 activation reinforced induction phase. Active Notch and CXCL12 signaling throughout specification and expansion phases balanced self-renewal of transplantable rEC-HSCs. This stepwise reprogramming approach provides a platform to decipher pathways involved in transition from ECs into hematopoietic cells. This will facilitate devising strategies to reprogram ECs into abundant autologous HSCs amenable to genetic modification for treatment of genetic and acquired hematological disorders.

ACCELERATION OF OLIGODENDROCYTE PROGENITOR CELL DIFFERENTIATION BY GENETIC ABLATION AND PHARMACOLOGICAL INHIBITION OF EXTRACELLULAR 6-O ENDOSULFATASES SULF1 AND SULF2

Hani J. Shayya[1], R. Ross Welliver[1], Suyog U. Pol[1], and Fraser J. Sim[1,2]

[1]Department of Pharmacology and Toxicology, [2]Neuroscience Program, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, New York 14214

Oligodendrocyte progenitor cells (OPCs) are a stem cell population that comprises roughly 3% of cells in the adult forebrain. When pathological insult to the central nervous system (CNS) results in the loss of myelin from neuronal axons, OPCs can infiltrate the demyelinated region, differentiate to myelin-producing oligodendrocytes (OLs), and restore myelin to axons in a regenerative process called remyelination. Though remyelination is typically efficient, it often fails in multiple sclerosis (MS) due to factors in the lesion microenvironment which inhibit OPC differentiation. Therefore, the identification of pharmacological compounds which promote OPC differentiation, particularly those which antagonize inhibitory signals in the lesion environment, is critically important for the clinical treatment of MS. Analysis of RNA-seq data from our laboratory and others reveals that SULF1 and SULF2, two genes encoding extracellular endosulfatases that remove 6-O sulfate groups from cell surface heparan sulfate proteoglycan (HSPG) side chains, are uniquely expressed in the oligodendrocyte lineage relative to other CNS populations and are highly down-regulated upon OPC differentiation to mature OLs. Using a lysolecithin-induced model of focal demyelination in the mouse spinal cord, we demonstrate that genetic ablation of the SULF genes, as well as pharmacological antagonism of the enzymes they encode, accelerates endogenous OPC differentiation *in vivo*. Mechanistically, we use a phage display antibody sensitive to heparan sulfate (HS) 6-O sulfation to demonstrate that pharmacological inhibition of Sulf enzymatic activity switches OPCs from a basal state of low HS 6-O sulfation to a highly sulfated state, abrogating cellular responsiveness to BMP and WNT signaling. Taken together, our findings define a novel therapeutic target for the acceleration of OPC differentiation, with potential translational applications in the treatment of demyelinating disease. [Supported in part by NYSTEM contract #C028108].

DIRECT CONVERSION OF HUMAN FIBROBLASTS TO INDUCED SEROTONERGIC NEURONS

Zhimin Xu[1,2,3], Houbo Jiang[2,3], Ping Zhong[2,3], Zhen Yan[2,3], Shengdi Chen[1,*], Jian Feng[2,3,*]

[1] Department of Neurology & Institute of Neurology, Ruijin Hospital affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China
[2] Veterans Affairs Western New York Healthcare System, Buffalo, NY 14215, USA.
[3] Department of Physiology and Biophysics, State University of New York at Buffalo, Buffalo, New York 14214, USA.

Serotonergic (5HT) neurons exert diverse and widespread functions in the brain. Dysfunction of the serotonergic system gives rise to a variety of mental illnesses including depression, anxiety, obsessive compulsive disorder, autism and eating disorders. Here we show that human primary fibroblasts were directly converted to induced serotonergic (i5HT) neurons by the expression of Ascl1, Foxa2, Lmx1b and FEV. The transdifferentiation was enhanced by p53 knockdown and appropriate culture conditions including hypoxia. The i5HT neurons expressed markers for mature serotonergic neurons, had Ca^{2+}-dependent 5HT release and selective 5HT uptake, exhibited spontaneous action potentials and spontaneous excitatory postsynaptic currents. Application of serotonin significantly increased the firing rate of spontaneous action potentials, demonstrating the functional utility of i5HT neurons for studying serotonergic neurotransmission. Furthermore, i5HT neurons transplanted in rat brains produced extensive neuronal processes. The availability of human i5HT neurons will be very useful for research and drug discovery on many serotonin-related mental disorders.

[Supported by Department of Veterans Affairs Merit Award I01BX002452, NYSTEM contracts C028129, C029556 and C026714, NIH grant NS061856, National Key Basic Research Program of China grants 2011CB504100 and 2011CB504104]

IMAGE-BASED GENERATION OF TISSUE ENGINEERED CARTILAGE USING MESENCHYMAL STEM CELLS

Lawrence Bonassar

Departments of Biomedical Engineering and Mechanical & Aerospace Engineering, Cornell University

ENGRAFTMENT AND FUNCTION OF HUMAN PLURIPOTENT STEM CELL-DERIVED HEPATOCYTE-LIKE CELLS IN MICE VIA 3D CO-AGGREGATION AND ENCAPSULATION

Wei Song[2], Yen-Chun Lu[2], Angela Frankel[1], Duo An[2], Minglin Ma[2], Robert E. Schwartz[1]

[1] Weill Cornell Medical College, New York, NY, United States. 2. Department of Biological and Environmental Engineering, Cornell University, Ithaca, NY, United States.

Cellular therapies for liver diseases and in vitro models for drug testing both require functional human hepatocytes, which have unfortunately been limited due to the paucity of donor liver tissues. Human pluripotent stem cells represent a promising and potentially unlimited cell source to derive human hepatocytes. However, the hepatic functions of these human pluripotent stem cells-derived cells to date are not fully comparable to adult human hepatocytes and are more similar to fetal ones. In addition, it has been challenging to obtain functional hepatic engraftment of these cells with prior studies having been done in immunocompromised animals. In this report, we demonstrated successful engraftment of human induced pluripotent stem cell derived hepatocyte-like cells in immunocompetent mice by pre-engineering 3D cell co-aggregates with stromal cells followed by encapsulation in recently developed biocompatible hydrogel capsules. Notably, upon transplantation, human albumin and α1-antitrypsin (A1AT) in mouse sera secreted by encapsulated induced pluripotent stem cell derived hepatocyte-like cells/stromal cell aggregates reached a level comparable to the primary human hepatocyte/stromal cell control. Further immunohistochemistry of human albumin in retrieved cell aggregates confirmed the survival and function of iPS-H. This proof-of-concept study provides a simple yet robust approach to improve the engraftment of induced pluripotent stem cell derived hepatocyte-like cells, and may be applicable to many stem cell-based therapies.

GENERATION OF THREE-DIMENSIONAL RETINAL TISSUES FROM HUMAN EMBRYONIC STEM CELLS

Albert Lowe, Raven Harris, Punita Bhansali, Ales Cvekl and Wei Liu§

Departments of Genetics and Ophthalmology and Visual Sciences, Albert Einstein College of Medicine, Bronx, NY 10461.

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Stem cell-derived retinal organoids are promising models for studying human retinal development and disease. Here we dissect retinal organoid morphogenesis in hESC-derived cultures and establish a method for isolating large quantities of retinal organoids that resemble embryonic optic cups. In long-term culture, the retinal organoids generated stratified retinal tissues with the major retinal cell types. The retinal cells were identified by immunohistochemistry with these markers: ISLET1/2 and BRN3 for ganglion cells, Tuj1 for ganglion cells and amacrine cells, Syntaxin for amacrine cells, Calbindin 28k for horizontal cells, PKCalpha for bipolar cells, RCVRN for photoreceptor cells, and LHX2 for Müller glial cells. Importantly, the photoreceptor cells highly expressed RHO, L/M-Opsin, S-Opsin, and displayed the ultrastructure of outer segments. Thus, our retinal differentiation system is capable of generating 3D retinal tissues and thus has multiple applications in modeling human retinal development and disease.

ENGINEERING HUMAN TISSUES FOR REGENERATIVE MEDICINE AND STUDY OF DISEASE

Gordana Vunjak-Novakovic

Mikati Foundation Professor of Biomedical Engineering and Medical Sciences
Columbia University

Tissue engineering is becoming increasingly successful with authentically representing the actual environmental milieu of the development, regeneration and disease. A classical paradigm of tissue engineering is related to the integrated use of human stem cells, biomaterial scaffolds (structural and logistic templates for tissue formation) and bioreactors (culture systems providing environmental control, molecular and physical signaling) in regenerative medicine. Today, living human tissues can be bioengineered from the autologous stem cells, and tailored to the patient and the medical condition being treated. A reverse paradigm is now emerging with the development of platforms for modeling of integrated human physiology, using iPS cell derived micro-issues of different types that are functionally connected by microvasculature. The biological fidelity of these “tissues on a chip” and the capability for high-throughput work and real-time measurement of physiological responses are poised to transform preclinical drug screening and modeling of human disease. This talk will discuss the biomimetic approach as the common underlying principle for tissue engineering, and some recent advances in the application of this approach to regenerative medicine, modeling of disease, and drug screening.

Funding: NYSTEM (C028119); NIH (EB17103; EB002520; HL076485)

REMAPPING HUMAN DENDRITIC CELL LINEAGE USING CLONAL DATA REVEALS COMMITMENT UPON INITIAL DIFFERENTIATION

Yu J Zhou[1]*, Jaeyop Lee[1]*, Wenji Ma[2]*, Arafat Aljoufi[1], Prakash Satwani[3], Govind Bhagat[4], Yufeng Shen[2#], Kang Liu[1#]

[1]Columbia University Medical Center, Departments of Microbiology and Immunology, New York, NY, [2]Columbia University Medical Center, Departments of Systems Biology and Biomedical Informatics, New York, NY, [3] Columbia University Medical Center, Departments of Pediatrics, Division of Hemotology-Oncology, New York, NY, [4]Columbia University Medical Center, Departments of Pathology and Cell Biology, Division of Hematopathology, New York, NY,

Despite decades of studies seeking marker-defined progenitors, a coherent model of dendritic cells (DC) hematopoietic development has proven elusive. In addition to the long-standing debate regarding the relationship between DC, myeloid and lymphoid lineages, the question of at what stage the three DC subsets diverge remains contentious. Disagreement about the DC lineages has been mirrored by similar confusion about other lineages. At the heart of all these debates are two common issues: first, repeatedly marker-purified progenitors are found to be heterogeneous; second, same progeny appear to descend from different progenitors. These issues have challenged the tree-like classic hematopoiesis model. However, no one has developed an approach capable of mapping hematopoietic lineages in a coherent developmental scheme. Here, we present a novel approach to solve this problem by combining mathematic modeling and clonal analysis. Using a clonal assay to resolve granulocyte (G), monocyte (M), lymphoid (L) and DC fates from single human CD34+ cells, we demonstrate that quantitative clonal output reliably indicates the developmental potential and direction that marker-defined phenotypes cannot. Using a diffusion model we mapped the fate of 2,247 single progenitor cells and visualized lineages of G, M, L, and three DC subsets. Our findings reveal that multi-potent progenitors in vivo commit to a specific lineage once differentiation begins, and abort other lineage potentials. We prove such commitment upon initial differentiation pattern by mapping bone marrow progenitors from healthy donors and DC-deficient patients. Our model demonstrates that commitment to specific blood lineage is made without going through sequential oligo-potent stages and that the “oligo-potent progenitors” that serve as key nodes of the standard hematopoietic model are in fact in vitro artifacts. We will use this new model as a framework to revisit the transcriptional regulation of DC lineage commitment. [Supported by NYSTEM contract # C029562].

*: Equal contribution
#: Corresponding authors

STEM CELL DIFFERENTIATION IS AN ACTIVE SELECTION PROCESS DETERMINED BY PI3K/TOR LEVELS

Marc Amoyel[1,2], Kenzo-Hugo Hillion[1], Shally R. Margolis[1] and Erika A. Bach[1,2]

[1]Department of Biochemistry and Molecular Pharmacology
[2]the Helen L. and Martin S. Kimmel Center for Stem Cell Biology, NYU School of Medicine, 550 1st Avenue, New York, NY 10016, USA.

Stem cell differentiation is perceived as a default fate that must be repressed by self-renewal signals. Stem cells reside in niches that provide these signals to maintain self-renewal and prevent differentiation. Newly-born cells that are displaced away from the niche differentiate as a result of losing access to self-renewal factors. Here we present results indicating that among cyst stem cells (CySCs) in the *Drosophila* testis, differentiation is actively promoted by signaling in the PI3K/TOR pathway. Even in the absence of self-renewal signals, CySCs cannot differentiate if TOR is not activated. Moreover, we show that differentiation is the result of a selection process: only cells with higher levels of PI3K/TOR activity than their neighbors are selected to differentiate, while the others remain at the niche. Thus leaving the stem cell niche and initiating differentiation is an active fate selection and not a default pathway.
[Supported by NYSTEM contract #C028132]

SELF-ORGANIZATION OF THE IN VITRO ATTACHED HUMAN EMBRYO

Gist F. Croft*[1], Alessia Deglincerti*[1], , Lauren N. Pietila[1], Magdalena Zernicka-Goetz[2], Eric D. Siggia[3], and Ali H. Brivanlou[1#]

[1] Laboratory of Stem Cell Biology and Molecular Embryology, Rockefeller University, New York, NY; [2], Department of Physiology, Development, and Neuroscience, University of Cambridge, Physiology building, Downing Street, Cambridge, UK; [3], Center for Studies in Physics and Biology, Rockefeller University, New York, NY. * equal contribution

Implantation of the blastocyst is a developmental milestone in mammalian embryonic development. At this time, a coordinated program of lineage diversification, cell-fate specification, and morphogenetic movements establishes the generation of extra-embryonic tissues and the embryo proper and determines the conditions for successful pregnancy and gastrulation. Despite its basic and clinical importance, this process remains mysterious in humans. Here we report the use of a novel *in vitro* system to study the post-implantation development of the human embryo. We show, for the first time, the extraordinary self-organizing abilities and autonomy of *in vitro* attached human embryos. We find human-specific molecular signatures of early cell lineages (epiblast, trophectoderm, and primitive endoderm), timing, and architecture. Embryos display key landmarks of normal development, including epiblast expansion, lineage segregation, bi-laminar disc formation, amniotic and yolk sac cavitation, as well as trophoblast diversification. Our findings highlight the species-specificity of these developmental events and provide a new understanding of early human embryonic development beyond the blastocyst stage. In addition, our study establishes a new model system relevant to early human pregnancy loss. Ultimately, this work will assist in the rational design of differentiation protocols of human embryonic stem cells (hESCs) to specific cell types for disease modeling and cell replacement therapy.

IMMUNOTHERAPIES SESSION

Isabelle Riviére
The Michael G. Harris Cell Therapy and Cell Engineering Facility
Memorial Sloan Kettering Cancer Center
CAR T Cell Therapy: the CD19 Paradigm and Beyond

The treatment of cancer patients with autologous T cells expressing a chimeric antigen receptor (CAR) is one of the most promising adoptive cellular therapy approaches. This immunotherapeutic strategy is predicated on gene transfer technology to instruct T lymphocytes to recognize and reject tumor cells. CARs are synthetic receptors that mediate antigen recognition, T cell activation, and costimulation to augment T cell functionality and persistence. We demonstrated over a decade ago that human T cells engineered with a CD19-specific CAR eradicated B cell malignancies in mice, and we were the first to report remarkable complete remission rates obtained with second generation CD19 CARs in adults with chemorefractory, relapsed ALL. Novel T cell engineering modalities hold the promise of further enhancing the effectiveness and safety of CAR therapy against a broad range of cancers including solid tumors. Various aspects of the MSKCC CAR T cell program will be discussed.

Michel Sadelain
Memorial Sloan Kettering Cancer Center
Alternative Cell Sources for Cancer Immunotherapy

Thinle Chodon
Roswell Park Cancer Institute
Combined Adoptive Transfer of Engineered Hematopoietic Stem Cell and T Cell Immunotherapy for Cancer

DISEASE STAGE-SPECIFIC HUMAN INDUCED PLURIPOTENT STEM CELLS (IPSCS) DELINEATE A PHENOTYPIC ROADMAP TO MYELOID TRANSFORMATION

Eirini P Papapetrou

Department of Oncological Sciences, Department of Medicine, Hematology and Medical Oncology, Tisch Cancer Institute, Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, New York.

While proof of principle of using iPSCs in disease modeling is now well established, the potential of iPSCs in cancer research is just beginning to be explored. A major strength of iPSCs over other patient-derived cancer models (organoids, patient-derived xenografts) is the possibility to capture early stages of malignant transformation, including premalignant and cancer predisposition states.

Myeloid malignancy is increasingly viewed as a disease spectrum, comprising hematopoietic disorders that extend across a phenotypic continuum, ranging from clonal hematopoiesis to myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). However the cellular events demarcating progression to overt leukemia through a premalignant myelodysplastic phase are not well defined.

By integrating cancer genetics with nuclear reprogramming, we derived a collection of iPSC lines capturing a range of disease stages, encompassing preleukemia (cells with predisposing mutations), low-risk MDS, high-risk MDS and secondary AML. We characterized the hematopoiesis derived from this panel of iPSC lines, compared to that derived from normal iPSCs, in terms of: size and timing of emergence of progenitor populations, clonogenic capacity, dysplastic morphologic changes, growth and viability. We find phenotypes of graded severity and/or stage specificity which delineate a phenotypic roadmap of disease progression.

As proof of principle that transitions between stages (cancer progression or reversal) can be modeled in our system, we show that a high-risk MDS-iPSC line can be phenotypically “reverted” to a premalignant state by spontaneous correction of a chr7q deletion, whereas a preleukemic iPSC line harboring a GATA2 mutation (associated with familial MDS) can “progress” to low-risk MDS following CRISPR/Cas9-mediated inactivation of the second GATA2 allele. We then use this model to test the effects of 5-AzaC – a drug used as first-line therapy in MDS with variable responses, the mechanisms of which remain elusive. We find that 5-AzaC rescues the emergence of erythroid and multilineage colonies in methylcellulose assays specifically in low-risk MDS, but not in normal, preleukemic, high-risk MDS or MDS//AML iPSCs, while in a cell competition assay it specifically inhibits the growth of hematopoietic progenitors derived from high-risk MDS. These results support a model whereby 5-AzaC may mediate a therapeutic effect by inducing differentiation in early disease stages, whereas in later stages its main action is to inhibit growth of the MDS clone.

In summary, this study provides insights into the pathophysiologic changes underlying the initiation and progression of myeloid transformation and a new platform to test genetic and pharmacologic interventions to reverse this process.

DEFINING CANCER-PRONE STEM CELL NICHE IN THE HUMAN TUBAL EPITHELIUM

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Stem cell (SC) niches of the female reproductive tract remain insufficiently elucidated. This precludes comprehensive understanding of the pathogenesis of the ovarian/extra-uterine high-grade serous carcinoma (HGSC), the most lethal gynecological malignancy. Previously we have identified a cancer-prone SC niche of the mouse ovarian surface epithelium (OSE). The OSE-SCs contribute to the OSE homeostasis in long-term lineage tracing experiments and give rise to carcinomas closely similar to human HGSC (Flesken-Nikitin et al., *Nature* 495: 241-245, 2013). Due to its different anatomy, the human equivalent of mouse cancer-prone SC niche remains unknown. It has been reported that likely precursor of HGSC, serous tubal intraepithelial carcinoma, is commonly found in the distal (fimbriated end) region of the fallopian tube. In search of putative SC niches for the human tubal epithelium (TE) we determined the expression of SC markers, such as KRT5/6 and LEF1, in the distal and proximal regions of the fallopian tube. We also sorted cells from both regions according to their ALDH enzymatic activity determined by ALDEFLUOR (AF), and examined their gene expression profiles. Both regions contained areas expressing SC markers and yielded AF+ cells, which preferentially expressed SC markers. Primary cultures of both distal and proximal TE cells were differentiation competent, as evidenced by growth of both ciliated (AC-α-TUB+, FOXJ1+) and secretory (PAX8+) cells. However, despite these similarities, primary distal cells showed lower cellular senescence and increased proliferative potential, as compared to proximal cells. Furthermore, distal but not proximal cells were immortalized by concurrent CRISPR/Cas9-mediated *TP53* knock out and *MYC* overexpression. In sum, these results support the notion of HGSC origin from the distal region of the fallopian tube, and suggest presence of two TE-SC pools marked by their disparate susceptibility to the malignant transformation.

[Supported by NIH/NICHD (P50HD076210) to AFN, NIH/NCI (CA182413), NYSTEM (C028125, C024174 and T028095), and the Ovarian Cancer Research Fund (327516) to AYN.]

PLURIPOTENT STEM CELL DISEASE MODELING OF GATA6 HAPLOINSUFFICIENCY IN HUMAN PANCREATIC DEVELOPMENT

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Human disease phenotypes associated with haploinsufficient gene requirements are not well recapitulated in animal models. For example, recent studies show that *GATA6* haploinsufficiency is the major cause of pancreatic agenesis in humans, while in mice pancreatic agenesis phenotype only occurs when the sister gene *Gata4* is simultaneously deleted. However, further studies observe a wide spectrum of disease phenotypes in human patients, including pancreatic agenesis and neonatal diabetes, adult-onset diabetes, and in some cases, no apparent pancreatic phenotypes. Coupling CRISPR/Cas-mediated genome editing with hPSC directed differentiation, this study investigates the association of *GATA6* haploinsufficiency with the wide-range of clinical phenotypes mentioned above. We report that one *GATA6* wild-type allele was both necessary and sufficient for the specification of human endoderm and subsequently PDX1^{+} early pancreatic progenitors (PP1). However *GATA6* heterozygous loss of function mutants (including frameshift mutations and a disease-specific mutation) could not efficiently form PDX1^{+}NKX6.1^{+} pancreatic endocrine progenitors (PP2). These findings suggest that *GATA6* heterozygous humans may have a compromised pancreas due to loss of one allele of *GATA6*. Compound mutant analysis by deleting *GATA4* in *GATA6* heterozygous mutants revealed that the formation of PP1 progenitors and the transcriptional expression of *PDX1* were exquisitely sensitive to the *GATA6* and *GATA4* gene dosage. Specific deleting GATA binding sites in the *PDX1* enhancer region also showed a clear dosage effect on *PDX1* transcription. Our findings not only reveal the importance of GATA factors in human endoderm and pancreas development, but also demonstrate the potential of genetic background and/or environmental factors may underlie the pancreatic disease severity associated with *GATA6* haploinsufficiency. This work also support the broader use of hPSCs and CRISPR/Cas technology for understanding multigenic traits such as type 2 diabetes and identifying disease modifying factors.

[Supported by NYSTEM C029567 and C029156 and NIH/NIDDK R01DK096239]

ACQUIRED LOSS OF THE GLUTAMATE TRANSPORTER SLC1A6 IN BLVRB-DEFICIENT PLURIPOTENT STEM CELLS IDENTIFIES A NOVEL MECHANISM FOR ALTERING CELLULAR GLUTAMATE METABOLISM

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We have previously identified a redox-defective mutation in biliverdin IXβ reductase (BLVRB) that causes accumulation of reactive oxygen species (ROS) as a putative metabolic trigger for enhanced hematopoietic stem cell lineage commitment leading to thrombocytosis in humans. BLVRB functions within the heme degradation pathway to catalyze the NADPH-dependent reduction of the biliverdin IXβ tetrapyrrole to bilirubin, thereby generating a potent antioxidant cycle with potential regulatory effects on stem cell quiescence and/or cellular stress. To further characterize metabolic consequences of BLVRB function, we introduced a mutation in exon 3 of the *BLVRB* gene in $CD34^+$-derived iPSC cells (NCRM1) using a lentivirus/CRISPR construct, with complete BLVRB absence as established by immunoblot analysis. RNAseq of two mutant subclones together with two controls demonstrated acquired and singular loss of the glutamate transporter gene (*SLC1A6*) in both *BLVRB*-null cell lines. *SLC1A6* reappearance was evident in genetic complementation experiments using either wild-type or redox-defective *BLVRB*, thereby establishing tight transcriptional regulation of *SLC1A6*-mediated glutamate transport with *BLVRB* expression. *BLVRB*-null iPSCs demonstrated decreased expression of genes essential for genome stability (*HIST1H4F*) and enhanced expression of stress response genes including *SHMT2* and extensive members of the metal-ion binding metallothionein 1 (*MT1*) gene family (*MT1E, MT1F, MT1G, MT1H, MT1X, MT2A*, 2-70-fold increase), collectively establishing a critical role in transcriptional regulation of stress response genes. In summary, our data establish a fundamentally important role for *BLVRB* in a heme-regulated pathway of pluripotent stem cell metabolism, presumably by regulating glutamate uptake and metabolism linked to NADPH production, cellular redox function, and/or macromolecular synthesis.

KLF4 REGULATES HUMAN PROSTATE STEM CELL DIFFERENTIATION AND MALIGNANT TRANSFORMATION

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We showed that prostate stem cells reside in the proximal region of murine prostatic ducts and express high levels of Sca-1 and KLF4. We also found that high KLF4 expression in primary human prostate tumors identifies individuals who do not relapse for up to 10 years, predicting for an excellent prognosis. To determine the effects of KLF4 on human prostate cells, we knocked-down KLF4 in a normal human stem cell line (WPE) and observe a more primitive, basal (CK5) phenotype and an increase in their self-renewal potential. Conversely, KLF4 overexpression with a doxycycline inducible Tet-ON system promotes a more differentiated, luminal (CK8) phenotype and decreases self-renewal and proliferation of WPE cells in a dose-dependent manner. As stem cells have significant proliferative potential and may be targets of transformation, KLF4 may prevent excessive stem cell proliferation. To determine the effects of KLF4 on human prostate cancer cells we overexpressed KLF4 in LNCaP and PC3 cells and obtain similar effects on proliferation to those noted in WPE cells. LNCaP cells, which express very low levels of endogenous KLF4, are extremely sensitive to KLF4 modulation, as demonstrated by almost complete inhibition of proliferation after induction of KLF4 with a low dose of doxycycline (1 ng/ml). Additionally, genomic ablation of KLF4 in PC3 cells increases their colony forming capacity in anchorage-independent conditions. In conclusion, our data show that KLF4 regulates normal human prostate stem cell differentiation and restrains their self-renewal potential thereby preventing uncontrolled proliferation. In addition, it inhibits tumorigenicity of human prostate cancer cells as evidenced by their decreased clonogenic potential. The identification of downstream effectors of KLF4 may provide additional genes that aid in prognosis prediction and that, in addition, could serve as targets for specific therapeutic interventions, thus having a significant impact on the well-being of prostate cancer patients.

This work was supported by NIH CA132641 and Kimmel Stem Cell Center at NYUSOM.

REGULATION OF mRNA BINDING PROTEIN AUF1 EXPRESSION BY CTCF CONTROLS MUSCLE STEM CELL DIFFERENTIATION

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Muscle stem cells, also known as satellite cells, are vital for muscle regeneration following wounding and for preservation of the regenerative potential of muscle with age. Understanding the key molecular mechanisms by which satellite cell fate is regulated and how satellite cells contribute to muscle maintenance with increasing age can advance our knowledge of the nature of myopathic diseases. Post-transcriptional regulation of mRNAs plays a crucial role in the control of the expression of genes that determine stem cell fate. The mRNAs encoded by some of the key genes in muscle stem cell determination are regulated by an AU-rich element (ARE), located in the 3' untranslated region (3'UTR), which controls their targeted rapid degradation.

Our lab found that the ARE binding protein AUF1 (hnRNP-D) plays crucial role in regulating stem cell fate. In our *auf1* knock out mouse model, we observed that *auf1*$^{-/-}$ mice undergo accelerated loss of skeletal muscle mass and develop severely increased muscle weakness with age. Our genome-wide mRNA analysis also showed that *auf1* mRNA is strongly (>70 fold) and specifically increased in expression only with activation of satellite cells, suggesting that the regulation of AUF1 expression is important for satellite cell fate determination. Using a transcription factor-wide siRNA screen, we found that transcription factor CTCF is essential for *auf1* expression. We also showed that CTCF binds to the promoter region of AUF1. Using mouse myoblast C2C12 cells as a model system, we found that CTCF is essential for transcriptional activation of AUF1 in this muscle myoblast system as well, and for myoblast differentiation in culture. Silencing CTCF results in down regulation of AUF1 protein and mRNA levels, and delay of myoblast differentiation. We demonstrate that CTCF regulates myoblast differentiation through AUF1, and restoration of AUF1 levels by ectopic expression in a CTCF silencing background rescues normal myoblast differentiation. Our results suggest that the CTCF/AUF1 axis is important in regulating muscle stem fate and could an important therapeutic target in late-onset myopathies and age-related sarcopenia.

IN VITRO OSTEOGENIC DIFFERENTIATION OF HUMAN PERIODONTAL LIGAMENT STEM CELLS (PDLSCS)

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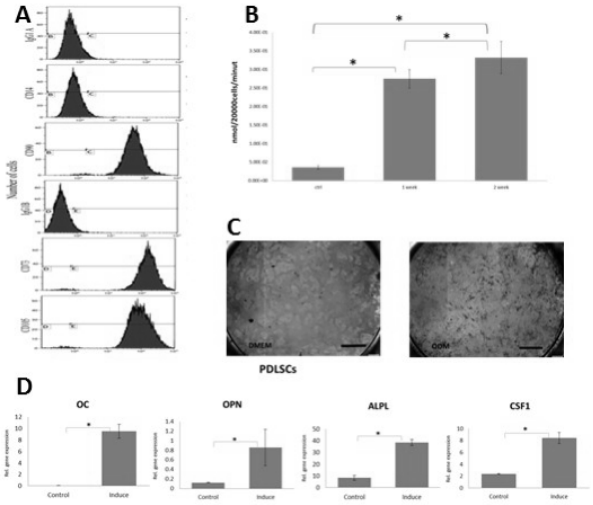
Besides its role in tooth anchoring, the periodontal ligament (PDL) provides progenitor cells for alveolar bone formation and remodeling. The PDL have also been shown to contain a population of stem cells (PDLSCs) which have the potential to differentiate into osteoblasts, chondrocytes and adipocytes. The aim of this study was to isolate cells from adult PDL tissue, to show that the isolated cells express mesenchymal stem cells markers, and finally to assessed their osteogenic potential *in vitro*.



Flow cytometric analysis of PDL cells. Histograms in figure A represent cells stained with fluorescent antibodies. The number of cells staining positive is shown according to the median fluorescence intensity. Analysis revealed that more than 95% of the cells were positive for CD90, CD73 and CD105 and less than 4 % expressed the surface markers CD14. Panel B shows, the effect of osteogenic medium on the osteogenic differentiation of PDLSCs was assessed by measuring the ALP activity after 7 and 14 days. All experiments included 11 replicates. All values represent means, plus minus standard error ($\delta/\sqrt{n}$). For statistical analysis, Student's T-test was applied; *p < 0.05 comparison of two groups. In panel C, cells were treated with ODM for 28 days and stained with alizarin red S staining. Micrographs show extracellular calcium deposition. Original magnification 10x, scale bar =100 µm. Panel D shows effect of osteogenic medium on gene expression of osteogenic markers, by PDLSCs. The total RNA was isolated from PDLSCs after 7days in osteogenic differentiation medium. All values represent means plus standard error ($\delta/\sqrt{n}$). For statistical analysis, Student's t-test was applied; *p < 0.05 comparison of two groups. OC, osteocalcin; OPN, osteopontin; ALPL, alkaline phosphatase; CSF1, colony stimulating factor 1; PDLSCs, periodontal ligament stem cells; Rel., Relative.

This study demonstrated the feasibility of *in vitro* osteogenic differentiation of PDLSCs. Our data suggests that PDLSCs could be used for stem cell-based therapy for bone tissue engineering applications as it is capable of increasing extracellular matrix mineralization. Consequently, there is a potential for regenerating lost bone tissue with PDLSCs in periodontics and oral implantology.

BIO-ENGINEERED ADULT-LIKE HEART TISSUE FROM HUMAN IPS DERIVED CARDIOMYOCYTES ENABLES PREDICTIVE MODELING OF TOXICITY AND DISEASE

Kacey Ronaldson[1], Stephen Ma[1], Timothy Chen[1], Keith Yeager[1], Dario Sirabella[1], Lujin Song[2], Masayuki Yazawa[2] and Gordana Vunjak-Novakovic[1]

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Cardiac tissues formed from human induced pluripotent stem (hiPS) cells have limited utility due to their immature, fetal-like phenotype. We report the development of adult-like human heart muscle from co-cultures of human induced pluripotent stem cell derived cardiomyocytes and supporting fibroblasts in native hydrogel subjected to three weeks of electromechanical conditioning at an increasing intensity. The resulting engineered tissues recapitulated many of the molecular, structural, and functional properties of adult human heart muscle, including well-developed registers of sarcomeres, networks of T-tubules, mature calcium homeostasis, comprehensive responses to beta-adrenergic stimulation, and a positive force-frequency relationship. The level of maturation was confirmed by comparison to human fetal heart tissue, and probed by studying physiological responses to drugs and by modeling heart disease. The measured drug EC_{50} values corresponded to plasma levels in patients, while the patient-specific model of Timothy Syndrome recapitulated the disease phenotype and the effects of clinically used drugs. We show here that adult-like human heart muscle can be engineered *in vitro* and used for predictive studies of drugs and modeling of cardiac disease.

DEVELOPING CARDIOMYOCYTE CONTRACTILITY BIOINFORMATIC INSIGHTS FROM ETHNICALLY AND EPIGENETICALLY DIVERSE IPSC LINES

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Stem cell based applications directed at improving the long-term therapeutic outcome of heart disease in many regards remains in its infancy. Cardiomyocyte therapies have potential to play a critical role in recovery from myocardial infarctions and their derivation has been explored from multiple adult, embryonic, and induced strategies. The stochastic nature of reprogramming generates differences in gene expression even amongst replicate lines derived from a single somatic cell source, the importance of which have yet to be understood in regard to downstream cardiomyocyte differentiation and function. Stem cell studies of cardiomyocytes that incorporate bioinformatics to analyze transcriptome and epigenome data are vital to identify signaling pathways underlying heart morphogenetic normal development and alterations relevant to heart disease models to advance patient-optimized therapies. Further such studies should begin to evaluate contributions of patient diversity in age or ethnicity, which has been slowed by availability of such resources. Previously we derived replicate iPSC lines of African American, Hispanic-Latino and Asian self-designated ethnically diverse (ED) origins with normal karyotype, qRT-PCR verified pluripotency biomarkers, teratoma formation and *in vitro* tri-lineage commitment. Here, bioinformatics of ED-iPSC RNA-Seq and ChIP-seq pluripotency data sets are compared along with differentiation to multiple neural, pancreatic, smooth muscle and cardiomyocyte multi-lineage cell types. Distinct among ED-iPSC ethnicities and replicates are differences in cardiomyocyte beating, accompanied by shared and distinct genes and contributing pathways. By evaluating reprogrammed epigenomes and transcriptomes across ethnicities coupled with comprehensive differentiation profiling this study enhances our ability to optimize iPSC technology for cardiac therapeutic potential and provides a longtime resource for broader iPSC studies of multilineage differentiation efficacy. [Supported in part by NYSTEM contract #C026186].

FOXA2 MARKS A VENTRICULAR-SPECIFIC PROGENITOR POPULATION DURING GASTRULATION

Evan Bardot[1,2,3], Damelys Calderon[1,2,3], Francis Santoriello[1,2,3], Songyan Han[1,3], Kakit Cheung[4], Bharati Jadhav[4], Heiko Lickert[5], Valerie Gouon-Evans[1,2,3], Andrew J. Sharp[4], Nicole C. Dubois[1,2,3]

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Major advancements in the field of heart development have led to the description of the first and second heart field populations; however, little remains known about the earliest specification events of the mammalian heart, or the mechanisms that direct atrial versus ventricular specification and differentiation. In this study, we provide the first evidence of a population of cells that gives rise selectively to cardiovascular cells of the ventricles, but not the atria, as well as epicardial cells. Using multiple lineage tracing models, we determined that this cell population is first specified during gastrulation, when it transiently expresses Foxa2, a pioneer transcription factor active during gastrulation. Using mixed chimera experiments we further illustrate a potential cell-autonomous role for Foxa2 in normal development of the heart. Translation of our findings to the mouse embryonic stem cell (mESC) system identifies an analogous Foxa2-derived cardiac mesoderm population during differentiation to cardiomyocytes and demonstrates reduced cardiac differentiation potential of Foxa2-deficient embryonic stem cells. Finally, Foxa2-derived cardiac mesoderm cells have been isolated and characterized by RNA sequencing, thus allowing for a detailed analysis of atrial-ventricular specification mechanisms. These findings not only advance our understanding of early cardiac cell fate specification to support better treatments for congenital heart disorders, but could also lead to improved strategies for the generation of specific cell types from pluripotent stem cells (PSCs) to benefit regenerative medicine approaches.

NICHE REGULATION OF MUSCLE STEM CELL QUIESCENCE BY CLASSICAL CADHERINS

Robert Krauss

Icahn School of Medicine at Mount Sinai

POSTER ASSIGNMENTS

(1) TRANPLANTED HUMAN RETINAL PIGMENT EPITHELIAL DERIVED STEM CELLS INTO THE SUBRETINA RESULTS IN LONG-TERM TREATMENT SUCCESS IN A RODENT MODEL OF AGE RELATED MACULAR DEGENERATION

(2) PROGRAMMING LOGIC OF CATECHOLAMINERGIC NEURONS

(3) HYPERTROPHIC CHONDROCYTE-BASED GRAFTS FOR ENHANCED BONE REGENERATION

(4) PROTEINS ASSOCIATED WITH OLFACTORY RECEPTOR NUCLEAR FOCI

(5) NON-CODING RNA REGULATES STEM CELL DIVISION

(6) IDENTIFYING A CARDIAC PROGENITOR POPULATION THAT ENHANCES VENTRICULAR CARDIAC PHENOTYPE IN THE HUMAN PLURIPOTENT STEM CELL SYSTEM

(7) UNDERSTANDING THE LONG-TERM OUTCOMES OF FETAL TISSUE GRAFTS FOR PARKINSON'S DISEASE

(9) ROLE OF LONG NON-CODING RNAS IN THE ESTABLISHMENT OF HEMATOPOIETIC STEM CELL IDENTITY

(10) DEVELOPMENT OF A CLINICAL-SCALE, cGMP-COMPLIANT CELL CULTURE PROCESS AND MANUFACTURE AND CHARACTERIZATION OF TISSUE-DERIVED RETINAL PIGMENT EPITHELIAL CELLS

(11) HUMAN ES-BASED MODELING OF PEDIATRIC GLIOBLASTOMA BY HISTONE MUTATIONS

(12) DEFINING THE PROSTATE LUMINAL STEM CELLS AND CELL OF ORIGIN OF PROSTATE CANCER

(13) RNA-BINDING PROTEINS AS A NOVEL CLASS OF THERAPEUTIC TARGETS IN GLIOMA STEM CELLS

(14) A DNA AND RNA-BINDING PROTEIN MODULATES TET- MEDIATED 2C-GENE REGULATION IN ESCS

(15) ENDOTHELIAL CELL-JAGGED2 REGULATES HEMATOPOIETIC STEM CELL MAINTENANCE, RECONSTITUTION AND LINEAGE SPECIFICATION

(16) CHARACTERIZING MOTOR FUNCTION IN FETAL TISSUE TRANSPLANT RECIPIENTS WITH PARKINSON'S DISEASE

(17) IDENTIFICATION AND CHARACTERIZATION OF LNCRNA FUNCTION IN LEUKEMIA STEM CELLS

(18) A CELL THERAPY FOR PARKINSON'S DISEASE

FACILITIES POSTERS

(1) TRANPLANTED HUMAN RETINAL PIGMENT EPITHELIAL DERIVED STEM CELLS INTO THE SUBRETINA RESULTS IN LONG-TERM TREATMENT SUCCESS IN A RODENT MODEL OF AGE RELATED MACULAR DEGENERATION

Nazia M. Alam[1] on behalf of the Retinal Stem Cell Consortium[1,2,3,4,5]
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Age-related macular degeneration (AMD) is the most common cause of blindness in the elderly. In AMD, retinal pigment epithelial (RPE) cells in the central retina become dysfunctional and degenerate, leading to neural retinal deterioration and visual decline. We are investigating whether RPE transplantation is a viable approach to treat AMD by transplanting healthy human derived RPE stem cells (hRPESCs) into the Royal College of Surgeons (RCS) rats- a model of AMD. Previously, we determined that the developmental stage of cultured RPE transplant is a key determinant of successful transplantation. RPE cells that are at an immature, or highly mature, stage of differentiation can preserve photoreceptors, but not vision; however, cells at an intermediate stage are able to longitudinally preserve vision and retinal anatomy for two months. Here we extend these results by evaluating the long-term benefit afforded by intermediate stage cultures. hRPESCs were differentiated for 4-5 weeks, prepared as suspensions, subretinally injected into rats at one month of age, and visual function and retinal structure was tracked for five months. Visual behavior was quantified, by assessing spatial frequency thresholds in a virtual optokinetic system (OptoMotry) and in the Visual Water Task (VWT). Optical coherence tomography (OCT) and histological analyses were also used to evaluate retinal anatomy. We demonstrate that transplanted hRPESC are able to prevent vision loss and preserve retinal anatomy in RCS rats from two to six months of age. The stage of differentiation that RPESC-RPE cells acquire prior to transplantation is critical for long-term efficacy. Our findings demonstrate that the developmental stage of cultured RPE transplant is a key determinant of successful transplantation. [Supported by NYSTEM contract # C028504]

(2) PROGRAMMING LOGIC OF CATECHOLAMINERGIC NEURONS

Begüm Aydin[1], Mireia Moreno-Estelles[2], Akshay Kakumanu [3], Nikathan S Kumar[1], Shaun Mahony[3], Nuria Flames[2], Esteban O Mazzoni[1]
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Direct programming of neurons by forced expression of transcription factors (TFs) holds promise for clinical applications of stem cell based therapies to cure neurodegenerative diseases. However, the mechanism by which TF combinations control diverse neuronal cell types remains obscure. Catecholaminergic –

dopaminergic, noradrenergic and adrenergic – neurons are a good model to tackle this question for two main reasons: (1) These neurons share common terminal features, even though they are not related by lineage, and (2) specification of different catecholaminergic neurons involves shared TFs in distinct combinations. To study how TF combinations control catecholaminergic fate, we generated inducible mouse embryonic stem cell lines with distinct TF combinations: the proneural gene Ascl1 (iA) alone and Ascl1 with Lmx1b (iAL). Initial characterization of these cell lines suggests: (1) Ascl1 expression by itself can efficiently induce mixed/generic neuronal fate, but fails to induce a specific catecholaminergic identity. (2) Addition of Lmx1b promotes catecholaminergic identity by upregulating the catecholaminergic marker, *Th,* and the noradrenergic marker, *Dbh.* We began investigating how Ascl1 coordinates its activity by analyzing Ascl1 binding events when it is induced in isolation (iA) and in combination with Lmx1b (iAL). Our ChIP-seq analysis shows that the vast majority of the Ascl1 binding events in iA and iAL does not change, which suggests that Ascl1 does not depend on Lmx1b to find its genomic targets. This data is consistent with previous findings on Ascl1 binding during direct programming of induced neurons from mouse fibroblasts. We are currently performing Lmx1b ChIP-seq in iAL to elucidate whether some of these shared Ascl1 binding sites are also bound by Lmx1b, or if Lmx1b binds independently of Ascl1 to regulate cell-type specific gene expression. We hope that uncovering this programming logic will improve ongoing efforts to generate patient specific catecholaminergic neurons from hiPSCs.
[Supported by NYSTEM Contract #C026880]

(3) HYPERTROPHIC CHONDROCYTE-BASED GRAFTS FOR ENHANCED BONE REGENERATION

Jonathan C. Bernhard[1], James Ferguson[2,3], Bernhard Rieder[3,4], Gabriele Leinfellner[2,3], Dominik Rünzler[3,4], Stefan Tangl[3,5], Heinz Redl[2,3], Gordana Vunjak-Novakovic[1]
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The body utilizes two bone formation processes in the development and maintenance of the skeleton, intramembranous and endochondral ossification. Intramembranous consists of a direct formation of bone, whereas endochondral ossification requires a cartilage intermediate before bone production. To better understand each process and its suitability for tissue engineering applications, each process was recapitulated using mesenchymal stem cells and the matrix deposition, mineral formation, and bone regeneration of each process were specifically studied. Based on the behavior measured *in vitro*, the hypothesis stated that endochondral-based tissue engineered grafts would facilitate faster bridging of critical sized rat femoral defects. Utilizing unique culture medium compositions, scaffolds seeded with human mesenchymal stem cells were differentiated to mimic the corresponding process over five weeks *in vitro*. After *in vitro* analysis, constructs were placed in five millimeter critical-sized

femoral defects, stabilized with an internal fixator attached to the native bone. After *in vitro* culture, endochondral constructs demonstrated significantly enhanced expression of key bone forming genes (*COL1A1, RUNX2, ALPL, IBSP*), and a broadened deposition of bone template proteins (collagen type I, bone sialoprotein). With regards to mineral deposition, the endochondral process produced significantly more mineral, at a significantly higher density. However, FTIR analysis showed that endochondral constructs contained immature, calcified cartilage-like mineral, whereas the intramembranous constructs produced mature, trabecular-like mineral. Interestingly, the localization of deposited mineral by endochondral was within the pore space, whereas intramembranous was along the scaffold walls. Analysis of the critical-sized defects demonstrated that endochondral constructs had enhanced regeneration compared to intramembranous, with 7/8 versus 2/8 constructs bridging the defect. The results elucidated key differences in the bone regeneration capabilities and provided clear evidence of the superior endochondral process for bone tissue engineering. Future work aims to determine the specific mechanisms responsible for the unique behavior of each recapitulated process.
[Supported by NYSTEM contract C028119]

(4) PROTEINS ASSOCIATED WITH OLFACTORY RECEPTOR NUCLEAR FOCI

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Previous work with 5-aryl-isoxazole-3-carboxyamide suggested that this molecule could induce mouse embryonic stem cells to differentiate to a cardiomyocyte lineage as well as trigger differentiation of neuronal progenitor cells into mature neurons. Subsequently, it was found that exposure of cell lysates to biotinylated isoxazole (b-isox) resulted in precipitation of RNA-binding proteins similar to the constituents of RNA granules.

In our work, we use b-isox to selectively isolate proteins that may participate in the formation of olfactory receptor nuclear foci and may mimic the formation of RNA granules. We used lysates of primary olfactory neurons from mouse and analyzed the protein composition of the b-isox precipitated proteins compared to control preparations with liquid chromatography/mass spectrometry (LC/MS/MS). Identifications were returned on 620 proteins with a 1% false discovery rate (predicted error). These proteins were represented by at least two peptides and provided 11,153 protein expression quantitative determinations.

Many RNA-binding or RNA-associated proteins were highly abundant in the b-isox samples compared to DMSO controls. One example was HNRPD_MOUSE, Heterogeneous nuclear ribonucleoprotein D0, which had a greater than 10-fold higher abundance in the B-isox samples compared to the DMSO controls. This protein has several repeats of a GYG motif that indicative of a low-complexity region characteristic

of proteins precipitated by b-isox. In the DMSO control, actin/tubulin cytoskeleton and ribosomal proteins predominated, indicating that this preparation represented a non-specific centrifugal enrichment of these structural components that contrasted markedly from the very specific enrichment for proteins containing low complexity sequence domains precipitated by b-isox. None of the RNA-binding proteins was prominent in the list of proteins for the DMSO control. The proteins reported in this study were nuclear, not cytosolic, suggesting a variety of novel functions not described previously.

[Supported by NYSTEM contract C029159]

(5) NON-CODING RNA REGULATES STEM CELL DIVISION

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Asymmetric cell division relies on spatial imbalance of cell fate determinant proteins to break symmetry. Here, we showed that asymmetric distribution of the microRNA miR-34a determines colon cancer stem cell (CCSC) symmetry by creating bimodal Notch signaling in daughter cells. Mechanistically, miR-34a, Notch and Numb forms an incoherent feedforward loop (IFFL) in which miR-34a directly targets Notch and Numb, while Numb suppresses Notch. The IFFL synergizes to create a unique regulatory scheme in order to separate stem cell and non-stem cell fates robustly. Perturbation of the IFFL leads to a new population of cells with more plastic and ambiguous identity. miR-34a also regulates normal colon stem cell asymmetric division. Lgr5+ colon stem cells normally undergo symmetric division. When trigged by inflammation, Lgr5+ colon stem cells can divide asymmetrically. Inflammation promotes colon stem cell self-renewal that expands the stem cell pool for regeneration, but miR-34a-mediated asymmetric division limits excessive colon stem cell accumulation, hence serving as a safeguard. Loss of miR-34a abolishes colon stem cell asymmetric division and further induces colon stem cell proliferation. Therefore, asymmetric division can be activated to counter stem cell proliferation at the onset of oncogenesis and remains active in early-stage CCSCs, until being eventually silenced by tumor progression in which miR-34a is silenced. Indeed, loss of miR-34a in CCSCs from late stage of CRCs promotes symmetric self-renewal.

(6) IDENTIFYING A CARDIAC PROGENITOR POPULATION THAT ENHANCES VENTRICULAR CARDIAC PHENOTYPE IN THE HUMAN PLURIPOTENT STEM CELL SYSTEM

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While much progress has been made in understanding early cardiogenesis, the relevant mechanisms that specify the different cardiomyocyte subtypes remain poorly understood. Electrophysiological analysis of cardiomyocytes derived from human pluripotent stem cells (hPSC) shows that atrial, ventricular and nodal-like cells are generated, but their respective isolation is not broadly established. Recent data from our lab have shown that transient *Foxa2* expression identifies a progenitor population with exclusive ventricular cardiac potential in the mouse embryo.

Here we have translated this novel specification event to the hPSC system. Using a *FOXA2-GFP* reporter cell line we analyzed expression of *FOXA2* during hPSC cardiac differentiation and found that a subset of cardiac mesoderm precursors expresses *FOXA2* transiently from day 3 to day 7 of differentiation.

Gene expression analysis of FOXA2+ and FOXA2- cardiac mesoderm revealed that both populations express early cardiac specification markers such as *PDGFRA*, *TBX5*, and *ISL1*. Interestingly *TBX20* and *GATA4* are significantly upregulated in the FOXA2+ population. Isolation and subsequent differentiation of FOXA2+ and FOXA2- populations demonstrates their comparable differentiation potential to both cardiomyocytes and epicardial cells. Moreover, cardiomyocytes derived from FOXA2+ precursors showed upregulated expression of MLC2v, a marker of ventricular cardiomyocytes, compared to cardiomyocytes derived from FOXA2- precursors. To identify the mechanisms that regulate ventricular specification, we performed small molecule screening and found that inhibition of the EGFR pathway strongly increased the cardiac mesoderm population in general, and the FOXA2+ precursors in particular. Finally, we have identified a combination of cell surface markers to specifically isolate FOXA2+ cardiac precursors.

Our results suggest that FOXA2+ cardiac mesoderm harbors ventricular-specific differentiation potential and isolation of these cells permits the generation of cultures enriched for ventricular cardiomyocytes. These data will be relevant for generating pure cell populations for regenerative medicine approaches, as well as for disease modeling from induced pluripotent stem cells.

(7) UNDERSTANDING THE LONG-TERM OUTCOMES OF FETAL TISSUE GRAFTS FOR PARKINSON'S DISEASE

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It is critical to study outcomes of early attempts at cell replacement as a new generation of stem cell technologies for neurorestoration nears clinical translation. Despite a substantial history of fetal ventral mesencephalic (fVM) tissue allotransplantation for Parkinson's disease (PD) through the 1990's, there is a dearth of data on very long term outcomes. We present clinical and radiological testing in patients who received bilateral fVM tissue grafts, without immunosuppression, into the bilateral putamen through a sham surgery-controlled clinical trial (Freed et al., 2001). This cohort was uniquely accessible for follow up through a study that tracked patients after clinical trial closure. We enrolled five patients for comprehensive evaluation of motor and non-motor function, and 11C-PE2i positron emission tomography (PET) imaging of the dopamine transporter (n=3). Subjects were 59 to 75 years old with PD duration of 28 to 42 years at the time of examination. LRRK2 mutations were present in 2 patients. All underwent fVM transplantation 16-18 years previously. Treatments ranged from 300mg to 1600 mg levodopa equivalents, and deep brain stimulator placement in 4/5. 3/5 participants could walk unassisted, and 2/5 ambulated with help. Graft-induced dyskinesias were present in the "off medication, off stimulation" state in a subset of participants, and were severe in one who as a result had undergone unilateral pallidotomy. 11C-PE2i PET imaging revealed robust but irregularly distributed signal in the bilateral putamen. In summary, very long term follow up suggests (1) graft maintenance up to 18 years post-transplant, (2) clinically manifest graft activity and (3) heterogeneous, yet collectively atypical outcomes for advanced PD with symptoms milder than expected. These findings highlight the importance of examining the long term outcomes of previous transplant trials and provisioning for long term up to lifetime follow up for recipients of living therapeutics/cell therapies. [Supported by NYSTEM Contract #C028503]

(9) ROLE OF LONG NON-CODING RNAS IN THE ESTABLISHMENT OF HEMATOPOIETIC STEM CELL IDENTITY

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Hematopoietic stem cells (HSCs) are tissue-specific stem cells that replenish all mature blood lineages during the lifetime of an individual and are widely used for transplantation-based therapies for leukaemias and congenital blood disorders. To date, the *in vitro* generation of HSCs from pluripotent stem cells has remained elusive and only leads to myeloid-restricted hematopoietic progenitor cells (HPCs). Long non-coding RNAs (lncRNAs) are non-coding RNAs that greater than 200 nucleotides in length and are emerging as a class of important regulators of stem cell self-renew and differentiation. Notably, reports have shown that lncRNAs contribute to the

survival and differentiation of long term HSCs. Here, I hypothesize that the impaired developmental potency of in vitro derived HPCs is most likely due to unidentified molecular differences between these cells and bona fide HSCs, and lncRNAs play an important role in establishment of HSC identity *in vitro*. To find such differences with high confidence, I developed protocols that allow the derivation of genetically matched HPCs (by in vitro differentiation from high quality murine iPSCs) and HSCs (by isolating Lin-Kit+Sca1+CD150+/- cells from E14.5 129XB6 fetal liver). I compared these two different types of cells using deep genome-wide RNA sequencing (RNA-seq) technology and identified even low abundant lncRNAs. [Supported by NYSTEM contract # C026880]

(10) DEVELOPMENT OF A CLINICAL-SCALE, cGMP-COMPLIANT CELL CULTURE PROCESS AND MANUFACTURE AND CHARACTERIZATION OF TISSUE-DERIVED RETINAL PIGMENT EPITHELIAL CELLS

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The University of Rochester Medical Center received a grant from NYSTEM for the construction of a facility enabling the production of human cell therapy materials for testing in early-phase human clinical trials. The Upstate Stem Cell cGMP Facility (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.

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 of RPE Comparability Master Cell Banks and Final Cell Product using the developed GMP-compliant, clinical-scale cell culture process. Comparability Batches are characterized with respect to proposed release specifications for cell number, viability, purity, identity, potency, sterility, endotoxin, mycoplasma, adventitious viruses, karyotype and stability.

In addition, the development of *in vitro* measures of phagocytosis and assessment as a potential potency assay are shown along with proof of principle efficacy studies in Royal College of Surgeons (RCS) rat models showing that RPESC-derived RPE cells demonstrate efficacy showing a clear visual preservation effect approximately 5 months past implantation.
(Supported by NYSTEM contract C026713 and C028504)

(11) HUMAN ES-BASED MODELING OF PEDIATRIC GLIOBLASTOMA BY HISTONE MUTATIONS

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Glioblastoma (GBM) is the most frequent and malignant primary brain tumor. Recent findings reveal recurrent mutations in histone H3.3 variant in pediatric glioblastoma. H3.3-G34R/V mutation is predominantly found in supratentorial gliomas in juvenile and young adult patients, whereas H3.3-K27M mutation occurs in younger age group and is largely confined to the midline, implying different biological entities. Concomitant with H3.3-G34R/V are mutations in p53 and ATRX as well as amplification and/or mutation of PDGFRA. However, the molecular mechanism how H3.3-G34R/V and other mutations contribute to gliomagenesis is still to be elucidated. To evaluate the effect of each mutation, we have derived neural precursors (NPCs) from human ES cells and performed a stepwise transduction of H3.3-G34R, H3.3-G34V, shp53, shATRX and constitutively active mutant (D842V) of PDGFRA. Our data demonstrate increased proliferation in the presence of shp53 (Ki67 index of 20.9 ± 4.2 % *vs* 11.4 ± 3.6 % in control NPCs). On the other hand, knockdown of ATRX leads to a rapid depletion of the cells 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 the cell death induced by ATRX knockdown is partially rescued by H3.3-G34R mutation, 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 mutation contributes to the resistance to DNA damage, rather than enhancing DNA damage repair. Furthermore, we have analyzed the impact of these oncogenes, alone or in combination, on neuronal and astrocytic differentiation. Our data show that G34R mutation could suppress the differentiation to both neuronal and astrocytic lineages, only if combined with shATRX and shp53. Taken together, these preliminary data indicate synergistic effects of these oncogenic mutations in the tumorigenesis of pediatric glioblastoma.
[Supported by NYSTEM contract # C026879]

(12) DEFINING THE PROSTATE LUMINAL STEM CELLS AND CELL OF ORIGIN OF PROSTATE CANCER

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The absence of basal cells along with luminal cell expansion is the definitive pathologic characteristic of prostate cancer. Although controversial, basal cells had been suggested to be the cellular origin of human prostate cancer. The prostate luminal stem cell and cell of origin of prostate cancer have been poorly understood. In a recent study, we developed the organoid culture technology for human and mouse prostate cell culture, and found that luminal cells can form organoids in vitro(Gao et al., 2014; Karthaus et al., 2014). Encouraged by these results and driven to find evidence for prostate luminal stem cells, we then analyzed mouse prostate luminal cell and identified a small luminal cell population which had higher efficiency to form organoids in vitro in the prostate luminal cell niche which were characterized by higher Trop2 expression. The Trop2-high luminal stem cells accumulate after castration and finally all the residual castration resistant luminal cells survived with high Trop2 expression. This observation indicates a novel luminal stem cell population which is an efficient target for oncogenic transformation in prostate cancer. This project will shed light on the cell origin of prostate cancer initiation and prostate development, which may provide a novel model to identify the molecular mechanism of hormone-refractory prostate cancer.
Acknowledgement: This work was supported by NYSTEM contract #C026879.

(13) RNA-BINDING PROTEINS AS A NOVEL CLASS OF THERAPEUTIC TARGETS IN GLIOMA STEM CELLS

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Glioblastoma multiforme (GBM) is the most malignant adult brain tumor and remains incurable despite aggressive treatment. Glioblastomas are highly heterogeneous tumors consisting of a small fraction of glioma stem cells (GSCs), which contribute to tumor initiation and show preferential resistance to existing therapies. Previous studies have mostly focused on the role of transcriptional and epigenetic regulators in GSCs, although significant oncogenic and tumor suppressor roles of RNA-binding proteins during cancer development have been reported. We propose that understanding the role of RNA-binding proteins in GSCs may provide further insight into the identification of novel therapeutic targets.

Here, we identify NOVA1 as the top upregulated RNA-binding protein in GSCs compared to *in vitro* differentiated GBM cells. We further show that NOVA1 is co-expressed with the stem cell transcriptional regulators SOX2 and OLIG2 in patient tumor sections as well as in patient cell lines *in vitro*. It is interesting to note that NOVA1 was previously thought to be selectively expressed in neurons.
To explore the role of NOVA1 in GSCs, we have conducted shRNA knockdown (KD) experiments in patient-derived cell lines that are enriched for the stem cell biomarker CD133. In vitro limiting dilution assays showed significant reduction of glioma sphere formation in NOVA1 KD cells compared to control GBM cells. Consistent with our hypothesis of a major regulatory role in GSC biology, we observed a significant reduction of $CD133^{+}$ cells in NOVA1 KD compared to control shRNA. We also find reduced proliferation of NOVA1 KD cells *in vitro*, potentially due to the loss of self-renewal capacity of NOVA1 KD GSCs. Furthermore, NOVA1 KD reduced SOX2 expression suggesting likely NOVA1 regulation of SOX2. We are in the process of generating CRISPR-Cas9 NOVA1 deletion in GBM cell lines for further functional validation and RNA-Seq splicing analysis.

(14) A DNA AND RNA-BINDING PROTEIN MODULATES TET- MEDIATED 2C-GENE REGULATION IN ESCS

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ESCs in culture are quite heterogeneous at the transcriptional level. While retaining their pluripotency, they differ in their differentiation potential and potency. Recently, a subpopulation of highly potent cells that express characteristic 2-cell stage embryo (2C) transcripts, such as endogenous retroviral elements (ERVs) has been described. Transcriptional and epigenetic mechanisms are involved in protecting the cells from the uncontrolled reactivation of ERVs, which could lead to cancer or genetic diseases. Among well-known epigenetic regulators, the TET family of proteins has been recently shown to be involved in the control of 2C and ERV transcription in ESC. However, the distinct contribution of Tet1 and Tet2 as well as the involvement of their catalytic dependent and independent functions in such regulation, remain to be defined. In order to gain further insight into retroviral silencing and stem cell pluripotency, we have constructed Tet1 and Tet2 interactomes in ESCs and uncovered many novel TET partner proteins. We have found a DNA and RNA-binding protein that is able to work in concert with Tet1 and Tet2 to balance 2C genes and ERV expression. Furthermore, through binding to Tet1 mRNA, but not Tet2, it regulates its translation and abundance as an additional layer of TET control. The discovery of factors that coordinate epigenetic regulators will, not only shed light into the mechanisms by which this 2C-like state is acquired and lost during ESC culture,

but may facilitate the definition of a 2C-state, and the requirements for the development of media conditions to maintain and propagate totipotent cells *in vitro.* [Supported by NYSTEM contract #C028121]

(15) ENDOTHELIAL CELL-JAGGED2 REGULATES HEMATOPOIETIC STEM CELL MAINTENANCE, RECONSTITUTION AND LINEAGE SPECIFICATION

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Defining the cellular and molecular components that regulate the self-renewal, regeneration and lineage differentiation of hematopoietic stem cells (HSC) can facilitate targeted interventions towards improved hematopoietic recovery post injury and the generation of sufficient numbers of HSC for transplantation. At the core of this dynamic cell-cell interaction is the instructive niche provided by bone marrow endothelial cells (BMEC), which deploys angiocrine signals such as Jagged1, KitL and CXCL12, to regulate HSC self-renewal and lineage differentiation under homeostatic and myelosuppressive conditions. Endothelial cells express four types of Notch ligands. The presented data will address the non cell-autonomous role of endothelial cell (EC)-jagged2 on HSC self-renewal and lineage differentiation under various experimental contexts. Utilizing EC-specific Jag2 deletion mouse models coupled with transplantation assays, we concluded that: (i) jagged2 is differentially expressed in different vascular beds at steady state, and its expression is dynamically regulated in cultured conditions and within a time window post injury; (ii) EC-jagged2 is minimally required to maintain HSC self-renewal and repopulating capacity under steady state; (iii) EC-jagged2 induced specific Notch targets expression in HSCs; (iv) EC-jagged2 is necessary for efficient recovery of HSCs under myelosuppressive conditions, in a relevant time window; (v) EC-jagged2 regulates HSC lineage differentiation post GCSF-mediated mobilization; (vi) EC-jagged2 is required for HSC expansion and early T cell development in ex vivo co-culture conditions; (vii) Functionally, although the development of gamma-delta T cells is not affected, EC-jagged2 regulates the activation status of T cells in secondary lymphoid organs. Compared with previous published data, the distinct role of EC-jagged2 compared with EC-jagged1 suggested differential functional readout as a consequence of ligand-receptor interaction in between EC and HSCs. Collectively, the presented experimental efforts demonstrated the functional relevance of Notch signaling in the context of EC-HSC interaction and the practicality of probing EC-based coculture platforms for cellular therapies. [Supported by NYSTEM contract C026878]

(16) CHARACTERIZING MOTOR FUNCTION IN FETAL TISSUE TRANSPLANT RECIPIENTS WITH PARKINSON'S DISEASE

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Transplantation of fetal ventral mesencephalic (fVM) tissue as a source of dopaminergic neuroblasts has been previously undertaken to replace striatal dopamine inputs lost in Parkinson's disease (PD). In published studies, while some recipients achieved improved motor function, some developed graft-induced dyskinesia (GID). We describe motor function at very long term follow up using a comprehensive battery of tests in 5 patients from a previously published clinical trial of fVM tissue transplantation performed between 1995-1998 (Freed et al 2001). Of these, 4/5 had also undergone subsequent deep brain stimulator implantation. Motor function was video-recorded and assessed using the Unified Parkinson's Disease Rating Scale. Quantitative measures of bradykinesia and dyskinesia were made using the Parkinson's Kinetigraph (PKG), a novel accelerometer-based motion tracker. Self-reported measures were collected using the Hauser Home Diary. In the medication off/stimulator off states, we recorded dyskinesias in 2 participants. PKG recordings will be presented, and provide temporal information linking dyskinesias to presence of graft as well as medication effects. Patient-reported "off" time was 2-15%; "on" time without dyskinesia was 4-63%; and "on" time with moderate-to-severe dyskinesia was 0-37%. GID in the subset of participants related to presence of engrafted tissue demonstrated by [11C]PE2i positron emission tomography (PET) imaging of the dopamine transporter. Nearly 2 decades after the original study we see a nuanced picture of motor function including: (1) GID, reported in 15% in the original cohort at 1 year. (2) subtle and heterogeneous evidence for grafts' clinical effects. Our findings support continuous monitoring of patients undergoing experimental cell replacement studies using sensitive and multivariate testing batteries, including well validated clinical scales and novel technologies such as PKG. It also reinforces the need for sustained surveillance for latent effects of therapy which may appear years or decades after an intervention. [Supported by NYSTEM Contract #C028503]

(17) IDENTIFICATION AND CHARACTERIZATION OF LNCRNA FUNCTION IN LEUKEMIA STEM CELLS

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In acute myeloid leukemia (AML), a subpopulation of leukemic cells are enriched for leukemic initiating stem cells (LSCs) that can transfer the disease, and a high

frequency of these cells at diagnosis predicts poor survival after chemotherapy due to high minimal residual disease. Long non-coding RNAs (lncRNAs) have emerged as important regulators of various cellular processes and may play a role in regulating LSC function. Annotation of LSC enriched lncRNAs is being carried out by RNA sequencing of sorted $CD34^{+}CD38^{-}$ cells from patient samples. We also performed RNA sequencing and histone mapping of four AML cell lines representing the two major subtypes of AML, M0-2 and M4-5, to generate a high confidence annotation of lncRNAs to help validate the primary patient data. A total of 40,365 non-coding transcripts were assembled from the sequencing, and using the lncRNA discovery pipeline that we developed, we identify 3,867 lncRNAs that are expressed in the cell lines, of which 1,215 are novel. In order to test the function of candidate lncRNAs, we have generated successfully a CRISPR inhibition system with dCas9 in two AML cell lines. This will allow us to conduct a negative selection screen for important lncRNAs that are expressed in LSCs and affect AML growth. LncRNAs identified from the screen represent new potential targets for treating AML. [Supported by NYSTEM contract #C026880]

(18) A CELL THERAPY FOR PARKINSON'S DISEASE

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In 2011, we demonstrated the generation of functional midbrain dopaminergic neurons (mDA) from human embryonic stem cells (hESCs). Based on this research, we are now developing a cell therapy for Parkinson's Disease (PD). This treatment, if available at scale, manufactured in defined conditions and at high purities could potentially bring life-long relief from the devastating symptoms of PD. Indeed, fetal cell transplantation studies provided proof-of-concept data and restored function in some patients for 18 years or more. Thus, cell therapy for PD is an attractive treatment option. Today, we have established robust and scalable manufacturing protocols and moved the cell production from the academic lab into MSKCC's cGMP production facility. The cell product can be cryopreserved and thawed at high viability. In addition, we now produce 1-2 billion cells in a single production run, theoretically treating several hundred patients. Further, this production scale allows us to run ALL safety and efficacy testing on the same batch of cells that will eventually serve for the first-in-human clinical trial. Currently, we are finalizing our release criteria and production runs and have scheduled a pre-IND meeting with the FDA to discuss our planned IND-enabling studies. If completed successfully, we will seek FDA approval for a clinical trial in 2017 (IND). In this presentation, we will show details of our differentiation and quality control protocols and present some of the challenges we faced to date during the move from bench to bedside. We will further describe our efforts in defining the best patient population for this trial and the global effort of the GForce-PD group to harmonize and control cell therapy for PD. Sharing our experience with the stem cell community will be of great value to all attendees and

will have broad applicability to stem cell therapies in general. [Supported by NYSTEM contract #C028503]

(19) SINGLE CELL ANALYSES REVEAL CELL STATE SPECIFIC ACTIVITIES OF FGFR1 AND FGFR2 FOR STABLE FATE CHOICE WITHIN THE MOUSE BLASTOCYST

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Dynamic FGF/ERK signaling promoted by FGF4 within the inner cell mass (ICM) of the mouse blastocyst drives lineage specification of pluripotent epiblast (EPI) and primitive endoderm (PRE). To investigate how FGF4 signaling is transmitted in individual cells within the ICM, we analyzed the cognate FGF receptor(s) using both single-cell transcriptome and quantitative wholemount *in situ* protein expression analyses. We find that despite the PRE-specific expression of *Fgfr2*, *Fgfr1* which is expressed by all ICM cells, is required in the ICM to respond to FGF4 for the establishment of a PRE identity. Furthermore, our data reveal that *Fgfr1* mutants exhibit elevated levels of NANOG expression in uncommitted ICM cells, while *Fgfr2* mutants downregulate NANOG and GATA6 expression in later EPI- and PRE-biased cells, respectively. Our studies identify distinct but combinatorial roles of *Fgfr1* and *Fgfr2* drive stable cell fate specification of EPI/PRE cells in the mouse blastocyst. Specifically, the signal from a single ligand, FGF4, diverges through distinct receptors (*Fgfr1* and *Fgfr2*) to control ICM cell states and direct cell fate choice during discrete temporal domains of pre-implantation mouse embryo development.

[Supported by NYSTEM contract C029568]

(20) THE G9a HISTONE METHYLTRANSFERASE INHIBITOR BIX01294 ACTS AS AN EXPANSION FACTOR FOR ENDOGENOUS CARDIAC PROGENITOR CELLS FROM THE ADULT HEART

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The pharmacological agent BIX01294 is a selective inhibitor of G9a histone methyltransferase activity. The present investigation was initially undertaken as a follow-up to our previous reports showing BIX01294 can enhance the cardiac potential of bone marrow stem cells. The initial objective of this study was to determine whether BIX01294 would have any adverse effects on cardiac function and/or differentiation that would compromise its utility in generating stem cells for use in cardiac repair.

Differentiated cardiomyocytes and adult heart tissue fragments were cultured in the presence and absence of BIX01294, and examined for changes in cardiac function and molecular expression. In addition, enriched populations of cardiac progenitor cells (CPCs) were harvested from non-treated control and BIX01294-treated cardiac tissue and assayed for changes in cell phenotype and differentiation potential.
While BIX01294 had no discernible effect on myocyte function and sarcomeric organization, treatment with this drug significantly increased CPC proliferation, as indicated by enhanced MTT metabolization and BrdUrd incorporation (4.1 and 2.0 fold, respectively) after 48 hour labeling, and increased Ki67 expression (4.8 fold) after seven days of culture. Heart explants exposed to BIX01294 generated 3.6 fold greater yields of CPCs by two weeks of culture. Importantly, CPCs obtained from non-treated and BIX01294-treated cultures did not differ in phenotype or differentiation potential.
Together these data demonstrate that the G9a histone methyltransferase inhibitor BIX01294 can act as an expansion factor for endogenous CPCs without changing their phenotype or compromising their cardiac competency. Thus, this drug may have utility as a reagent for generating large number of CPCs for cardiac tissue repair and regeneration.

(21) FOXC1 GOVERNS HAIR FOLLICLE STEM CELL QUIESCENCE AND NICHE MAINTENANCE TO PRESERVE LONG-TERM TISSUE-REGENERATING POTENTIAL

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Adult stem cells (SC) are critical for making and repairing tissues throughout the lifetime of the organism. This is exemplified by SCs residing in the bulge (Bu) of the murine hair follicle (HF), where they are kept quiescent and activated only cyclically to fuel hair regeneration. Whether such tight regulation of SC activity is essential to conserve their long-term tissue-regenerating potential remains poorly understood. We addressed this by conditionally ablating a key Bu-HFSC transcription factor, FOXC1. We show that FOXC1-deficient HFs are perturbed in two ways: 1) their Bu-HFSCs exit quiescence prematurely to make new hairs; and 2) they lose their old Bu while making a new one in every hair cycle. Transcriptomic profiling revealed that perturbation of Bu-HFSC quiescence may cause downstream changes in cellular adhesion. Indeed, during regeneration, both WT and FOXC1-deficient Bu-HFSCs down-regulate E-cadherin when proliferating. However, FOXC1-deficient Bu-HFSCs fail to re-establish quiescence and restore E-cadherin levels promptly. As a result, when the new hair grows past them, the old Bu, along with its poorly adherent Bu-HFSCs and SC-inhibitory factors, becomes shed from the HF. This altered SC niche in FOXC1-deficient HFs, coupled with their SC-intrinsic propensity to proliferate, further lowers the threshold for SC activation, causing excessive hair cycling and aberrant SC expenditure. Consequently, FOXC1-deficient Bu-HFSCs decline in numbers and in their ability to maintain the hair coat for the aging animal. Together, our findings suggest that Bu-HFSCs conserve their limited potential by coupling quiescence to adhesion-mediated niche maintenance in order to achieve long-term tissue homeostasis.

(22) IDENTIFICATION OF NOVEL REGULATORS OF HUMAN DEFINITIVE ENDODERM DIFFERENTIATION BY GENOME-WIDE CRISPR SCREEN

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Studying the molecular mechanism of definitive endoderm (DE) differentiation is very important to understand the very first step of human development. DE cells are the common precursor cells giving rise to most cells all the digestive and respiratory systems. Elucidating the developmental signaling pathway also has profound implications for the development of stem cell in regenerative medicine. hESCs also offer unique advantages in using in vitro differentiation and functional genomics to model human embryonic development. Therefore, we used a genome-wide CRISPR knockout screen approach to identify genes playing functional roles in the process of DE differentiation. Using a Z-score ranking method, we discover a list of 189 positive and 176 negative regulators for DE differentiation from the genome-wide screen. TGFb pathway components (ACVR1B/ALK4, SMAD2, SMAD4, FOXH1) and key endoderm transcription factors (e.g. EOMES, MIXL1) are ranked top in the positive regulators list. Out of the 23 top positive regulator hits that we examined so far, 21 genes were required for efficient endoderm formation. More importantly, we also validated 13 genes that are not known to be involved in endoderm development. Ongoing experiments seek to individually validate more top hits and study the molecular mechanism in the context of DE differentiation.

(23) EPIGENETIC PROFILES SIGNIFY CELL FATE PLASTICITY IN UNIPOTENT MAMMALIAN SPERMATOGONIAL STEM AND PROGENITOR CELLS

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Mammalian spermatogonial stem and progenitor cells (SSCs) are precursors of all subsequent germ cells in the adult male gonad. During *in vitro* expansion, these unipotent stem cells can spontaneously convert to multipotent adult spermatogonial-derived stem cells (MASCs) without ectopic expression of any transcription factors.

The underlying mechanisms of this spontaneous conversion process are still poorly understood. Here, we report an integrative analysis of both the transcriptomes and histone modification-defined epigenomes of mouse SSCs and MASCs. We found in SSCs that many genes essential to maintenance and differentiation of embryonic stem cells (ESCs) are enriched with both histone H3 lysine 4 and lysine 27 trimethylation modifications (K4me3+K27me3) at promoter regions. Quantitative epigenomic analysis suggested that these 'bivalent' modifications are maintained at most somatic gene promoters after conversion, except for changes at a few signature genes for ESCs and germ cells. Thus, MASCs are bestowed with ESC-like promoter chromatin states. This phenomenon distinguishes MASC derivation from induced pluripotent stem (iPS) cell reprogramming, which involves global epigenetic changes and reconstitution of promoter bivalency. At enhancer regions, the core pluripotency circuitry was activated partially in SSCs and completely in MASCs, concomitant with global erasure of germ cell-specific enhancer activity and initiation of an embryonic-like program. Furthermore, long-term *in vitro* cultured SSCs maintained epigenomic characteristics reflective of germ cells *in vivo*. Our observations suggest that unipotent SSCs encode their innate developmental flexibility by means of the epigenome and that both the conversion of promoter chromatin states and the activation of cell type-specific enhancers are prominent features of SSC reprogramming. [Supported by NYSTEM contract # C029156]

(24) IDENTIFICATION OF STEM CELL POPULATIONS IN SWEAT GLANDS REVEALS ROLES IN HOMEOSTASIS AND WOUND REPAIR

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Sweat glands are abundant in the body and essential for thermoregulation. Like mammary glands, they originate from epidermal progenitors. However, they display few signs of cellular turnover, and whether they have stem cells and tissue-regenerative capacity remains largely unexplored. Using lineage tracing, we here identify in sweat ducts multipotent progenitors that transition to unipotency after developing the sweat gland. In characterizing four adult stem cell populations of glandular skin, we show that they display distinct regenerative capabilities and remain unipotent when healing epidermal, myoepithelial-specific, and lumenal-specific injuries. We devise purification schemes and isolate and transcriptionally profile progenitors. Exploiting molecular differences between sweat and mammary glands, we show that only some progenitors regain multipotency to produce *de novo* ductal and glandular structures, but that these can retain their identity even within certain foreign microenvironments. Our findings provide insight into glandular stem cells and a framework for the further study of sweat gland regeneration.

(25) STEM CELLS OF THE SUTURE MESENCHYME IN CRANIOFACIAL BONE DEVELOPMENT, REPAIR AND REGENERATION

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Development of the skeleton is mediated through two distinct ossification mechanisms. Craniofacial bones are formed mainly through intramembranous ossification, a mechanism different from endochondral ossification required for development of the body skeleton. The skeletal structures are quite distinct between the two, thus they are likely to have their unique stem cell populations. The sutures serve as the growth center critical for healthy development of the craniofacial skeleton. Defects in suture morphogenesis cause its premature closure, resulting in development of craniosynostosis, a devastating disease affecting 1 in ~2,500 individuals. The suture mesenchyme has been postulated to act as the niche of skeletal stem cells essential for calvarial morphogenesis. However, very limited knowledge is available for suture biology and suture stem cells (SuSCs) have yet to be isolated. Here we report the first evidence for identification and isolation of a stem cell population residing in the suture midline. Genetic labeling of SuSCs shows their ability to self-renew and continually give rise to mature cell types over a 1-year monitoring period. They maintain their localization in the niches constantly produce skeletogenic descendants during calvarial development and homeostastic maintenance. Upon injury, SuSCs expand drastically surrounding the skeletogenic mesenchyme, migrate to the damaged site and contribute directly to skeletal repair in a cell autonomous fashion. The regeneration, pluripotency and frequency of SuSCs are also determined using limiting dilution transplantation. In vivo clonal expansion analysis demonstrates a single SuSC capable of generating bones. Furthermore, SuSC transplantation into injured calvaria facilitates the healing processes through direct engraftments. Our findings demonstrate SuSCs are bona fide skeletal stem cells ideally suited for cell-based craniofacial bone therapy as they possess abilities to engraft, differentiate into skeletogenic cell types, generate bones and enhance repair processes. Future study of SuSCs also promises new insights into pathogenic mechanisms of skeletal disease.
[Supported by NYSTEM contract # C029558]

(26) 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 *C elegans*, 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. Yet, niche formation is poorly understood. This is because in most models, the cells participating in the niche are not well identified prior to niche assembly, and because formation is difficult to

observe *in vivo*. In *C elegans*, however, the gonad primordium is a simple, easily observed organ consisting of just four cells – two primordial germ cells, and two somatic niche cells. The primordium forms via a fascinating morphogenetic process in which the two niche cells migrate posteriorly, recognize the germ cells, and wrap almost completely around them. This process recapitulates many key steps that must occur more generally in niche formation. Previously, it was reported that ablation of niche precursors results in a loss of the primordial germ cells. However, no mechanism has been reported for this phenomenon. Using genetics, fluorescent imaging and embryological manipulations we are testing the hypothesis that signaling or adhesion between stem and niche cells are needed for stem cells to survive. Initially, we repeated the niche ablations using fluorescent reporters to follow cells in the primordium. We too found the germ cells are frequently lost in these animals. Surprisingly, we also observed that unprotected germ cells appear to be consumed by neighboring endodermal cells. We then repeated these ablations in mutants lacking endoderm. As predicted, germ cells persist in these mutants. Earlier studies show that germ cells express a high level of E-Cadherin. We are currently testing if niche cells ability to protect germ cells from endodermal engulfment requires E-Cadherin and if so whether it acts through an adhesion or signaling based mechanism.

Supported by NYSTEM contract # IIRP C029561

(27) TCF7L1 MODULATES COLORECTAL CANCER GROWTH BY INHIBITING EXPRESSION OF THE TUMOR-SUPPRESSOR GENE EPHB3

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Dysregulation of the Wnt pathway leading to accumulation of β-catenin (CTNNB1) is a hallmark of colorectal cancer (CRC). Nuclear CTNNB1 acts as a transcriptional coactivator with TCF/LEF transcription factors, promoting expression of a broad set of target genes, some of which promote tumor growth. However, it remains poorly understood how CTNNB1 interacts with different transcription factors in different contexts to promote different outcomes. While some CTNNB1 target genes are oncogenic, others regulate differentiation. Here, we found that TCF7L1, a Wnt pathway repressor, buffers CTNNB1/TCF target gene expression to promote CRC growth. Loss of TCF7L1 impaired growth and colony formation of HCT116 CRC cells and reduced tumor growth in a mouse xenograft model. We identified a group of CTNNB1/TCF target genes that are activated in the absence of TCF7L1, including EPHB3, a marker of Paneth cell differentiation that has also been implicated as a tumor suppressor in CRC. Knockdown of EPHB3 partially restores growth and normal cell cycle progression of TCF7L1-Null cells. These findings suggest that while CTNNB1 accumulation is critical for CRC progression, excessive Wnt pathway activation in certain contexts, such as the loss of TCF7L1, may in fact inhibit tumor growth by inducing expression of tumor suppressor genes.
[Supported by NYSTEM contract # C026880]

(28) FACILITATED REPROGRAMMING OF HUMAN FIBROBLASTS USING SOX2-VP16

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Reprogramming of human fibroblasts with OSK yields very few iPS-like colonies. The efficiency of reprogramming is ordinarily dependent on the inclusion of cMYC in the reprogramming mix, and is lower with cells taken from older donors. How may we improve the efficiency of reprograming of human cells, especially those derived from older donors? We present our findings on reprogramming human cells with an array of chimeric variants of each of the four OSKM factors. We find that by substituting a fusion variant bearing a strong activation domain (VP16) attached to SOX2, for its wild-type counterpart, we enhanced the efficiency of reprograming of fibroblasts derived from both young, and especially of those from older donors. SOX2-VP16 (S(v)) can decrease, and in one scenario eliminate, the requirement for cMYC for reprogramming. SOX2-VP16 elicits early and strong expression of certain pluripotency genes including *NANOG*. A genome-wide assay shows that where SOX2 and OCT4 (but not KLF4 and cMYC) bind to DNA outside of existing active enhancers, new enhancers are almost always created. These enhancers are 'stronger' with SOX2-VP16. 'Super-enhancers' active in fibroblasts are readily extinguished following ectopic expression of OSKM or OS(v)KM. (Supported by NYSTEM contract # N09G217 and N11G262)

(29) IN VIVO IMAGING OF DENTATE GYRUS IN FREELY MOVING MICE DURING EXPLORATION OF NOVEL ENVIRONMENTS

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The Dentate gyrus (DG) is one of the few brain structures that is capable of generating new neurons from neural stem cells throughout life. This process of adult neurogenesis is thought to facilitate pattern separation allowing for discrimination of small differences in environments. To address how the DG performs this computation, we used population Ca imaging to monitor the activity of granule cells in the dorsal DG during free exploration of three environments (A-B-C) that had distinct contextual cues. We examined the unit activation rate by converting the number of activations per cell into z score matrices for three environments and identified a similar number of cell subsets (109 in A; 104 in B; 102 in C, pooled cells=1041, n=3 mice, $z > 2.1$) that displayed strong activations in one environment but not in others. A large portion ($22.1 \pm 5.4\%$) of identified cells were highly active in one environment but completely silent in both of the other environments. In fact, there was only a small number of overlapping cells (n=7) active in all three contexts. We next determined the spatial tuning of DG GCs. Standard place cell analysis determined 156/1041, 176/1041, and 218/1041 exhibited significant spatial tuning in environments A, B, and C, respectively. There was only a small portion ($15.2 \pm 7.2\%$) of spatially tuned cells that overlapped with the cells that were highly active based on their z score. Lastly, we investigated the anatomical locations of highly active cell subsets determined in A-B-

C environments. Pairwise inter-cell distances of each cell subsets were calculated as 115±20.3μm, which showed no distinct spatial layout for the functional cell groups. However, we noted a significant fraction of cell units (31.1±10%) in each subset that were within a 20 μm radius. These results suggest the DG is a sparse structure that recruits different population of cells to encode different environments with similar number of cellular units that can exhibit distinct anatomical layouts in the closer distances.

(30) PROPHYLACTIC KETAMINE RESCUES ABNORMAL STRESS BUFFERING FOLLOWING ABLATION OF ADULT HIPPOCAMPAL NEUROGENESIS

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Stress exposure is a major risk factor for mood disorders, such as major depressive disorder (MDD). However, not all individuals develop psychopathology following stress. This ability to adapt to stress, known as stress resilience, is putatively mediated by adult hippocampal neurogenesis, the birth of new neurons in adulthood. Here, we aim to identify the mechanisms and neural circuits by which neurogenesis buffers stress responses by visualizing the whole brain neural activity that mediates behavior in mice with arrested adult hippocampal neurogenesis. We ablated adult hippocampal neurogenesis using a reversible, genetic method in which glial fibrillary acidic protein (GFAP)-positive neural progenitor cells are ablated with ganciclovir (GCV). We found that GFAP-thymidine kinase (TK) transgenic (Tg) mice show an elevated fear response when compared with control (Ctrl) mice following contextual fear conditioning (CFC), supporting the notion that adult hippocampal neurogenesis is required for basal stress resilience. Moreover, we found that a single injection of ketamine administered prior to CFC rescued this impaired fear response in GFAP-TK Tg mice. To identify the neural circuits mediating this improved stress response by ketamine, we processed all brains following stress exposure using an iDISCO-based protocol for detecting whole brain neural activity by c-fos immunolabeling and microscopic imaging at single cell resolution. It remains unknown if ketamine converges downstream of neurogenesis in a common canonical resilience pathway or instead induces a novel parallel pathway. Therefore, we aim to identify the neural ensembles involved in ketamine-mediated neurogenesis-independent resilience, and whether they converge with or diverge from those of neurogenesis-dependent resilience. This work will elucidate the mechanisms underlying stress resilience and guide the development of effective treatments for mood disorders. [This work was supported by NYSTEM contract # C-029157].

(31) POLYCOMB-MEDIATED REPRESSION AND SONIC HEDGEHOG SIGNALING INTERACT TO REGULATE MERKEL CELL SPECIFICATION DURING DEVELOPMENT

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During development, embryonic epidermal progenitor cells give rise to the epidermis, hair follicles, and Merkel cells. Merkel cells are mechano-receptor cells, innervated by sensory neurons, located in touch sensitive areas of the mammalian skin and are involved in recognition of the texture and shape of objects. Merkel cells are essential for these tactile discriminations, as they generate action potentials in response to touch stimuli and induce the firing of innervating afferent nerves. While it has been recently shown that Merkel cells originate from epidermal stem cells, the cellular and molecular mechanisms of their development are largely unknown.
In the murine dorsal skin, Merkel cells are organized in touch domes, which form around only one hair follicle type, the guard hairs. We have found that the developing hair follicle functions as a niche for Merkel cell formation. We show that Sonic hedgehog (Shh) signaling, initiated by the production of Shh ligand in the developing hair placodes, is required in the epidermis for Merkel cell specification. Shh signaling is common to all hair types, raising the possibility that restrictive mechanisms regulate Merkel cell specification. Indeed, we find that loss of Polycomb repressive complex 2 (PRC2) in the epidermis results in the formation of ectopic Merkel cells that are associated with all hair types. Interestingly, PRC2 loss does not alter the inductive signals that specify Merkel cells. Instead, it expands the field of cells competent to produce Merkel cells through the upregulation of key Merkel-differentiation genes, which are known PRC2 targets. Merkel cell specification, however, can only occur around hair follicles, which provide necessary Shh signaling. Our study exemplifies how the interplay between epigenetic repression and signaling pathways regulates cell fate specification.

[Supported by NYSTEM IDEA grant #N11G-152]

(32) HETEROGENEOUS EPITHELIAL POPULATIONS CONTAIN STEM CELLS IN THE ADULT ESOPHAGUS

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Basal cells in the adult esophagus are considered as stem/progenitor cells of the stratified epithelium. We previously demonstrated that these basal cells are the cell-of-origin for esophageal squamous cell carcinoma (Liu et al., 2013) and that BMP signaling is critical for the self-renewal and differentiation of basal cells in the adult

mouse and human esophagus (Jiang et al., 2015). However, controversy remains regarding whether basal cells are a homogeneous population. Doupé et al. (2012) used lineage tracing to show that basal cells possess equivalent potential to self-renew and differentiate. By contrast, Croagh et al. (2007), Kalabis et al. (2008) and Aaron et al., (2014) suggested that basal cells are composed of heterogeneous populations and certain subpopulations (e.g. Itga6/Itgb4high) are thought as stem cells. Intriguingly, we recently found that a subpopulation of basal cells express Bone morphogenetic protein (BMP) 4 as detected by the *Bmp4-lacZ* reporter mouse line and in situ. Our preliminary data showed that these BMP4+ve basal cells are at quiescent state. We are now using a novel strategy to perform lineage-tracing, and preliminary data suggested heterogeneous populations exist in the basal cell layer.

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Supported by NYSTEM contract C029555A

(33) ASYNCHRONOUS FATE DECISIONS BY INDIVIDUAL CELLS COLLECTIVELY ENSURE LINEAGE SPECIFICATION IN THE MOUSE BLASTOCYST

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Intercellular communication is essential to coordinate individual behaviors during organismal development. The preimplantation mammalian embryo is a paradigm of tissue self-organization and regulative development; however, the cellular basis of these regulative abilities has not been established. We have undertaken a high-resolution, single-cell analysis of lineage specification in the mouse blastocyst, using a quantitative image analysis pipeline. We show that a consistent ratio of epiblast and primitive endoderm lineages in the blastocyst is achieved through incremental allocation of cells from a common progenitor pool. Timed modulation of the FGF-MAPK pathway showed that individual cells commit to either fate asynchronously, and suggest that a seed of pluripotent epiblast cells may be necessary to trigger differentiation. These

data support the notion that lineage divergence in the blastocyst is a continuum resulting from the coordinated behavior of individual cells and suggest that a tissue size control mechanism operates to ensure the generation of lineages of appropriate size.

[Supported by NYSTEM contract #N13G-236]

(34) SOX17 DRIVES REGERATIVE POTENTIAL IN ENDOTHELIUM CONVERTED FROM NONVASCULAR CELLS

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Transplanting vascular endothelial cells (ECs) to support metabolism and express regenerative paracrine factors is a strategy to treat vasculopathies and to promote tissue regeneration. However, transplantation strategies have been challenging to develop because ECs are difficult to culture and little is known about how to sustain their vascular identity and direct them to form long-lasting new vessels or engraft into existing ones. We found that multiple non-vascular cell types transiently expressed EC markers after enforced expression of the transcription factors, Etv2, Erg, and Fli1. However, only mid-gestational amniotic cells could be converted to cells that maintained EC gene expression and proliferated in culture to yield billions of vascular cells. Even so, these converted cells performed sub-optimally in assays of EC function. To circumvent this, we used constitutive Akt signaling, which, in ECs, mimics the shear forces of the vascular environment and promotes EC survival. Akt signaling enhanced EC function in converted cells and shifted the genomic targeting of Fli1 to favor nearby Sox consensus sites. Enforced expression of Sox17 was dispensable for broad EC gene activation, but indispensable for transplantation that led to vascular engraftment and reperfusion of ischemic tissue. Our results identify a transcription factor network comprised of Ets and Sox17 factors that specifies and sustains endothelial cell fate and function. This work shows that the commonly used criterion of transcriptional similarity for cell conversion can fail to predict in vivo vascular function. Our approach shows that stringent functional testing in vitro and in vivo is necessary to validate engineered endothelial cell grafts.

(35) IN VIVO ANALYSIS OF ADULT SPERMATOGONIAL STEM CELL FATE IN MICE

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Analysis of molecular markers indicates that spermatogonial stem cells in vivo (SSCs) are heterogeneous. To characterize this heterogeneity, we employed comparative lineage tracing of SSC clones labeled by different markers. We traced clonal SSC lineages using mouse strains that express a tamoxifen-inducible (CreERT2), including those from the endogenous Sox2 and Pax7 promoters, which have both been shown previously to mark stem cells in the mammalian testis. We carried out side-by-side comparisons under the same conditions, i.e., same reporter, tamoxifen dose, and pulse-chase periods. Both drivers revealed long- and short-lived stem cell populations. Although Sox2 and Pax7 were previously considered as “bona fide” stem cell markers, only a small fraction of labeled clones survived beyond 6 months. The clonal dynamics described here are different from that of a published Gfra1-CreERT2 driver that not only labeled a large fraction of SSCs but also exhibited a greater number of clones surviving after one year. Interestingly, the Pax7 Cre driver marked not only a rare stem cell population, as previously published, but also more differentiated cells at early time points as well. This suggests that our Pax7 CreERT2 reporter system is highly sensitive, resulting in robust labeling of differentiating progenitors that may be undetectable with weaker reporters. At longer time points, both the Sox2 and Pax7 Cre drivers produced similar numbers of SSC clones and clonal size. In conclusion, our lineage tracing system is useful to characterize and compare SSC clonal dynamics. The molecular differences that underlie the differential longevity of short- and long-lived stem cell populations are under further investigation. Supported by NYSTEM contract # C029156.

(36) MATRIX METALLOPROTEINASE-12 REGULATES THE POSTNATAL DEVELOPMENT OF SUBVENTRICULAR ZONE NEURAL STEM CELL NICHE

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The subventricular zone (SVZ) houses the largest neural stem cell niche in the mammalian brain. In the adult SVZ, apical regions of neural stem cells are encircled by multiciliated ependymal cells, forming a pinwheel-shaped niche. Ependymal cells arise postnatally from neural stem cells, and the movement of ependymal motile cilia directs the flow of cerebrospinal fluid to ensure proper neurogenesis. Ciliary orientation is coordinated by the planar cell polarity of ependymal cells. The apical adult SVZ neural stem cell niche contains a unique extracellular matrix (ECM), which we have recently found to develop concurrently with the formation of niche pinwheels. At birth, this niche ECM is diffuse, but by adulthood becomes highly polarized to discrete regions in niche pinwheel centers, which we have termed “ECM hubs”.
Since a dramatic cellular and ECM remodeling occurs during postnatal SVZ development, we hypothesized that matrix metalloproteinases (MMPs), a family of extracellular endopeptidases that regulate ECM spatial organization in many tissues,

may play a role in niche reorganization. Here we report that MMP-12 expression in particular is highly upregulated during ependymal cell maturation. We find that MMP-12 inhibition and knockdown in neonatal SVZ cultures impedes the maturation of ependymal cells and that MMP-12 knockout in mice leads to an abnormal SVZ niche structure, characterized by aberrant numbers of cells per pinwheel, and reductions in the number and size of ECM hubs. In addition, the establishment of translational planar cell polarity in niche ependymal cells is significantly delayed. Intriguingly, we find that in developing ependymal cells, MMP-12 may regulate the expression of several genes involved in ependymal cell planar cell polarity, possibly due to MMP-12's recently discovered function as a transcription factor. Together, these findings indicate that MMP-12 may regulate the development of both cellular and extracellular structures of the SVZ neural stem cell niche.

(37) DYSTROGLYCAN: A NOVEL NOTCH REGULATOR IN NEURAL STEM CELL NICHE DEVELOPMENT AND FUNCTION

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The extracellular matrix (ECM) is a key regulator of stem cell niche organization and function. One such niche, the subventricular zone (SVZ), is the largest germinal niche in the adult mammalian brain. The SVZ generates much of the cortical plate during embryogenesis, the majority of forebrain glia during postnatal development, and continues to produce neurons and glia throughout adulthood. Despite the SVZ's critical role in brain development as well as local enrichment of ECM in the SVZ, the role that the ECM plays in this niche remains almost entirely unknown. We now report that during the first weeks of postnatal development, VZ/SVZ ECM is reorganized into laminin-rich "hubs" at the interface between neural stem cells and ependymal cells. This process is regulated by the ECM receptor dystroglycan and appears to be important for the establishment of proper niche organization. Either genetic removal of dystroglycan or blockade of dystroglycan binding engenders several defects in neural stem cell function in the perinatal SVZ: (i) dysregulation of the proliferation and differentiation of embryonic neural stem cells, leading to delayed emergence of their progeny, the ependymal cells that serve as niche support cells in the adult SVZ; (ii) perturbed niche organization characterized by alterations in ependymal planar cell polarity and niche cellular organization into pinwheel-like structures; (iii) increased gliogenesis, with increases in both the production and proliferation of oligodendrocyte progenitors, although interestingly, not at the expense of neuroblast production. However, while increased numbers of oligodendrocyte progenitors are generated, myelination by oligodendrocytes appears to be delayed, indicating that dystroglycan loss attenuates oligodendrocyte maturation. Finally, we find that dystroglycan is a novel regulator of notch signaling as its functional effects are due at least in part to suppression of notch signaling in neural stem cells, niche supporting ependymal cells, and, ultimately, in oligodendrocyte precursor cells.

(38) 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. Our group recently reported the establishment of a human pluripotent stem cell-based platform to model pediatric gliomas, and used this platform to model tumors with *PDGFRA* activation, p53 loss, and an $H3^{K27M}$ mutation. 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*, and that both of these mutations drive elevated proliferation in these NPCs. We have also found that expression of activated *FGFR1* increases expression of *LIN28B*, a stem cell-related gene associated with early neuroectodermal cells but not highly expressed in NPCs. 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 will aim to more thoroughly characterize this model *in vitro* and through cell grafting *in vivo*, with the goal of providing novel insights into the biology and potential treatment of these refractory pediatric brain tumors. [Supported by NYSTEM contract # C026879 (K.F.)]

(39) ACTIVE AND INACTIVE ANTIGEN RECEPTOR ENHANCERS CO-OPERATE TO EXERT LOCALIZED AND LONG-RANGE CONTROL OF GENE REGULATION

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Given their essential role in adaptive immunity, antigen receptor (AgR) loci have been the focus of analysis for many years and are among a handful of the most well studied genes in the genome. Their investigation led initially to a detailed knowledge of linear structure and characterization of regulatory elements that confer control of their rearrangement and expression. However, advances in DNA FISH and imaging combined with new molecular approaches that interrogate chromosome conformation have led to a growing appreciation that linear structure is only one aspect of gene regulation and in more recent years the focus has switched to analyzing the impact of 3D nuclear organization on control of V(D)J recombination.
Recombination propels development of B and T lymphocytes. It relies on the presence of proximal enhancers that activate the antigen receptor (AgR) loci in a lineage and stage specific manner. Unexpectedly we find that both active and inactive AgR enhancers co-operate with each other to disseminate their effects in a localized and long-range manner. We demonstrate the importance of short-range contacts between active enhancers that constitute an *Igk* super-enhancer in B cells. Deletion of one element reduces the interaction frequency between other enhancers in the hub, which in turn compromises the transcriptional output of each component. We further establish that in T cells long-range contact and co-operation between the inactive *Igk* enhancer, MiEκ and the active *Tcrb* enhancer, Eβ alters enrichment of CBFβ binding in a manner that impacts *Tcrb* recombination. These findings indicate that localized and long-range enhancer-sharing between active and inactive elements can impact gene regulation in a lineage and stage specific way.

[Supported by NYSTEM contract # C026880]

(40) UNDERSTANDING BETA CELL DYSFUNCTION USING PATIENT INDUCED PLURIPOTENT STEM CELLS

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During the progression of type 1 and type 2 diabetes, a decreasing number of beta cells have to provide the insulin to meet metabolic needs. This imposes a stressful environment on the beta cell. Our question is whether the high demand for insulin contributes to beta cell failure. Loss of function mutation in SUR1 gene induces excessive insulin secretion. These patients are at high risk for developing diabetes later in their life. Derivation and differentiation of induced pluripotent stem cells from the patient with this type of SUR1 mutation serve as a good model to study and understand how excessive insulin production contributes to the beta cell dysfunction. Firstly, we modified the pancreatic differentiation protocol for the efficient generation of functional beta cells from human induced pluripotent stem cells. Secondly, a stem cell line has been derived from the patient with SUR1 mutation, and genetic editing approach Crispr/Cas9 was used to create an isogenic control. The patient cell line

and isogenic control were differentiated into pancreatic beta cells in vitro using newly developed functional protocol.
We were able to obtain around 50% c-peptide positive cells, half of which were monohormonal and co-expressed the mature beta cell transcription factors. In addition, the derived cells showed glucose-regulated c-peptide secretion in vitro and upon transplantation into mice. For the cell line derived from the patient with SUR1 mutation, an isogenic control was generated by correction of mutation via Crispr/Cas9 induced homologous recombination with repaired single strand targeting DNA. The differentiation efficiency towards pancreatic lineage has no significant difference in terms of the percentage of PDX1 and NKX6.1 coexpressing pancreatic progenitors and c-peptide positive beta like cells. We observed an increased secretion of insulin and proinsulin and decreased proinsulin processing in SUR1mutated beta cells. Further characterization of derived beta cells will be performed. This study will add to our understanding how diabetes progresses, and potentially lead to new ways to prevent and treat the disease.

(41) CRITICAL ROLE OF WNT SIGNALING IN FOLLICULAR MELANOCYTE STEM CELLS

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Wnt signal controls stem cell behavior during homeostasis, regeneration and cancer in a variety of tissues in the body. How this signaling pathway impacts stem cells in the melanocytic lineage is not completely understood. Our study reveals that extrinsic Wnt ligands produced by epithelial niche cells are required for the function of melanocyte stem cells (McSCs) that are responsible for hair pigmentation. Upon exposure to Wnt ligands at the initiation of hair regeneration, McSCs give rise to mature melanocytes that produce pigment for the hair. Loss and gain of function of Wnt signaling in McSCs revealed that temporal activation of Wnt signaling promotes McSC differentiation and simultaneously maintains their self-renewing capacity. We also found that McSCs undergo prolonged Wnt activation during aging. This causes the loss of their self-renewing capacity similar to what is seen with constitutive Wnt activation in McSCs. In contrast, once McSCs undergo malignant transformation induced by oncogenic mutations, constitutive Wnt activation promotes their differentiation without diminishing the self-renewing capacity. This results in an aggressive melanoma with a highly differentiated melanocytic phenotype. These results show that the Wnt signaling pathway is critical to fine-tune the balance between self-renewal and differentiation of McSCs, and these regulatory mechanisms are dysregulated during cancerous transformation of McSCs. [Supported by NYSTEM contract #C026880]

(42) DISSECTING MICRORNA FUNCTION IN HESC PLURIPOTENCY AND DIFFERENTIATION USING CRISPR GENOME EDITING

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MicroRNAs regulate gene expression and control a wide variety of physiological processes such as cell division and differentiation. Their maturation is dependent on Dicer1. Previous studies have shown Dicer1 to be essential for mouse development. However, mouse ESCs (mESCs) deficient in Dicer1 can survive in culture, and show decreased proliferation and an inability to differentiate into all three embryonic lineages. It has not yet been determined whether this holds true for human ESCs (hESCs).

To probe the role of microRNAs in hESC maintenance and differentiation, we used CRISPR genome editing to knockout *DICER1* in hESCs for the first time. We targeted a sequence upstream of the catalytic domain of *DICER1* to generate biallelic frameshift mutants. However, after screening 464 clones we were not able to generate any true null DICER1 KOs. Interestingly, though, we were able to generate hESC lines with hypomorphic alleles that expressed DICER1 protein at extremely low levels. Upon analysis of the mRNA sequences, we discovered a rare transcript that effectively restored the reading frame, resulting in a slightly shorter transcript that can still produce a functional protein. Despite lower levels of mature microRNAs, these lines retain pluripotency markers, give rise to teratomas displaying all three embryonic lineages, and are able to differentiate into neuroectoderm. We are currently performing directed differentiation into endoderm, as well as generating embryoid bodies with these mutants. Additionally, to determine whether DICER1 is essential in hESCs, we are developing a rescue strategy to obtain DICER1 homozygous-knockouts.

This is the initial investigation of DICER1 function in hESCs. In addition to providing evidence that DICER1 is unexpectedly essential for survival in hESCs, we have generated DICER1 hypomorphs that can be used as a tool to study microRNA function in hESC pluripotency and/or differentiation.

(43) CARDIOMYOGENIC POTENTIAL OF PLACENTAL CDX2 CELLS

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The placenta serves as a reserve of stem/progenitor cells, which are likely superior to other adult stem cells in terms of stemness and plasticity. We have previously shown that injury to the maternal heart in pregnancy elicits migration of fetal-derived cells and their differentiation into cardiomyocytes and vascular cells. Interestingly, 40% of these cells expressed Cdx2, a trophoblast stem cell marker known solely to

form placenta. We hypothesize that placental Cdx2 could be a novel cell target that may have cardiomyogenic potential. In the current study, we confirmed the expression of Cdx2 in the murine end-gestation placenta and isolated these cells using a transgenic mouse model that labels fetal-derived Cdx2 cells with eGFP. When cultured on neonatal cardiomyocyte feeders, these cells differentiated into spontaneously beating cardiomyocytes and expressed cardiac structural proteins cardiac troponin T and alpha sarcomeric actinin. In addition, cdx2 cells clonally expand and give rise to endothelial (CD31) and smooth muscle (αSMA) cells implying a multipotent nature. We further demonstrated that Cdx2 cells exhibit a unique proteome that confers roles in cell movement, migration, growth and survival compared to the embryonic stem cells. Furthermore, Cdx2 cells displayed a gene signature suggesting an ability to evade host immune surveillance. In summary, we have uncovered a multipotent role for Cdx2 cells from placenta in that they appear to have cardiomyogenic and vasculogenic potential. Their immune signature suggests that Cdx2 cells may be a favorable source for allogeneic cell therapy for cardiac regeneration, endowed with a unique proteome that facilitates homing and survival.
*Supported by NYSTEM contract C029565

(45) THE ROLE OF TET PROTEINS IN HESC PLURIPOTENCY AND DIFFERENTIATION

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Previous studies have suggested a role of TET proteins during early development. However, their significance and mechanism of action during the specification of particular lineages is incompletely understood. Here we have generated hESC lines with inactivating mutations in *TET1*, *TET2* and *TET3* (TKO hESCs). We have characterized the genome-wide 5hmC and 5mC distribution in WT and TKO hESCs and correlate the loss of 5hmC with the gain of 5mC at particular loci in TKO hESCs. Notably TKO hESCs show hypermethylation at the PAX6 promoter region, which impairs activation of gene expression and leads to a strong defect in neuroectoderm differentiation. This hypermethylation correlates with increased binding of DNMT3B at the promoter region. We are currently investigating if knockout of DNMT3B or targeted hydroxymethylation using a dCas9-TET1CD fusion enables us to reverse hypermethylation at the *PAX6* P0 promoter and rescue the TKO differentiation defect. In addition to being the initial investigation of the role of TET proteins in hESCs, our study provides mechanistic evidence for the importance of TET proteins during hESC cell fate specification.

(46) OPTOGENETICS FOR TISSUE ENGINEERING APPLICATIONS

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Optogenetics can be described as light-based actuation through genetic engineering. The classical example is light-gated membrane depolarization through the use of channelrhodopsin-2 (ChR2), a cation channel that opens in response to blue light. Its counterpart is halorhodopsin (eNpHR), a chloride pump that hyperpolarizes the membrane in response to green light. While first used *in vivo* by Deisseroth, the precise spatiotemporal control enabled by optogenetics makes it promising for *in vitro* applications in tissue engineering. With that in mind, we have created induced human pluripotent stem cell (hiPSC) lines that express optogenetic proteins, from which we have created light-activated tissues.

Neuromuscular junctions (NMJ) are the synapses formed between motor neurons and skeletal muscle fibers, and are early targets of several motoneuron diseases. Since repeated visualization/manipulation of human NMJs *in vivo* is not technically possible, *in vitro* models represent the best next solution. Light-activated motoneurons were obtained from our ChR2 hiPSCs lines and cocultured with skeletal muscle cells to induce NMJ formation. Synapse function in our system was probed by measuring myotube contraction in response to motoneuron activation by blue light.

IPS-derived cardiomyocytes expressing ChR2 and eNpHR were generated for the study of arrhythmias. Light is easily patterned onto cardiac tissues through the use of projectors. We created localized conduction blocks of arbitrary spatiotemporal parameters through patterned illumination of halorhodopsin-expressing tissue. Similarly, we are able to dynamically pace cardiac monolayers expressing channelrhodopsin, creating arbitrary wavefronts. This allows us to rapidly and reproducibly prototype novel spatiotemporal control schemes in biological systems.

This in vitro approach to optogenetics adds an intriguing twist to the narrative of tissue engineering and stem cell research. The capabilities demonstrated here are easily adapted to disease models created from patient-derived stem cell lines. These systems hold enormous promise for better understanding clinically relevant diseases in a personalized manner.

(47) ER-POSITIVE AND ER-NEGATIVE LUMINAL LINEAGES ARE INDEPENDENTLY MAINTAINED BY DISTINCT UNIPOTENT STEM/PROGENITOR CELLS IN THE POSTNATAL MAMMARY GLAND

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A clear understanding of mammary epithelial lineage differentiation is important for uncovering the origin of breast cancer cell and for elucidating the mechanism of malignant transformation of these cells. The mammary gland is a branching ductal tree consisting of two primary lineages, inner luminal cells and outer basal cells. Recent reports showed that luminal and basal lineages can be maintained independently by their own respective unipotent stem cell, although bipotent stem cells also exist and contribute to both lineages.
Lack of specific markers for stem/progenitor cells has been a major obstacle for studying the lineage hierarchy of mammary gland. Our previous work showed that the transcriptional factor Sox9 can convert differentiated luminal cells into bipotent mammary stem cells together with Slug. To investigate whether Sox9-expressing cells act as stem/progenitor cells, a tamoxifen-inducible lineage tracing approach in Sox9-CreERT2/ROSA26-tdTomato reporter mice was used. It is found that no matter traced from prepuberty or puberty stage, tdTomato-labeled Sox9-positive cells specifically contributed to the generation of luminal cells over long terms (>25 weeks). Surprisingly, these cells are specifically ER- luminal cells, a subpopulation being implicated in basal-like breast cancer development. Also by genetic lineage tracing, we further showed that in postnatal CD133-CreERT2/ROSA26-tdTomato mice, ER+ cells can be maintained by CD133+ unipotent progenitors even after multiple pregnancies. Finally, the ER- progenitor role of Sox9+ cells and the ER+ progenitor role of CD133+ cells are confirmed through serial transplantation of tdTomto-labeled fragments into the cleared fat pad of NOD-SCID mice. All together, our results support that postnatal mammary gland contains distinct long-lived unipotent luminal stem cells that can respectively develop into ER+ or ER- luminal cells. This reveals unsuspected divergence in luminal lineage development and will have important implications for understanding the origin of different types of breast cancer.
[Supported by NYSTEM contract # C028109 and C029571]

(48) ZFP281 COORDINATES OPPOSITE FUNCTIONS OF TET1 AND TET2 FOR ALTERNATIVE PLURIPOTENT STATES

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Embryonic stem cells (ESCs) provide a powerful system for dissecting the molecules and mechanisms that regulate mammalian cell state. Pluripotency spans a spectrum encompassing two distinct pluripotent states, naive and primed, which are represented by mouse ESCs and epiblast stem cells (EpiSCs), respectively. Conventional human ESCs share defining features of primed pluripotency with mouse EpiSCs, both of which can be reverted to naive pluripotency by an epigenetic reprogramming process involving DNA demethylation that is incompletely understood, although naive hESCs were established with multiple versions among laboratories, possibly reflecting alternative pluripotent states. It is well established that a set of transcription factors centered on the triumvirate of Oct4, Sox2 and Nanog and a number of epigenetic regulators including DNA and histone modification enzymes play significant roles in controlling stem cell pluripotency. What other factors are involved and how they are regulated and targeted to specific genomic loci in transcriptional and epigenetic control of naïve versus primed pluripotency are pressing questions in the field.

Here we define a pluripotent cell fate gene signature associated with naive and primed pluripotency acquisition, and identify Zfp281 as a key transcriptional regulator for primed pluripotency and also as a barrier to achieve the naive pluripotency of both mouse and human ESCs. Mechanistically, we find that Zfp281 interacts with Tet1, but not Tet2, and that Zfp281 and its direct transcriptional target miR-302/367 negatively regulate *Tet2* expression in establishing and maintaining primed pluripotency. Conversely, ectopic Tet2 alone, but not Tet1, efficiently reprograms primed EpiSCs towards naive pluripotency. Our study reveals a previously uncharacterized molecular circuitry encompassing Zfp281, miRNAs, and the opposing functions of Tet1 and Tet2 in controlling alternative pluripotent states. Studying the molecular mechanisms underlying naive/primed pluripotency and their inter-conversion by developmental transition or molecular reprogramming will yield important insights into early development and mammalian cell fate determination. Ultimately, it will facilitate the application of human pluripotent stem cells in disease therapeutics and regenerative medicine. [Partly supported by NYSTEM contract #C028121]

(49) A P53-WNT-NODAL NETWORKN DRIVING MESENDODERM SPECIFICATION

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The p53 family is well known as a mediator of DNA damage response and guardian of the genome. However, their role in embryonic stem cell differentiation and development remains controversial. People are aware of the differentiation effects of p53 on ES and iPS cells, but view these effects as interesting oddities of ES and iPS cells in culture, not as a reflection of the physiologic role of p53. Here, we delineated a regulatory network involving the p53 tumor suppressor family and the Wnt pathway, acting together with the TGF-β pathway, to drive mouse mesendoderm differentiation. Knockout of all three members, p53, p63 and p73, shows that the family is essential for mesendoderm specification as cells exit pluripotency *in vivo* and in culture. *Wnt3* and its receptor *Fzd1* are among a small set of p53 target genes that are specifically activated in this context. Induction of Wnt signaling by p53 is critical for activation of mesendoderm differentiation genes. Globally, we show that Wnt3-activated Tcf3 and nodal-activated Smad2/3 transcription factors depend on each other for co-occupancy of target enhancer elements in master differentiation loci. Our results reveal a selective interdependence between signal-activated Tcf and Smad transcription factors. Thus, the p53 family governs a regulatory network that integrates essential Wnt-Tcf and nodal-Smad inputs for mesendoderm differentiation in the early embryo.

[Supported by NYSTEM contract # C026879]

(50) THE POTENTIAL ROLE OF THE FAR UPSTREAM BINDING PROTEIN 1 IN EMBRYONIC STEM CELLS AND DIFFERENTIATION

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The Far Upstream Element Binding Protein 1 (FUBP1) was identified as a transcriptional regulator of the proto-oncogene *c-myc* and other target genes. Recently it was shown that FUBP1 is highly expressed in several solid tumor entities and it plays an important role in tumor development and progression. The functional knockout of FUBP1 in mice resulted in embryonic lethality and a severe anemic phenotype around embryonal day E15.5. Detailed

analysis of the fetal liver showed significantly reduced numbers of FUBP1-deficient hematopoietic stem cells (HSCs) compared to WT controls. Long-term HSCs lacking FUBP1 activity suffered from a diminished self-renewal capacity *in vivo* as well as a decelerated proliferation and increased cell death in *ex vivo* cell culture due to deregulated FUBP1 target gene expression.
Screening different stem cell types, we found a substantial FUBP1 expression in embryonic stem cells (ESCs). Furthermore, immunohistochemical analysis during embryonic development revealed that FUBP1 is expressed in almost every cell type at day E13.5, especially in liver and brain, hinting at a potential role of FUBP1 in early embryonic development.
To investigate a potential role of Fubp1 in ESC maintenance, we established *Fubp1* knockout ESC clones using the CRISPR/Cas9 system, which do not demonstrate more apoptosis or a change in proliferation. However, analysis of three germ layers during EB formation showed a significant defect or delay in mesoderm marker expression (*Brachyury, Snail, FLK1, Snai2/Slug, Foxa2*). Furthermore, we are using the ESCs in an OP9 co-culture assay to test their differentiation potential towards the proximal lateral mesoderm (FLK1+), the hemangioblasts (FLK1+/VE-Cadherin+) and hematopoietic progenitors (FLK1-/VE-Cadherin-/CD45+/c-kit+). Currently, we are using the *Fubp1* knockout mouse model at day E9.5 for the analysis of the mesoderm marker expression and tissue morphology.
Our aim is elucidate a potential of Fubp1 during the development of the early mesoderm towards the early hematopoietic compartment.

(51) CHIRALITY OF EMBRYONIC STEM CELLS DURING LINEAGE SPECIFICATION

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The development of asymmetries in the left-right axis has been widely studied using embryonic animal models. Recently, examinations of asymmetric bias have been performed *in vitro* at the cellular level through the use of micropatterning technologies, and cellular chiral bias is found to be cell-type dependent. Therefore, the left-right breaking of biological tissue may occur during the stem cell lineage specification in embryo development. In this study, we hypothesized that stem cell differentiation changes cellular chiral bias. To test this hypothesis, the RUES2 cell line was used, and differentiated along several developmental paths, and the chirality of differentiated cells was evaluated with both 2D micropatterning assay and 3D cell rotation assay. Our results indicate that the RUES2 cell line shows no significant preference between clockwise and counterclockwise biases. However, differentiated cells display increasing levels of biases towards either clockwise or counterclockwise direction. RUES2 cells differentiated to a mid/hindgut phenotype display an increasing level of counterclockwise bias while stem cells driven to a cardiomyocyte phenotype display a bias towards clockwise direction. Ongoing work includes determination of associated alterations in cytoskeleton during differentiation, and examination of signaling pathways responsible for these changes. The establishment of a human *in vitro* model for left-right asymmetry allows for more human specific characterization related to left-right axis formation and could also allow for easier evaluation of

compounds which could be potentially disruptive to normal human embryonic development.
(Research is supported by NIH DP2HD083961(PI: Wan). Part of work was performed in the RPI Stem Cell Research Facility, funded by NYSTEM C026717)

(52) OPTIMIZED MODULATION OF SIGNALING PATHWAYS TO ENHANCE HUMAN INDUCED PLURIPOTENT STEM CELL DIFFERENTIATION INTO CARDIOMYOCYTES

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Introduction: Lately, cell replacement using human induced pluripotent stem cells (hiPSCs) is considered a promising therapeutic alternative for cardiovascular diseases. hiPSCs are endowed with the potential to produce a huge number of functional cardiomyocytes from autologous cell sources without the concern of ethical problems or immunological rejection. However, existing protocols for cardiac differentiation of hiPSCs are inefficient limiting their applications. Therefore, the objective of our study was to examine the modulation of signaling pathways to efficiently enhance cardiac differentiation of NP0040 hiPSCs into cardiomyocytes.
Methods: Cardiac differentiation was assessed by temporally modulating the regulatory elements of the signaling pathways: Wnt, BMP-4, FGF-2 and Ascorbic acid in a monolayer-based culture system under serum-free, feeder-free conditions with subsequent purification by “Lactate method”.
Results: Here, we showed that sequential treatment with glycogen synthase kinase-3 (GSK-3) inhibitor, bone morphogenetic protein-4 (BMP-4), fibroblast growth factor-2 (FGF-2) and Ascorbic acid followed by inhibitor of wnt production-2 (IWP-2) and Ascorbic acid produced a high yield of pure (up to 92%) cardiac troponin-T (cTnT)-positive cardiomyocytes that contracted spontaneously as coordinated sheets in multiple (>15) independent experiments. These cardiomyocytes were maintained as spontaneously contracting cells in culture for more than 1 month. The cardiomyocytes exhibited normal cardiac sarcomere organization expressing sarcomeric α-actinin, cTnT, MLC2v and the gap junction protein, Connexin 43. Reverse-transcriptase PCR revealed the expression of Isl-1, NKx2.5, Tbx5, Tbx20, GATA-4, Mef2c, Hand1, MYH6, MYL2, MYL7, TNNT2 and TNNI3). Field Potential recording indicated that cardiomyocytes were electrically coupled to one another. The characteristics of the recorded action potential showed mainly ventricular-like waveforms.
Conclusion: Our data indicate that induction of mesoderm and its subsequent specification into cardiac fate from hiPSCs requires well-orchestrated interaction between Wnt, BMP-4, FGF/MEK and MEK/ERK signaling pathways. Controlling these pathways allows efficient production of cardiomyocytes that may provide a possible source for cell-based therapy.

Acknowledgements: German Academic Exchange Service (DAAD) scholarship 2015 (GERSS).
Keywords: Human induced pluripotent stem cells, Cardiomyocytes, Wnt, BMP-4, FGF-2, Ascorbic acid.

(53) THE ROLE OF BEND6 IN REGULATING NEURAL STEM CELLS SPECIFICATION AND DIFFERENTIATION

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Our previous study showed that Bend6 physically interacts with CBF1, the mammalian Notch downstream CSL-class transcription factor, and antagonizes Notch-dependent activation. Ectopic overexpression of Bend6 inhibited neural stem cells proliferation. Reciprocally, knockdown of BEND6 attenuates neurogenesis. Moreover, the depletion of BEND6 counteracts with the consequences of Notch signaling inhibition and enhances the Notch activation. Together, previous work revealed a novel mammalian CSL co-repressor in the nervous system (Qi *et al.*, 2013).
The BEN domain has been recently recognized as a DNA binding domain. It is still unknown whether BEND6 could directly target to specific DNA sequence or function as a transcription factor. In this study, we used protein binding microarray (PBM) and found BEND6 preferentially binds YCCACCCYY site, which is distinct to any previously identified BEN motif. We then performed ChIP-seq for BEND6 and CBF1 using embryonic mouse brains and demonstrated that BEND6 binds to Notch targeting enhancers throughout genome. We will investigate whether the BEND6 PBM motif is enriched in BEND6 chromosomal binding religions. We will further test Bend6 transcriptional activity with reporter assay. We next extended our previous shRNA based study to *in vivo* analysis. We have made BEND6 mutant mouse with CRISPR technique. We will use this mouse to investigate the change of global gene expression after BEND6 knockout using RNA-seq. By combining ChIP-seq and RNA-seq data, we will further understand the role of BEND6 as a transcription factor during neurogenesis. We will also cross BEND6 mutant mouse with another Notch pathway co-factor, MINT, conditional knockout mouse line, we will characterize the BEND6 knockout phenotype with and without MINT knockout background, which will help us to reveal how BEND6 is integrated to Notch pathway and regulates neural development.

[supported by NYSTEM contract #C026879]

(54) A NOVEL STATE OF PLURIPOTENCY - METABOLICALLY NAÏVE HUMAN EMBRYONIC STEM CELLS

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Traditionally, two states of pluripotency are described: naïve and primed. They are defined by distinct transcriptional, epigenetic, metabolic features and growth factor-responsiveness. Mouse pluripotent stem cells (PSC) are in a naïve or ground state, while human PSC display primed features. Numerous efforts were directed at attaining human naive pluripotency, believed to harness higher developmental potential. These studies mainly employed ectopic factor expression or manipulation of cytokine environment, resulting in “alternative pluripotent states” with different combinations of naïve and primed elements. These findings suggest that pluripotency may be a metastable cellular state that exists in multiple, rather than two, distinct entities.

Here we present a novel form of human pluripotency found in cells cultured in the minimalistic E(ssential)8™ medium. E8-grown hPSC display a unique set of naïve and primed traits. While cytokine requirements are consistent with primed cells, E8 cells show dramatically altered and naïve-like mitochondrial morphology and activity. Epigenetic and transcriptional profiles have more naïve-like characteristics and finally, E8-hPSC show strong neurectodermal differentiation propensity.

Cellular metabolism is known to drive epigenetic and transcriptional changes, a phenomenon best studied in cancer but newly described also for PSC. We hypothesize that differential energy production brought about by medium composition underlies the novel pluripotency of E8-cultured cells. In support this, we have identified a specific metabolite known to regulate epigenetic factors that is at least in part responsible for the altered differentiation potential of E8-hPSC. Importantly, this transition occurs under conventional and primed state-promoting growth-factor conditions and without ectopic gene expression.

Our findings indicate that nutrient composition of the culture medium can modify the pluripotent state and profoundly impact downstream differentiation.

This mechanism could be harnessed to design tailored media for various differentiation routes and help eliminating basal lineage biases of hPSC lines. Such standardization will be crucial for the use of allogeneic cell banks for stem cell therapy.

The work was supported by NYSTEM contract # C026879

(55) GLUTAMINE INDEPENDENT SURVIVAL IS A HALLMARK OF CELLULAR STEMNESS

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The overwhelming majority of mammalian cells are exquisitely dependent on glutamine for cellular viability and proliferation, despite the fact that glutamine is a non-essential amino acid. This holds true for the majority of cancer cells as well as embryonic stem cells grown *in vitro* in media containing serum and leukemia inhibitory factor (Serum/LIF). Recently, culturing embryonic stem cells in media containing inhibitors of GSK3b and MEK1 (2i/LIF) was found to enable growth in the absence of exogenous glutamine due to a rewiring of cellular metabolism promoting increased glucose catabolism and less dependence of glutamine for anapleurosis. Whether this alteration in cellular metabolism required inhibition of GSK3b and MEK1, or rather is an intrinsic feature of cellular "stemness" remains unknown. Furthermore, whether a "stem-like" phenotype due to upregulation of pluripotency transcription factors, seen in many cancers, promotes glutamine independent survival has not been characterized.

In this work, we demonstrate that transient glutamine deprivation selects for the survival of embryonic stem cells (ESCs) most strongly expressing pluripotency transcription factors, such as Oct4 and Nanog. ESCs that survive in the absence of glutamine show enhanced self-renewal. Ectopic overexpression of Nanog, but not Klf2, promotes glutamine independent survival. Both ESCs surviving transient glutamine deprivation and overexpressing Nanog are less dependent on glutamine for TCA cycle anapleurosis. Finally, overexpression of Nanog in cancer cell lines promotes a similar metabolic shift, enabling glutamine independent survival. This work reveals that rewiring of cellular metabolism to support glutamine-independent survival is a hallmark of cellular stemness, and acquisition of stemness can enable cancer cell survival under conditions of nutrient deprivation. In future work, we plan to explore whether transient glutamine withdrawal promotes inducible pluripotent stem cell generation. [Supported by NYSTEM contract # #C026879]

(57) STEM CELLS ACROSS THE CURRICULUM: MODULES THAT INTEGRATE BIOLOGY AND SOCIAL JUSTICE LEARNING USING VISUAL NARRATIVES & CASE-BASED TEACHING

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STEM education must integrate the biological and social dimensions of emerging technologies if we intend to promote scientific innovation and socially just practices

and policies. *Stem Cells Across the Curriculum (SCAC)* is a modular collection of educational resources designed to achieve this learning goal. Our project was informed by common student misconceptions about the temporal and spatial relationships of scientific experimentation involved in stem cell research (SCR) including intracellular v. extracellular interactions and location of experimentation (animal, human, cell culture, industry, research, etc.). Additionally, we sought to broaden students' views of SCR using a social justice framework to highlight the emergence of a bioeconomy, differential funding and regulation in the public and private sectors, and differential access to stem cells and therapies. Early renditions of these learning tools were used in seminar courses and a lecture course serving liberal arts and design students. Directed content analysis of exams revealed that students were able to identify multiple ethical issues related to SCR including those that address issues of inequity. Additionally, to varying degrees, students applied case-based analysis to categorize stem cell types, describe experimental manipulations, propose next steps, and comment on the ethical and legal issues of translational research. Qualitative data suggest that the case studies were effective in promoting empathetic thinking and ethical reasoning. These data were used to refine the SCAC resources (animated slide sets, timelines, and information designs, case teaching notes). As an example, a downloadable interactive pdf depicting the provenance, access, and use of various stem cell types will be on display. We present this open access online curriculum for others to adopt and adapt, and reflect on the use of infographic thinking in the design of stem cell related curricula to improve science education in a variety of courses across the curriculum. [Supported by NYSTEM Contract #C026077]

(59) BIOMIMETIC SELF-ASSEMBLED SCAFFOLDS ENHANCE MUSCLE STEM CELL TRANSPLANTATION

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Muscle stem cells (also known as satellite cells) are a potent population of myogenic progenitors dedicated to efficacious skeletal muscle regeneration, but their therapeutic utility is currently limited by mode of delivery. In particular, MuSCs display inefficient survival, self-renewal, and differentiation after injection into muscle tissue. To improve the contributions of MuSCs post-transplant, we developed biomimetic scaffolds based on synthetic peptide amphiphiles (PAs) that assemble to encapsulate cells and growth factors within a muscle-like unidirectionally-ordered environment of long and aligned nanofibers. The stiffness of the PA scaffolds,

controlled through amino acid sequence, was found to determine the macroscopic degree of cell alignment templated by the nanofibers. Furthermore, these PA scaffolds support myogenic progenitor cell survival and proliferation *in vitro,* and they can be optimized, by tuning their stiffness, to induce progenitor differentiation and maturation. We engineered an *in vivo* delivery system to assemble the scaffolds immediately upon injection of a fluid MuSC-PA solution into damaged muscles of mice. The injected scaffold self-assembled around MuSCs and yielded co-alignment of scaffold nanofibers with endogenous myofibers to pattern muscle progenitor differentiation efficiently. These scaffolds displayed characteristic degradation rates *in vivo* matching the time course of tissue regeneration. This biomimetic encapsulation approach demonstrated profound improvements in the engraftment of MuSCs after transplantation, resulting in enhanced muscle repair outcomes. This work establishes a new stem cell encapsulation technology, which self-assembles to aid muscle cell therapies. Importantly, this scaffold system self-assembles without the need for *in vivo* polymerization and has emergent nanofiber alignment to template muscle cell maturation.

(F1) SHARED RESOURCE: LARGE-SCALE BIOCHEMICAL PROFILING FOR STEM CELL RESEARCH IN NEW YORK

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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. Recent work using data-independent acquisition on the Orbitrap Q Exactive HF platform and Spectronaut software from Biognosys AG has achieved some improved results in quantitative protein profiling multiplying the impact of this technology. This facility should have an important impact on stem cell research, and will serve as an important addition to the statewide stem cell infrastructure.

This work was supported by NYSTEM contract #C029159.

(F2) THE STEM CELL PATHOLOGY UNIT AT CORNELL UNIVERSITY

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The Stem Cell Pathology Unit supports internal and external researchers with project planning assistance, animal phenotyping, and digital slide scanning and analysis. The unit is a part of Cornell's Stem Cell Modeling and Phenotyping Core, which assures a tight 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 species and include evaluation of stem cell niches. Stem cells and their niches are crucial for normal life functions, and are frequently affected during pathological conditions. Importantly, stem cell defects are frequent causes of some diseases, such as cancer. 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. The spectrum of services by the Stem Cell Pathology Unit ranges from slide consultation to complete necropsy and histologic examination of experimental and control animals. All phenotyping services are performed by board-certified veterinary anatomic pathologists. Digital slide preparation for both bright field and fluorescent samples is performed with

Leica/Aperio ScanScopes and includes viewing software, image analysis algorithms and one month of image storage. Glass slides are scanned at giga-pixel resolutions and the resulting digital slides allow for highly efficient evaluation of histologic and cytologic materials without the use of a microscope. The Stem Cell Pathology Unit continues to evolve to meet the needs of the stem cell research community both within and outside of the Cornell University. (Supported by NYSTEM contract # C024174).

(F4) HIGH THROUGHPUT BIOLOGY CORE AT NYULMC: AN OPEN ACCESS, AUTOMATION, IMAGING, AND ANALYSIS RESOURCE FOR STEM CELL BIOLOGY

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High throughput and high content screening technologies represent a valuable resource for understanding the biology and regulation of stem cells. The High Throughput Biology Core (HTB Core) at NYU Langone Medical Center (formerly the NYU RNAi Core) is a state-of-the-art, high throughput/high content screening platform that supports projects related to stem cell biology, including functional genomic investigation of the molecular/genetic regulation of embryonic stem cells (ESCs), induced pluripotent cells (iPSCs), and adult stem cells.

The HTB Core offers a diverse array of services ranging from cell-based assay development in high density microplates, screening of siRNA/miRNA/synthetic CRISPR reagents, automation of routine assays, to the development of novel imaging and analysis protocols, with the goal of increasing throughput, overall data quality and reproducibility. The Core includes space, instrumentation, and software for a streamlined workflow from optimization to data analysis and visualization for both plate reader and high content imaging assays. Available screening libraries include human and mouse whole genome (with custom updated RefSeq targets) siRNA, and miRNA mimic and inhibitors. Completely unique to the HTB Core are fully customizable RNAi subsets, based on the investigator's favorite genes of interest, using an automated high density -80C freezer.

A summary of stem cell related projects along with the development of new protocols will be presented together with our best practices for assay development and optimization. Examples of data analysis and visualization workflows used to archive and assess results and calculate plate, well, and cell-based metrics will also be described.

The major goal of the HTB Core is to support stem cell research to all New York State investigators through open access, standard operating protocols and efficient workflows for stem cell projects.

[Supported by NYSTEM contract #C026719]

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PROGRAM AT A GLANCE

TUESDAY, MAY 10, 2016

8:00 – 9:00	REGISTRATION & POSTER I SETUP
9:00 – 9:15	OPENING REMARKS
9:15 – 10:45	PLENARY I: CONTROLLING CELL FATE
10:15 – 10:45	BREAK
10:45 – 12:15	Plenary II: BIOENGINEERING IN STEM CELL BIOLOGY
12:15 – 1:15	LUNCH and POSTER VIEWING
1:15 – 2:00	PLENARY III: STEM CELL BIOLOGY
2:00 – 3:30	IMMUNOTHERAPIES
3:30 – 4:00	BREAK
4:00 – 5:30	PLENARY IV: MODELING DISEASES
5:30 – 7:00	POSTER SESSION I & RECEPTION

WEDNESDAY, MAY 11, 2016

8:30 – 9:15	CONTINENTAL BREAKFAST & POSTER II SETUP
9:15 – 11:00	PLENARY V: MESODERMAL LINEAGES
11:00 – 12:30	POSTER SESSION II and LUNCH
12:30 – 1:30	KEYNOTE
1:30 – 1:45	POSTER AWARDS & CLOSING REMARKS
1:45	ADJOURN