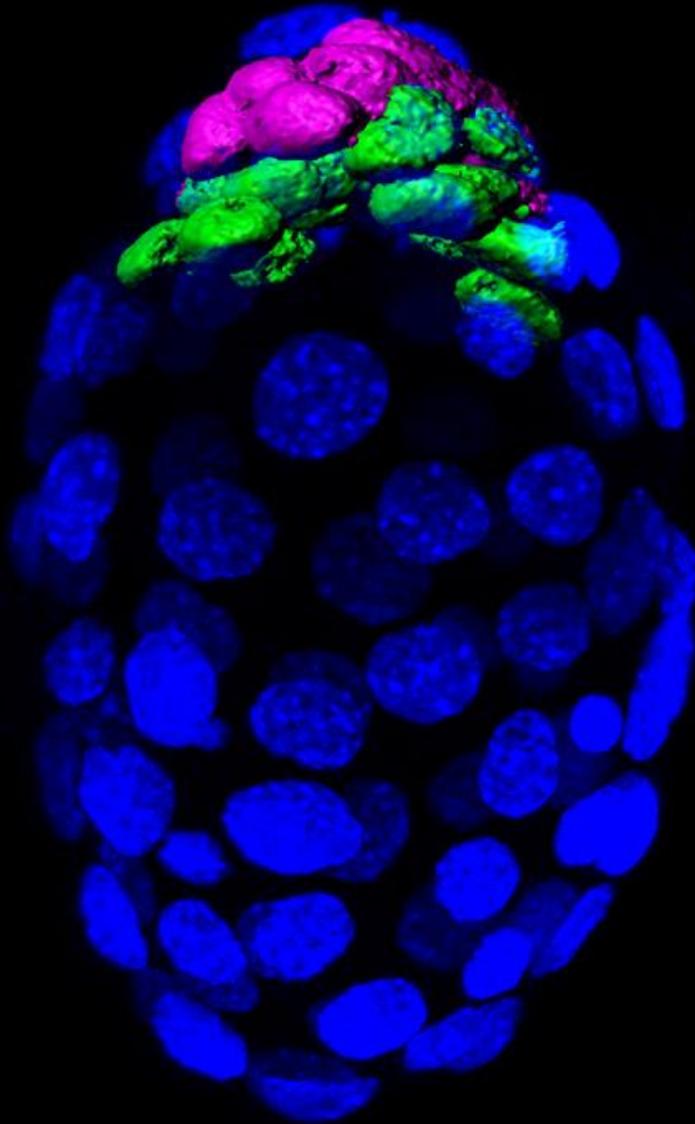


# NYSTEM 2015



N. Saiz, K. Hadjantonakis, Sloan Kettering Institute

## May 14 & 15, 2015

The Rockefeller University  
1230 York Avenue, New York, NY



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Cover photo: **Visualizing Cell Types in the Early Mouse Embryo**  
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## GENERAL

### **INFORMATION Sessions**

All sessions will take place in the Carson Family Auditorium.

### **Meals**

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

### **Posters**

Odd numbered posters should be displayed throughout the day Thursday, presented during the evening reception, and taken down immediately following the reception. Even numbered posters should be displayed throughout the day Friday and presented at the Friday Poster Session, 11-12 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

### THURSDAY, MAY 14, 2015

- 1:30 – 2:00      **REGISTRATION and POSTER I SETUP**
- 2:00 – 2:05      **OPENING REMARKS**
- 2:05 – 4:05      **PLENARY I: NEUROBIOLOGY AND CANCER**  
Chair: Paul Frenette, *Albert Einstein College of Medicine*
- 2:05– 2:35      Grigori Enikolopov, *Cold Spring Harbor Laboratory*  
Modes of Division and Differentiation of Adult Neural Stem Cells
- 2:35 – 2:50      Bastian Zimmer, *Memorial Sloan Kettering Cancer Center*  
Human Pluripotent Stem Cell Based Cell Therapy for Hypopituitarism
- 2:50 – 3:05      Liheng Wang, *Columbia University*  
Understanding the Neurophysiology of Obesity Caused by PC1/3 Deficiency with hESC-Derived Hypothalamic Neurons
- 3:05 – 3:20      N. Sumru Bayin, *New York University School of Medicine*  
Defining Glioblastoma Stem Cell Heterogeneity
- 3:20 – 3:50      Viviane Tabar, *Memorial Sloan Kettering Cancer Center*  
Human ES Cells as a Platform for Modeling Cancer
- 3:50 – 4:05      Xiling Shen, *Cornell University*  
Modeling Human Colon Cancer in Immunocompetent Mice Using Chemokine-Targeted Cancer Stem Cell
- 4:05 – 4:30      **BREAK**
- 4:30 – 6:00      **NYSTEM CONSORTIA II**  
Moderator: **Mahendra Rao**, *NYSCF*
- 4:30 – 4:50      Ronald Hoffman, *Icahn School of Medicine at Mount Sinai*  
Commercialization of Valproic Acid Expanded Cord Blood Stem Cells as Allogeneic Grafts for Adults with Refractory Hematological Malignancies
- 4:50 – 5:10      Kunle Odunsi, *Roswell Park Cancer Institute*  
Programming Hematopoietic Stem Cells for Long-Term Targeted T Cell Therapy of Patients with Relapsed Ovarian Cancer

#### 4 | PROGRAM

- 5:10 – 5:30 Shahin Rafii and Joseph Scandura, *Weill Cornell Medical College*  
Vascular Niche Platform to Expand Hematopoietic Stem and Progenitor Cells Engineered to Cure Sickle Cell Disease
- 5:30 – 6:00 **MODERATED Q&A**
- 6:00 – 7:30 **POSTER SESSION I AND RECEPTION**

#### **FRIDAY, MAY 15, 2015**

- 8:30 AM **REGISTRATION, BREAKFAST & POSTER II SET-UP**
- 9:00 – 11:00 **PLENARY II: LINEAGES**  
Chair: **Chris Fasano**, *Neural Stem Cell Institute*
- 9:00 – 9:30 Songhai Shi, *Memorial Sloan Kettering Cancer Center*  
Deterministic Behavior of Neural Progenitors in the Mammalian Neocortex
- 9:30 – 9:45 Valerie Gouon-Evans, *Icahn School of Medicine at Mount Sinai*  
Identification of an Endoderm-Derived VEGFR+ Progenitor with a Bi-Potent Hepatic and Endothelial Fate
- 9:45 – 10:00 Néstor Saiz, *Memorial Sloan Kettering Cancer Center*  
Asynchronous Single-Cell Fate Decision Events Drive Lineage Commitment in the Mouse Blastocyst
- 10:00 – 10:15 Michael Rendl, *Icahn School of Medicine at Mount Sinai*  
Stem Cell Niche Control of Hair Regeneration
- 10:15 – 10:30 Takamitsu Maruyama, *University of Rochester*  
Signaling Crosstalk in Skeletal Cell Fate Determination and Differentiation
- 10:30 – 11:00 Todd Evans, *Weill Cornell Medical College*  
Generation of Clinically Relevant but Rare Cell Types from ESCs
- 11:00 – 12:00 **POSTER SESSION II**
- 12:00 – 12:30 **STEMCELL TECHNOLOGIES PRESENTATION:** Introduction to Intestinal Organoid Culture by Ryan Conder
- 12:00 – 1:00 **LUNCH**
- 1:00 – 1:20 **PROGRAM UPDATES, POSTER WINNERS ANNOUNCED**

- 1:20 – 2:20      **KEYNOTE ADDRESS – HANS CLEVERS**  
Wnt Signaling, Lgr5, Stem Cells and Cancer
- 2:20 – 2:50      **BREAK**
- 2:50 – 4:20      **PLENARY III: POTENCY**  
Chair: **Alexander Nikitin**, *Cornell University*
- 2:50 – 3:20      Craig Thompson, *Memorial Sloan Kettering Cancer Center*  
Metabolic Insights Into Stem Cell Pluripotency
- 3:20 – 3:35      Jianlong Wang, *Icahn School of Medicine at Mount Sinai*  
Tex10 Coordinates Epigenetic Control of Super-Enhancer Activity  
for Pluripotency and Reprogramming
- 3:35 – 3:50      Marco Seandel, *Weill Cornell Medical College*  
Dynamic Control of FGFR-Mediated Self-Renewal Signaling  
Mediates Positive Selection of Mutant Spermatogonial Stem Cells  
with Advanced Paternal Age
- 3:50 – 4:20      Emily Bernstein, *Icahn School of Medicine at Mount Sinai*  
Epigenetic regulation of breast tumor initiating cells
- 4:20 – 4:30      **CLOSING REMARKS**
- 4:30                **ADJOURN**



**Keynote Address by Hans Clevers, M.D., Ph.D.**  
 Professor of Medical Genetics  
 Hubrecht Institute  
 Utrecht, the Netherlands

**Wnt Signaling, Lgr5, Stem Cells and Cancer**

The intestinal epithelium is the most rapidly self-renewing tissue in adult mammals. We originally defined *Lgr5* as a Wnt target gene, transcribed in colon cancer cells. Two knock-in alleles revealed exclusive expression of *Lgr5* in cycling, columnar cells at the crypt base. Using lineage tracing experiments in adult mice, we found that these *Lgr5*<sup>ve</sup> crypt base columnar cells (CBC) generated all epithelial lineages throughout life, implying that they represent the stem cell of the small intestine and colon. *Lgr5* was subsequently found to represent an exquisitely specific and almost 'generic' marker for stem cells, including in hair follicles, kidney, liver, mammary gland, inner ear tongue and stomach epithelium. Single sorted *Lgr5*<sup>ve</sup> stem cells can initiate ever-expanding crypt-villus organoids, or so called 'mini-guts' in 3D culture. The technology is based on the observation that *Lgr5* is the receptor for a potent stem cell growth factor, R-spondin. Similar 3D cultures systems have been developed for the *Lgr5*<sup>ve</sup> stem cells of stomach, liver, pancreas and kidney. Using CRISPR/Cas9 technology, the CFTR locus has been corrected in intestinal organoids of cystic fibrosis patients.



Hans Clevers received his M.D. in 1984 and his Ph.D. in 1985 from Utrecht University in the Netherlands. He was a postdoctoral fellow from 1986 to 1989 with Cox Terhorst at the Dana-Farber Cancer Institute of Harvard University.

Since 1991 Dr. Clevers has been a Professor at Utrecht University, first in Immunology and, since 2002, in Molecular Genetics. From 2002 to 2012 he served as Director of the Hubrecht Institute in Utrecht. Since 2012, he has been President of the Royal Netherlands Academy of Arts and Sciences. Dr. Clevers has been a member of the Royal Netherlands Academy of Arts and Sciences since 2000, a member of the American Academy of Arts and Sciences since 2012, and a member of the National Academy of Sciences since 2014.

Dr. Clevers is the recipient of numerous awards and honors, including the Dutch Spinoza Award in 2001, the Swiss Louis Jeantet Prize in 2004, the Memorial Sloan-Kettering Katharine Berkan Judd Award in 2005, the Israeli Rabbi Shai Shacknai Memorial Prize in 2006, the Dutch Josephine Nefkens Prize for Cancer Research in 2008, the German Meyenburg Cancer Research Award in 2008, the Dutch Cancer Society Award in 2009, the United European Gastroenterology Federation Research Prize in 2010, the German Ernst Jung-Preis für Medizin in 2011, the Association pour la Recherche sur le Cancer Léopold Griffuel Prize in 2012, the Heineken Prize in 2012, and the Breakthrough Prize in Life Sciences in 2013. In 2008 he received a European Research Council (ERC) Advanced Investigator grant. In 2005 he was named a Chevalier de la Legion d'Honneur and in 2012 was conferred knighthip in the Order of the Netherlands Lion.

## **MODES OF DIVISION AND DIFFERENTIATION OF ADULT NEURAL STEM CELLS**

Grigori Enikolopov

Cold Spring Harbor Laboratory

Dynamic regulation of adult neurogenesis underlies cognitive function, response to therapies, and brain repair. Continuous decline in neuronal production may limit plasticity and repair of the nervous system and underlie age-related cognitive deficits. Many agents, including widely used drugs and treatments, can modulate neurogenesis and may potentially ameliorate age-related cognitive impairment. By developing novel approaches to investigate division and signaling in neural stem cells we have arrived at a new model of stem cell maintenance and division in the adult hippocampus. This model implies that the disappearance of neural stem cells is a direct consequence of their production new neurons and that the decline of the stem cell pool may be the price paid for the ability to produce new neurons as adults. The model also implies that various modes of division and differentiation of stem and progenitor cells may have profound consequences for the cognitive function and the long-term effects of drugs and therapies. We will discuss the therapeutic implication of our findings, the role of the stem cell niche, and the prospects of preserving or restoring the pool of stem cells in the adult and aging brain.

## HUMAN PLURIPOTENT STEM CELL BASED CELL THERAPY FOR HYPOPITUITARISM

Bastian Zimmer<sup>1,2</sup>, Jinghua Piao<sup>2,3</sup>, Jason Tchieu<sup>1,2</sup>, Kiran Ramnarine<sup>1,2</sup>, Mark Tomishima<sup>1,2</sup>, Viviane Tabar<sup>2,3</sup> and Lorenz Studer<sup>1,2,3</sup>

<sup>1</sup>Developmental Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY, 10065, USA

<sup>2</sup>Center for Stem Cell Biology, Memorial Sloan Kettering Cancer Center, New York, NY, 10065, USA

<sup>3</sup>Department of Neurosurgery Memorial Sloan Kettering Cancer Center, New York, NY, 10065, USA

Human pluripotent stem cells (hPSCs) are currently used in many different applications ranging from basic developmental biology to disease modeling, drug discovery toxicological risk assessment and regenerative medicine. In the present study we use hPSCs-derived pituitary cells to treat a rat model of hypopituitarism.

By generating a TALEN-based knock-in reporter line for the pan-placodal marker SIX1 we were able to transfer our recently published protocol to generate cranial placode from hPSCs<sup>1</sup> into a fully defined, xeno-free cGMP-ready culture and differentiation environment. By recapitulating key signaling pathways known from *in vivo* pituitary development we generated human pituitary cells *in vitro*. The cells were characterized using whole transcriptome RNASeq, single-cell RT-qPCR as well as standard RT-qPCR and immunohistochemistry. Adding patterning morphogens to our “default” differentiation changed the phenotype of the cells in our culture system from dorsal pituitary cell types to more ventral pituitary cell types.

Functionality of the cells was confirmed *in vivo* by measuring release of key anterior pituitary hormones such as ACTH and GH. We showed that our cells are responsive to external stimuli such as somatocrinin and nafarelin. Since hormone release from the pituitary gland is tightly regulated by positive and negative feedback mechanisms, a functional response to external stimuli is crucial for a cell replacement therapy in the future. By transplanting the cells into a rat model of hypopituitarism, in which the entire pituitary gland was removed surgically, we demonstrate that our cells survive and restore hormone levels in the animal.

We therefore believe that our cell replacement strategy could provide a better and more natural hormone therapy for e.g. cancer survivors (radiation therapy) or congenital hypopituitarism (e.g. Prader–Willi syndrome) than the current static “external” delivery of hormones.

[Supported by NYSTEM contract C026879]

<sup>1</sup>Zehra Dincer, Jinghua Piao, Lei Niu, Yosif Ganat, Sonja Kriks, Bastian Zimmer, Song-Hai Shi, Viviane Tabar, Lorenz Studer, Specification of Functional Cranial Placode Derivatives from Human Pluripotent Stem Cells, Cell Reports, Volume 5, Issue 5, 12 December 2013, Pages 1387-1402

## **UNDERSTANDING THE NEUROPHYSIOLOGY OF OBESITY CAUSED BY PC1/3 DEFICIENCY WITH HESC-DERIVED HYPOTHALAMIC NEURONS**

Liheng Wang<sup>1</sup>, Kana Meece<sup>2</sup>, Claudia A. Doege<sup>1</sup>, Sharon L. Wardlaw<sup>2</sup>, Dieter Egli<sup>1</sup>, Rudolph L. Leibel<sup>1</sup>

<sup>1</sup>Departments of Pediatrics and the Naomi Berrie Diabetes Center, Columbia University, New York, NY, USA and <sup>2</sup>Department of Medicine and the Naomi Berrie Diabetes Center, Columbia University, New York, NY, USA.

Hypomorphism for prohormone convertase 1/3 (PC1/3) results in a complex endocrine-metabolic phenotype that includes obesity, malabsorptive diarrhea and systemic endocrinopathies. To understand the molecular neurophysiology of the obesity in PC1/3 deficient subjects, we generated PC1/3 deficient hESC lines with CRISPR or by knocking down PC1/3 with shRNA. These cells were differentiated into hypothalamic arcuate nucleus (ARC)-like neurons using a protocol recently developed by us (Wang et al. JCI 2015). The 90% reduction of proprotein convertase subtilisin/kexin type 1 (PCSK1) mRNA and the absence of mature PC1/3 protein in these neurons confirmed the PC1/3 deficiency. PC1/3 plays a critical role in processing neuropeptides and prohormones such as proopiomelanocortin (POMC), proinsulin, proglucagon in the brain, islets, intestine and elsewhere. POMC neurons in the ARC are the source of  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$ MSH) which acts on paraventricular nucleus (PVN) neurons to suppress food intake, as well as endorphins such as  $\beta$  endorphin (BEP) that also affect ingestive behaviors. The percentage of POMC neurons in terminally-differentiated cells was not different between control and PC1/3-deficient lines, suggesting that lacking of PC1/3 did not affect neuronal differentiation. However, the ratios of  $\alpha$ MSH/POMC and BEP/POMC proteins were decreased by 67% to 75% in PC1/3 deficient hESC-derived neurons, indicating that POMC processing was impaired by the imposed PC1/3 insufficiency. Levels of mRNA expression of POMC, NHLH2 and CPE, as well as total amounts of unprocessed POMC neuropeptide, were increased 1.7 to 7 fold in the PC1/3 deficient neurons. These findings provide mechanistic insight into the molecular and functional consequences of hypothalamic PC1/3 deficiency, and demonstrate the potential of stem cell-derived hypothalamic-like neurons for investigation of the neurophysiology and pharmacology of body weight regulation.

[Supported by NYSTEM contract C029552]

**DEFINING GLIOBLASTOMA STEM CELL HETEROGENEITY**

N. Sumru Bayin<sup>1,5</sup>, Sheng Si<sup>1</sup>, Rajeev Sen<sup>1</sup>, Aram S. Modrek<sup>1</sup>, Valerio Ortenzi<sup>2</sup>, David Zagzag<sup>2</sup>, Igor Dolgalev<sup>3</sup>, Adriana Heguy<sup>3</sup>, Dimitris G. Placantonakis<sup>1,4,5</sup>

Departments of <sup>1</sup>Neurosurgery, <sup>2</sup>Pathology and, <sup>3</sup>Genome Technology Center, <sup>4</sup>Brain Tumor Center, <sup>5</sup>The Helen L. and Martin S. Kimmel Center for Stem Cell Biology, NYU School of Medicine

Glioblastoma Multiforme (GBM) is a deadly brain malignancy with extensive cellular heterogeneity. Glioblastoma stem cells (GSCs) self-renew, differentiate into tumor lineages and are resistant to current therapies.

Using human GBM biospecimens engineered to express GFP upon activation of Notch signaling, we observed only partial overlap between cells expressing cell surface CD133, a well-characterized GSC marker, and cells with activated Notch signaling, contrary to expectations based on prior literature. To further investigate heterogeneity within the GSC population, we isolated distinct GSC populations and characterized them.

We found that CD133+ (CD133+/Notch-) and Notch+ (CD133-/Notch+) GSCs differ vastly in their transcriptome, metabolic preferences and differentiation capacity, thus giving rise to histologically different tumors. CD133+ GSCs have increased expression of hypoxia-regulated and glycolytic genes, and are able to expand under hypoxic conditions by activating anaerobic glycolysis. In contrast, Notch+ GSCs are unable to activate glycolysis under hypoxic conditions, leading to decreased tumorsphere formation ability. Furthermore, CD133+ GSCs give rise to histologically homogenous hypoxic tumors devoid of large tumor vessels. In contrast, tumors initiated by Notch+ GSCs are marked by large perfusing vessels enveloped by pericytes. Using a lineage tracing system, which allows tracking of the progeny of Notch+ GSCs, we showed that pericytes are derived from Notch+ GSCs. In addition, Notch+ cells are able to give rise to all tumor lineages, including CD133+/Notch-cells, as opposed to Notch- populations *in vitro* and *in vivo*, which have restricted differentiation capacity and do not generate Notch+ lineages. This finding suggests that Notch+ GSCs lie at the apex of GBM's cellular hierarchy and are more multipotent than CD133+ GSCs.

Overall, our results suggest that GBM's cellular heterogeneity of GBM manifests in its stem cell population. Discreet GSC subtypes are able to support tumor growth either by surviving hypoxic conditions or supporting tumor angiogenesis by differentiating into pericytes.

[Supported by NYSTEM contract C026880]

## **HUMAN ES CELLS AS A PLATFORM FOR MODELING CANCER**

Viviane Tabar  
Professor of Neurosurgery and Neuroscience  
Memorial Sloan Kettering Cancer Center

Over 70% of diffuse intrinsic pediatric gliomas, an aggressive brainstem tumor, harbor heterozygous mutations that create a K27M amino acid substitution (methionine replaces lysine 27) in the tail of histone H3.3. The role of the H3.3K27M mutation in tumorigenesis is not fully understood. Here, we use a human embryonic stem cell system to model this tumor. We show that H3.3K27M expression synergizes with p53 loss and PDGFRA activation in neural progenitor cells derived from human embryonic stem cells, resulting in neoplastic transformation. Genome-wide analyses indicate a resetting of the transformed precursors to a developmentally more primitive stem cell state, with evidence of major modifications of histone marks at several master regulator genes. Transformed cells establish brainstem tumors in mice that bear histological characteristics of human tumors. Drug screening assays identified a compound targeting the protein menin as an inhibitor of tumor cell growth in vitro and in mice. The drug is being validated in patient derived xenografts. Human ES cells thus offer an opportunity to model human tumors in the appropriate developmental stage and may be useful drug screening tools.

**MODELING HUMAN COLON CANCER IN IMMUNOCOMPETENT MICE USING CHEMOKINE-TARGETED CANCER STEM CELL**

Huanhuan Joyce Chen<sup>1</sup>, Steven M. Lipkin<sup>2</sup>, and Xiling Shen<sup>1</sup>

<sup>1</sup>Department of Biomedical Engineering, Cornell University.

<sup>2</sup>Departments of Medicine, Genetic Medicine, Weill Cornell Medical College.

A major challenge for cancer research is the lack of adequate animal models. Genetically engineered mouse models do not entirely capture the subtypes and genetic drivers of human cancer, while xenograft models require immunodeficient mouse hosts and often grow tumors at non-orthotopic sites, hence depriving tumor cells of the proper immune and microenvironment contexts. To address these challenges, we want to engineer immunocompetent mice to grow orthotopic human colorectal cancer (CRC), by leveraging our previous discovery of the CCL25-CCR9 chemokine axis [1-2] in patient-derived colon cancer stem cells (CCSCs) [3].

Here, we utilize early-stage mouse blastocyst microinjection techniques to model primary human CRC in immunoproticient mouse hosts. Using essentially the same techniques for mouse embryonic stem cell microinjection, we inject 10-15 CCR9+ CCSCs into e3.5 blastocysts of wild-type Swiss-Webster or C57B/L6 mouse. These chimeric mice are born without defects and grow up normally. Notably, after 6 months, locally invasive tumors from injected human CCSCs that can penetrate the bowel wall are detected, and these tumors are penetrated by T and B cells. Remarkably, these mice develop central immune tolerance towards human cells, as subcutaneous inoculation of 3 month old chimeras with their matched blastocyst injected cell lines result in subcutaneous tumors at the injection site.

Examination of the mouse embryos revealed that CCL25 is expressed in e12.5 embryonic thymus and e14.5 intestine. RFP-labeled CCSCs first migrate to thymus, allowing the mouse host to develop central tolerance towards human CCSCs, and then to newly formed mid- and hind-gut, where they eventually develop into tumors in adult chimeras.

Overall, we have developed a system to study human CRC in the GI microenvironments of immunoproticient mouse hosts. This technology has the potential to enhance our understanding of interactions between CRC and the immune system, which may lead to CRC immunological therapies.

[Supported by NYSTEM contract # C029543]

[1] Chen, H. *et al.*, JCI 122(9), 3184-96. PMID: PMC3428084.

[2] Chen, H. *et al.*, Nature Biotechnology, in press.

[3] Bu, P. *et al.*, Cell Stem Cell, 12(5), 602-15. PMID: PMC3646336.

**COMMERCIALIZATION OF VALPROIC ACID EXPANDED CORD BLOOD STEM CELLS AS ALLOGENEIC GRAFTS FOR ADULTS WITH REFRACTORY HEMATOLOGICAL MALIGNANCIES**

Ronald Hoffman

Icahn School of Medicine at Mount Sinai

Stem cell transplantation represents the only potential cure for most patients with blood cancers that are refractory to chemotherapy as well as patients with genetic disorders that involve blood cells (sickle cell anemia, thalassemia). Only 30% of patients who require a stem cell transplant will have a matched sibling donor. Although there are over 20 million adult volunteer unrelated donors in the National Marrow Donor Program and affiliated registries, many patients, especially individuals of diverse racial/ethnic backgrounds, will not have a donor identified. Another source of transplantable stem cells is cord blood (CB) that is present within the placenta of every child born. There are limited numbers of stem cells within CB collections, which leads to their being useful in children but unfortunately having limited use in adults. We have developed a process to expand in the laboratory the number of stem cells within a single CB collection so as to allow one to safely transplant adult patients. By treating CB-CD34+ cells in a serum free culture system containing a combination of cytokines and a histone deacetylase inhibitor, valproic acid (VPA) we have been able to increase 35 fold the numbers of stem cells. In this proposal we will move this approach from the research laboratory to the clinic which will allow adults who are transplant candidates who presently lack an appropriate stem cell donor to receive such expanded grafts which hopefully will provide them with a chance for cure of their blood disorder.

**PROGRAMMING HEMATOPOIETIC STEM CELLS FOR LONG-TERM TARGETED T CELL THERAPY OF PATIENTS WITH RELAPSED OVARIAN**

Kunle Odunsi

Roswell Park Cancer Institute

The goal of our studies is to generate robust and long-lasting tumor-specific T cell responses for durable tumor regression in patients with epithelial ovarian cancer (EOC). While the majority of women with advanced stage EOC initially respond to surgery and first-line chemotherapy, more than 70% of patients eventually die of recurrent disease within 5 years of diagnosis. In an effort to generate tumor-associated antigen (TAA) specific T cells for the treatment of EOC patients, our team has identified NY-ESO-1 as the prototypic TAA for immunotherapy in ovarian cancer. In clinical trials of cancer vaccines conducted by our group, although active immunization targeting NY-ESO-1 can generate TAA specific effector T cells, the long term control of ovarian cancer is infrequent. This is primarily because of the relatively low magnitude and short *in vivo* lifespan of the vaccine-elicited T cells limited long-term tumor control in the patients. Consequently, we have focused on genetically re-engineering T cells to express a NY-ESO-1 specific CD8+ T cell receptor (CD8TCR), followed by adoptive transfer of these cells into EOC patients. Although this approach led to large numbers of circulating tumor antigen specific T cells and tumor regression, the strategy is hampered by the relatively short lifespan of the effector CD8+ T cells. These results indicate that lack of sustained expansion of long lasting, durable tumor specific T cells is a major obstacle for successful immunotherapy

In preliminary studies, we have focused on CD4+ T cells as the major driver of anti-tumor immunity. In order to augment the *in vivo* persistence of the engineered CD8TCR cells, we have cloned a distinct subset of human CD4+ Th1 cells that directly recognize NY-ESO-1 naturally presented by MHC class II on cancer cells. In addition, these tumor-recognizing CD4+ T cells (TR-CD4TCR) potentially provide help to CD8TCR cells in an antigen-presenting cell (APC) independent fashion and amplify the anti-tumor effects of CD8+ T cells. In contrast, their conventional TAA-specific CD4+Th1 counterparts (non-tumor recognizing, NTR-CD4), require APCs for their activation. Direct cognate interaction between TR-CD4 and cancer cells triggers the release of an array of effector molecules which inhibit tumor growth and amplify the anti-tumor effects of CD8+ T cells. We have also demonstrated that human hematopoietic stem/progenitor cells (hHSC) can be genetically re-programmed for continuous generation of long lived tumor-specific T cells in an NSG mouse model. In our consortia, we propose a novel clinical application of hHSCs for the continuous (possibly lifelong) source of anti-cancer immune cells that will provide sustained attack against cancer cells.

Our central hypothesis is that TR-CD4TCR engineered hHSCs will provide a durable *in vivo* supply of mature TR-CD4TCR cells with anti-tumor activity and sustained help to CD8TCR effector T cells, thereby leading to long-lasting tumor rejection in patients with EOC. Our proposal is highly significant because it will culminate in a clinical trial that addresses an unmet need in the treatment of chemotherapy-resistant EOC by the innovative use of re-programmed tumor-specific hHSCs. Moreover, the combined adoptive transfer of TR-CD4TCR cells and CD8TCR cells for targeting cancer is, to our knowledge, completely novel.

## VASCULAR NICHE PLATFORM TO EXPAND HEMATOPOIETIC STEM AND PROGENITOR CELLS ENGINEERED TO CURE SICKLE CELL DISEASE

Shahin Rafii and Joseph Scandura  
Weill Cornell Medical College

**Abstract:** The long-term goal of this consortia project is to develop innovative cell therapies to cure more patients with life-threatening hematological and stem cell disorders than is currently feasible. The main objectives of this proposal are to greatly expand the availability of suitable donors for hematopoietic stem cell transplantation (HSCT), to establish a new approach that limits the duration of cytopenias—the leading cause of treatment-related death—and to vertically advance the potential for gene correction of hematopoietic stem cells as well as other acquired or genetic organ-specific stem cell disorders.

Toward this end, our research consortium leverages the expertise of Rafii/Scandura/Butler/Evans at Weill Cornell Medical College (WCMC) along with Sadelain/Rivière/Baker/Giralt/Boulad at Memorial Sloan Kettering Cancer Institute (MSKCC) and proposes to develop a platform for *ex vivo* clinical-scale expansion of hematopoietic stem and progenitor cell (HSPC) using a novel physiological vascular niche platform, developed by the members of this consortium. These vascular niche expanded HSPCs are anticipated to have unprecedented capacity to accelerate hematopoietic recovery and avoid life-threatening cytopenias in patients treated with myelosuppressive therapies and to enable genetic correction of inherited hematological disorders, such as sickle cell disease, by allowing expansion of bona fide hematopoietic stem cells (HSCs) with the capacity for long-term engraftment in humans. This approach is based on the discovery that endothelial cells (ECs) are the physiologic chaperones of HSCs *in vivo*: responsible for maintaining life-long HSC self-renewal and governing their timely differentiation to all blood cell types. Having established an *ex vivo* vascular niche platform that recapitulates the full physiological repertoire of stimulatory and inhibitory factors (angiocrine factors) that these essential niche cells provide *in vivo*, we now plan to use this platform to expand cocultured umbilical cord blood (UCB) HSPCs and adult bone marrow for human therapies.

We have consulted with FDA through two pre-IND meetings and have obtained detailed FDA guidance for two INDs for clinical trials translating the potential of vascular niche to the clinical setting. Our strategy leverages the capabilities of an MSKCC GMP facility that is deeply experienced with clinical-grade preparation of grafts for transplantation in humans. Additionally, our approach provides the critical first-steps in a continuum of research that is expected to lead to the transplantation of gene targeted/corrected long-term HSCs for the treatment of sickle cell disease and this ultimately sets the stage for the treatment of other organ-specific acquired or genetic stem cell disorders.

We plan to achieve our goals by completion of the following interrelated projects:

**Project 1:** Develop a clinical GMP grade manufacturing process for *ex vivo* expansion of UCB-derived HSPCs using the human vascular niche platform. This is a necessary first step to provide the necessary human safety data required for the proposed gene therapy for sickle cell disease.

**Project 2:** Autologous bone marrow derived HSCs from sickle cell patients will be gene corrected by FDA-approved lentiviral insertion of a beta-globin anti-sickling vector and then expanded on the vascular niche platform before transplantation back into sickle cell patients.

Well-defined milestones mark a clear path to the initiation of two Phase 1 trials within the tenure of this grant and to lay the groundwork for planned HSC-based gene therapies. In Year 2, we will file for IND approval to use expanded allogeneic UCB-derived

HSCs in the first phase 1 trial to 1) *test the safety of the vascular niche expansion model* and 2) *limit duration of myelosuppression and toxicity in subjects* receiving intensive chemotherapy for acute leukemia by shortening the time to neutrophil *and in particular platelet engraftment* after UCB transplantation. In Year 3, we plan to file a second IND for genetically correcting and marking autologous HSCs from sickle patients *to not only cure these patients but also track long-term engraftment of expanded barcoded HSCs*. This is going to be the first time that human barcoded stem cells ex vivo expanded will be transplanted to assess their long-term engraftment potential.

Our approach will benefit underserved populations of patients, including ethnic and racial minorities, elderly patients and those, who require efficient genetic engineering of HSCs for correction of genetic disorders. The novel *ex vivo* vascular niche HSPC expansion approach that we propose is expected to provide more cures, limit toxicity, and diminish the current high cost of transplantation, thereby extending access and to a greater number of patients in need.

**DETERMINISTIC BEHAVIOR OF NEURAL PROGENITORS IN THE MAMMALIAN NEOCORTEX**

Songhai Shi

Memorial Sloan Kettering Cancer Center

Radial glial progenitors (RGPs) are responsible for producing nearly all neocortical neurons, yet a quantitative understanding of RGP division, and neuronal production and organization is lacking. We recently analyzed excitatory neuron genesis in the mouse neocortex using Mosaic Analysis with Double Markers (MADM), which provides unprecedented single-cell resolution of progenitor division pattern and potential *in vivo*. Remarkably, once they enter the asymmetric neurogenic division phase, individual RGPs across different regions of the developing neocortex produce ~8-9 neurons, suggesting a unitary output in neuronal production. Concordantly, symmetric proliferative divisions of RGPs result in multiplication of the unit. Virtually all RGPs generate both superficial and deep layer neurons organized in vertical clusters with variable topology. Moreover, ~1/6 neurogenic RGPs proceed to produce glia, indicating a coupling between gliogenesis and neurogenesis. These results reveal definitive ontogeny of neocortical excitatory neurons and glia, and suggest a deterministic nature of RGP behavior in the mammalian neocortex.

**IDENTIFICATION OF AN ENDODERM-DERIVED VEGFR2+ PROGENITOR WITH A BI-POTENT HEPATIC AND ENDOTHELIAL FATE**

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<sup>3</sup> These authors contributed equally to this work.

Using the human and mouse embryonic stem cell differentiation system and early fetal livers, we have identified a novel human hepatic progenitor based on the unexpected expression of KDR (VEGFR2/Fik-1). Indeed, KDR expression was thought to be restricted to mesodermal derivatives including endothelial, hematopoietic, cardiac and skeletal muscle precursors. However, our in vitro studies provide evidence for the novel concept that KDR also marks a conserved mouse and human endoderm-derivative. We provide a definitive in vivo proof for the contribution of the KDR+ progenitors to liver development by a lineage tracing study in mice that marked all cells expressing KDR as well as their progeny. Analyses of fetal and adult livers identified a large subset of fetal hepatoblasts and subsequently adult hepatocytes that were generated from the KDR+ progenitors (Goldman *et al.*, Cell Stem Cell, 2013).

More recently, we demonstrated that KDR+ progenitors give rise not only to hepatic cells but also endothelial cells. The in vivo functionality of the endothelial cells was shown by the visualization of many human CD31+ endothelial cells integrated in the repaired host vessels following transplantation into injured muscles of mice that underwent a femoral artery ligation. These results introduce the concept that endothelial cells can originate from endoderm, which was supported in an endoderm specific-lineage tracing mouse model. Similar endothelial cells were found in human fetal liver specimens suggesting that endoderm-derived endothelial cells are relevant to human fetal liver development (Goldman *et al.*, Stem Cell Reports, 2014).

Taken together, analyses of lineage tracing mouse models, human ESC culture systems and human fetal liver specimens reveal the existence of a conserved endoderm-derived KDR+ liver progenitor with a unique bi-potential hepatic and endothelial fate. We are currently investigating the clinical impact of the KDR+ bi-potent progenitors and their hepatocyte and endothelial cell progeny during liver regeneration.

**ASYNCHRONOUS SINGLE-CELL FATE DECISION EVENTS DRIVE LINEAGE COMMITMENT IN THE MOUSE BLASTOCYST**

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Lineage specification in the mouse blastocyst occurs in two sequential steps that lead to the segregation of the extraembryonic trophectoderm (TE) and primitive endoderm (PrE) from the embryonic epiblast (EPI). Differential activation of the FGF4-MAPK signaling axis results in the establishment of distinct gene expression patterns within the inner cell mass (ICM), restricting GATA6 to PrE cells and NANOG to the EPI. We have applied a single-cell, quantitative approach to analyze PrE and EPI specification with high spatiotemporal resolution. Functional assays and time-lapse analyses revealed a gradual loss of plasticity within the ICM, arising from individual, asynchronous cell fate decision events. Individual cells become irresponsive to FGF signaling as they commit to either EPI or PrE, thus ensuring the stability of both lineages. We also observed that the ratio of PrE to EPI cells needs to be tightly regulated for the embryo to be viable. We propose that lineage specification within the ICM of the mouse blastocyst is a cumulative process, where incremental allocation of precursors to either lineage creates a window of opportunity to achieve balanced proportions of PrE and EPI cells.

[Supported by NYSTEM contract C029568]

**STEM CELL NICHE CONTROL OF HAIR REGENERATION**

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To coax adult tissue stem cells (SCs) towards specific cell fates for replacement therapy, we need to understand their regulation by signals from the SC niche. Adult hair follicle (HF) regeneration is an excellent model system for studying activation of adult tissue SCs by niche signals, since the spatial and temporal aspects of the physiological destruction, rest and re-growth during the normal hair cycle are well-defined. Specialized dermal papilla (DP) cells act as niche cells that instruct adult HF SCs to switch from a resting to an activated fate during the regeneration phase of the hair cycle. However, the specific activating niche signals are largely unknown due to the lack of genetic tools to systematically isolate, characterize DP niche cells and target them for gene ablation. Here we use genetic drivers for DP signature genes *Tbx18*, *Sox2* and *Crabp1* to establish inducible cre-mediated gene ablation in the adult HF SC niche. We systematically define their molecular features at a genomic scale in comparison to regular non-niche fibroblasts and contrast them with bulge/germ HF SCs at all key stages. Finally, we interrogate the functional role of PDGF signaling in the niche for SC activation during adult HF regeneration. This work provides essential new tools and insights for the HF SC niche, as it identifies the essential signal(s) from the DP niche that activate SCs during HF regeneration, a prerequisite for improving our ability of manipulating adult skin SCs for future hair regenerative therapies.

[Supported by NYSTEM contract C029574]

## **SIGNALING CROSSTALK IN SKELETAL CELL FATE DETERMINATION AND DIFFERENTIATION**

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The Wnt signal transduction pathway controls development of various stem cell types essential for proper formation of multiple organs. In the skeleton, Wnt signaling regulates bone formation and remodeling through modulation of osteoblasts, chondrocytes and osteoclasts. Genetic inactivation of *CTNNB1* encoding  $\beta$ -catenin indicates its importance in mesenchymal cell fate determination. Interplay of Wnt with other skeletogenic signaling pathways including FGF and BMP is also critical for skeletal lineage specification. We have identified *Gpr177*, the mouse orthologue of *Drosophila Wls/Evi/Srt*, essential for Wnt sorting and secretion. *Gpr177* regulates intracellular trafficking of Wnt proteins in signal-producing cells. Human genetic analysis further reveals that *Gpr177* is a susceptibility locus for bone-mineral-density and osteoporosis. The loss of *Gpr177* impairs expansion of the skeletal precursors and their differentiation into osteoblasts and chondrocytes, demonstrating its requirement in intramembranous and endochondral ossification during skeletal development. Mesenchymal but not osteoblast production of Wnt is essential for development of the skeletal precursors into osteoblast lineage. However, Wnt proteins produced by chondrocytes are necessary for the chondrogenic lineage. Our findings not only identify the sources of Wnt essential for osteogenesis and chondrogenesis, but also reveal the function of *Gpr177* in modulating the interplay of Wnt signals across distinct cell types. However, the disruption of Wnt secretion does not result in mesenchymal cell fate change. The discrepancy might be attributed to a dual role of either *Gpr177* in canonical and non-canonical Wnt pathways, or  $\beta$ -catenin in signal transduction and cell-cell interaction. To test these possibilities, we have created several new mouse strains and utilized proteomic analyses to elucidate the regulatory mechanism underlying lineage-specific development of the skeletogenic mesenchyme. The results suggest that cellular signaling downstream of  $\beta$ -catenin-mediated cell adhesion is essential for fate determination of the mesenchymal stem cells.

[Supported by NYSTEM contract C029558]

**GENERATION OF CLINICALLY RELEVANT BUT RARE CELL TYPES FROM ESCS**

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In the past decade, much progress has been made in the directed differentiation of tissue-specific progenitors and their derivatives from pluripotent stem cells in vitro. However, for most organ systems, including the heart and pancreas, the derivatives are often heterogeneous and major challenges remain for generating functionally pure populations of certain cell types, which may be quite rare, yet have major clinical significance. This is important with regard to scalable generation of cells for regenerative therapies and even for modeling human disease “in a dish”. We have pursued several complementary approaches for overcoming the hurdles, including directed differentiation by defined transcription factors, the use of reporters, and most importantly the power of coupling differentiation with chemical screens to enhance the generation of defined cellular phenotypes. Several specific examples are presented including for otherwise rare cells of the cardiac conduction system and the pancreatic ductal program.

**METABOLIC INSIGHTS INTO STEM CELL PLURIPOTENCY**

Craig Thompson

Memorial Sloan Kettering Cancer Center

## TEX10 COORDINATES EPIGENETIC CONTROL OF SUPER-ENHANCER ACTIVITY FOR PLURIPOTENCY AND REPROGRAMMING

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Super-enhancers (SEs) are large clusters of transcriptional enhancers that are co-occupied by multiple lineage specific transcription factors driving expression of genes that define cell identity. In embryonic stem cells (ESCs), SEs are highly enriched for Oct4, Sox2, and Nanog (OSN) in the enhanceosome assembly and express a group of non-coding RNAs known as eRNAs. We sought to understand the dynamic epigenetic control of SE activity and eRNA transcription for both maintenance and establishment of pluripotency. Starting from a protein interaction network surrounding Sox2, a key pluripotency and reprogramming factor that guides the ESC-specific enhanceosome assembly and orchestrates the hierarchical transcriptional activation during the final stage of reprogramming, we discovered Tex10 as a novel pluripotency factor that is essential for ESC self-renewal, pluripotency, early embryo development, and somatic cell reprogramming. Mechanistically, we uncovered a unique mode of action of Tex10 in epigenetic control of SE activity driving downstream target gene and eRNA transcription. Specifically, we found that Tex10 is enriched at ESC SEs in a Sox2-dependent manner and that it recruits the histone acetyltransferase p300 and the DNA hydroxylase Tet1 to promote H3K27 acetylation and SE hypomethylation, respectively. We also defined the functional conservation of human TEX10 in maintaining pluripotency and promoting reprogramming in human cells. Our study thus discovers an evolutionally conserved and functionally significant novel pluripotency regulator and sheds new light on mechanistic understanding of the intrinsic relationship between the tight control of SE activity and the pluripotent identity encompassing ESC self-renewal, somatic cell reprogramming, and early embryo development.

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**DYNAMIC CONTROL OF FGFR-MEDIATED SELF-RENEWAL SIGNALING MEDIATES POSITIVE SELECTION OF MUTANT SPERMATOGONIAL STEM CELLS WITH ADVANCED PATERNAL AGE**

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Spermatogonial stem cells (SSCs) maintain testicular homeostasis through carefully orchestrated changes in growth factor signaling. Recently, anatomical clustering of pathogenic mutations was found in older men's testes, suggesting a clonal process. Thus, positive selection of SSCs was proposed to explain the higher frequency of disorders among children of older fathers (i.e., the paternal age effect [PAE]). We recently demonstrated that SSCs expressing an Apert syndrome (activating) mutation in FGFR2 (referred to as AS SSCs) exhibited enhanced competitiveness, contingent upon growth factor dose, indicating that the niche influences phenotypic effects of a mutation. Interestingly, PAE disorders do not include oncogenic mutations, suggesting hyperactivation is incompatible with gene transmission to children. Herein, we hypothesize that SSC fate depends on FGF/FGFR pathway activation in conjunction with tight negative feedback. To test this, we studied SSCs in varied growth factor conditions. We found AS SSCs exhibited increased sensitivity to very low FGF2, manifested by enhanced MAPK signaling (~50% increased pErk1/2 vs. control), consistent with gain-of-function. Moreover, AS SSCs proliferated long-term in suboptimal GDNF conditions despite reduced FGF2, suggesting enhanced FGF signaling preserves SSC activity when growth factors are scarce. Paradoxically, we found a biphasic response in pERK1/2 to FGF2 in wildtype SSCs (>30% reduction at high dose). We also profiled mRNA at varying chronic doses of FGF2 and found stably increased expression for certain stem cell markers at lower doses. Moreover, sustained MAPK signaling (>2-fold increase at 2 hr) occurred only with lower FGF doses. Additionally, canonical and non-canonical signals mediating negative feedback downstream of FGFRs were found in SSCs. These data suggest that tight regulation of FGF/FGFR signaling is required to preserve both normal SSCs and those with pathogenic mutations and may explain the spectrum of human disease mutations that occur due to positive selection.

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**EPIGENETIC REGULATION OF BREAST TUMOR INITIATING CELLS**

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Breast tumor-initiating cells (BTICs) have been implicated in driving tumorigenesis and resistance to therapy, however the mechanisms underlying BTIC behavior remain poorly understood. Using mammosphere (MS) culture as functional readout for BTIC properties, we performed a loss-of-function screen targeting sixty epigenetic regulators. We identified the Polycomb protein Cbx8 as a key regulator of MS formation and tumorigenesis *in vivo*. Consistent with these findings, Cbx8 is amplified and overexpressed in breast cancer patients and correlates with poor survival. Our genomic analyses revealed that Cbx8 positively regulates a Notch signaling network by maintaining H3K4me3 levels on Notch-network gene promoters. Because Cbx8 depletion results in decreased transcriptional output and associates with non-PRC1 complexes, our study implicates a non-canonical function for Cbx8 as a positive regulator of Notch signaling. Finally, we show that Notch ectopic expression rescues MS formation in Cbx8-depleted cells. Collectively, we report a novel role for Cbx8 in regulating tumor-initiating properties through activating Notch expression in breast cancer cells.



**POSTER ASSIGNMENTS**

- (1) THE ABILITY OF HUMAN RPE-DERIVED STEM CELLS TRANSPLANTS TO IMPROVE VISUAL FUNCTION IN A RAT MODEL OF RETINAL DEGENERATIVE DISEASE DEPENDS ON DIFFERENTIATION STATE AND DOSAGE
- (2) DIFFERENTIAL LEVELS OF PI3K/TOR SIGNALLING DETERMINE THE OUTCOME OF COMPETITION BETWEEN STEM CELLS FOR DIFFERENTIATION
- (3) BREAST CANCER STEM CELL QUANTIFICATION IN PATIENTS UNDERGOING NEOADJUVANT CHEMOTHERAPY FOR PREDICTION OF THERAPY RESPONSE
- (4) CREB EXPRESSION IN THE HUMAN DENTATE GYRUS AND ADULT NEUROGENESIS IN THE CONTEXT OF MAJOR DEPRESSIVE DISORDER
- (5) MAPK-ACTIVATED VASCULAR NICHE INHIBITS FUNCTIONAL HEMATOPOIESIS AND ENHANCES LEUKEMIC PROGRESSION
- (6) IMMUNOSUPPRESSION FOR STEM CELL-DERIVED TREATMENTS IN NEURODEGENERATIVE DISEASE: IF, WHY, WHAT, AND WHEN?
- (7) iPS-DERIVED ASTROCYTES: A ROLE FOR THERAPY IN PARKINSON'S DISEASE?
- (8) TRANSIENT NICHE DERIVED SIGNALS REGULATE SUBVENTRICULAR ZONE TYPE B NEURAL STEM CELL PROPERTIES TO PROMOTE INJURY REPAIR
- (9) GENOMIC INSTABILITY LIMITS REPROGRAMMING FOLLOWING NUCLEAR TRANSFER
- (10) MICRORNA REGULATION OF ADULT NEURAL STEM CELL STATES
- (11) IDENTIFICATION OF PUTATIVE ADULT STEM CELL NICHES BY COMPARATIVE EVALUATION OF THE HUMAN AND MOUSE OVARIAN SURFACE AND TUBAL EPITHELIA
- (12) BMP MEDIATED CROSS-TALK BETWEEN POLARIZED MACROPHAGES AND HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES
- (13) HUMAN ES-BASED MODELING OF PEDIATRIC GLIOBLASTOMA BY HISTONE MUTATIONS
- (14) MODY3 iPSC-DERIVED  $\beta$ -CELLS TO MODEL DIABETES DUE TO HNF1A DEFICIENCY
- (15) TUMOR MICROENVIRONMENT OF METASTASIS (TMEM) REPRESENTS A STEM CELL NICHE IN BREAST CANCER PATIENTS

- (16) ENDOTHELIAL CELL-JAGGED2 REGULATES HEMATOPOIETIC STEM AND PROGENITOR CELL MAINTENANCE AND RECONSTITUTION
- (17) A NEW APPROACH TO FOR THE TREATMENT OF PARKINSON'S DISEASE – AN UPDATE
- (18) FGF RECEPTOR SIGNALING DURING THE EMERGENCE OF PLURIPOTENCY IN VIVO
- (19) PHARMACOLOGICAL REGULATION OF EPIGENETIC MECHANISMS EFFECTS THE REGENERATIVE CAPACITY OF CARDIAC PROGENITOR CELLS
- (20) KERATIN 14 IS A POTENTIAL MARKER OF STEM/PROGENITOR CELLS IN ADULT SALIVARY GLAND
- (21) ELECTRICAL CONDITIONING ESTABLISHES FUNCTIONAL HUMAN STEM-CELL DERIVED CARDIOMYOCYTES
- (22) CRITICAL ROLE OF ENDOTHELIN RECEPTOR SIGNALING IN FOLLICULAR MELANOCYTE STEM CELLS IN ADULT SKIN
- (23) TARGETED BLVRB GENE KNOCK-OUT IN IPSC CELLS AS MODELS FOR LINEAGE-RESTRICTED HEMATOPOIETIC DIFFERENTIATION
- (24) VASCULAR NICHE DEPENDENT CONVERSION OF ADULT MURINE ENDOTHELIAL CELLS INTO LONG-TERM ENGRAFTABLE HEMATOPOIETIC STEM CELLS
- (25) GENERATION OF RETINAL TISSUES FROM HUMAN EMBRYONIC STEM CELLS (hESCS) THROUGH SELF-ASSISTED CELL SORTING
- (26) IMPROVING THE INFORMED CONSENT PROCESS FOR FIRST-IN-HUMAN STEM CELL TRANSPLANT TRIALS FOR PARKINSON'S DISEASE: WHAT ARE THE BARRIERS?
- (27) CANCER-ASSOCIATED POT1 MUTATIONS LEAD TO TELOMERE DYSFUNCTION AND PROMOTE GENOME INSTABILITY
- (28) NF-KB INHIBITION IN ENDOTHELIAL CELLS ENHANCES SELF-RENEWAL AND REGENERATION OF THE HEMATOPOIETIC SYSTEM
- (29) CYCLIN A2 MEDIATES REPROGRAMMING IN ADULT MAMMALIAN CARDIOMYOCYTES
- (30) DYNAMICS OF PROLIFERATIVE GERM CELLS DURING EXPANSION AND MAINTENANCE

- (31)** 3-D CONSTRUCTS—MOLDED VS. PRINTED: THE DIFFERENCES FROM A STEM CELL BASED PERSPECTIVE
- (32)** CO-OCCUPANCY OF ENDOTHELIAL GENES BY FLI1 AND SOX17 REPROGRAMS MOUSE AMNIOTIC FLUID CELLS INTO FUNCTIONAL ENDOTHELIUM
- (33)** UNDERSTANDING BETA CELL DYSFUNCTION USING PATIENT INDUCED PLURIPOTENT STEM CELLS
- (34)** METABOLOMIC ANALYSIS FOR THE OPTIMIZATION OF CHONDROCYTE DIFFERENTIATION OF MESENCHYMAL STEM CELLS IN 3D PELLET CULTURE
- (35)** PROTEIN PROFILING (HDMS<sup>E</sup>) FOR MONITORING OF CHONDROCYTE DIFFERENTIATION OF MESENCHYMAL STEM CELLS IN 3D PELLET CULTURE IN A MULTI-OMIC APPROACH
- (36)** NOVEL ROLE OF PLACENTAL CDX2 CELLS IN CARDIOMYOGENESIS
- (37)** P53 ACTS AS A SWITCH FACTOR TO LICENSE MASTER DIFFERENTIATION GENES EXPRESSION BY TGF-BETA SIGNALS
- (38)** ANALYSIS OF LGR5-POSITIVE CELLS IN MOUSE MAMMARY GLAND
- (39)** LINEAGE-DEPENDENT FUNCTIONAL ORGANIZATION OF NEOCORTICAL INTERNEURONS
- (40)** MODELING THE GENETICS OF HUMAN PANCREATIC DEVELOPMENT AND DISEASE THROUGH GENOME EDITING IN EMBRYONIC STEM CELLS

**FACILITIES POSTERS (even numbers Session I, odd numbers session II)**

- (F1)** SHARED RESOURCE: LARGE-SCALE BIOCHEMICAL PROFILING FOR STEM CELL RESEARCH IN NEW YORK
- (F2)** NYSTEM HIGH-THROUGHPUT SCREENING AND CHEMISTRY SHARED FACILITY AT COLUMBIA UNIVERSITY
- (F3)** THE STEM CELL PATHOLOGY UNIT AT CORNELL UNIVERSITY
- (F4)** THE SKI STEM CELL RESEARCH FACILITY
- (F5)** THE NYU RNAI CORE: AN OPEN ACCESS, HIGH-THROUGHPUT SCREENING RESOURCE FOR FUNCTIONAL GENOMICS



**(1) THE ABILITY OF HUMAN RPE-DERIVED STEM CELLS TRANSPLANTS TO IMPROVE VISUAL FUNCTION IN A RAT MODEL OF RETINAL DEGENERATIVE DISEASE DEPENDS ON DIFFERENTIATION STATE AND DOSAGE**

Nazia M. Alam<sup>1</sup> on behalf of the Retinal Stem Cell Consortium

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Non-exudative age-related macular degeneration (AMD) is an insidious and growing cause of untreatable vision loss. Research over the past decade has culminated in the first clinical evaluations of cell therapies for retinal disease. Transplantation of the retinal pigment epithelium (RPE), which includes human embryonic stem cell (hESC) and induced pluripotent stem cell (iPSC)-derived RPE, is being developed as a cell-replacement therapy for AMD. We evaluated the adult human RPE stem cell (hRPESC)-derived RPE as an alternative and renewable source of RPE for transplantation. hRPESC-derived RPE cell lines at different states of differentiation (duration in culture) and dosage (number of cells), were transplanted into the sub-retinal space of juvenile, pigmented Royal College of Surgeons (RCS) rats under immunosuppression. Visual behavior was quantified at two and three months of age, by assessing spatial frequency thresholds for optokinetic tracking in a virtual optokinetic system (OptoMotry). Optical coherence tomography of retina, and retinal histology was also used to evaluate retinal anatomy. Control RCS rats showed near normal visual function at the time of surgery, which declined 13% by two months, and 27% by three months. hRPESC transplanted animals, however, were able to maintain 95% of normal function out to three months. The beneficial effect of hRPESC transplantation was maximized using cells maintained in culture for 4-5 weeks, and with a dosage of 47K-100K cells, and was largely independent of cell line. Anatomical measures indicated that retinal structure was better in the region of the transplant in animals with preserved visual function. These data thus indicate that the retinal transplantation of hRPESCs holds promise as a treatment for human AMD.

[Supported by NYSTEM contract C028504]

**(2) DIFFERENTIAL LEVELS OF PI3K/TOR SIGNALLING DETERMINE THE OUTCOME OF COMPETITION BETWEEN STEM CELLS FOR DIFFERENTIATION**

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While stem cell self-renewal has been extensively studied, it is generally presumed that differentiation is a default pathway that occurs upon removal of self-renewal factors. Stem cell niches provide the requisite signals for self-renewal. Upon division, a cell that is pushed out of the niche is no longer exposed to this permissive environment and initiates differentiation. However we show that differentiation is an active process that requires signalling in the PI3K/Tor pathway in somatic stem cells (CySCs) in the *Drosophila* testis. We show that differentiating cells display elevated PI3K activity and that knock down of the PI3K effector Akt1

prevents normal CySC differentiation, suggesting that elevated metabolic activity is required during differentiation. Finally, although we previously showed that clones with elevated PI3K or Tor activity differentiate, hyper-activation of PI3K throughout the somatic lineage does not lead to forced differentiation. These results suggest that stem cells are normally competing with each other to differentiate and that the outcome of this competition is determined by the relative levels of PI3K/Tor activity between somatic cells. Thus we establish enhanced metabolism as a central regulator of differentiation.

### (3) BREAST CANCER STEM CELL QUANTIFICATION IN PATIENTS UNDERGOING NEOADJUVANT CHEMOTHERAPY FOR PREDICTION OF THERAPY RESPONSE

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The ultimate goal of cancer treatment is individualized therapy aimed at specific molecular targets to prevent or treat metastatic and drug resistant disease. Both metastasis and chemotherapy resistance are characteristics of cancer stem cell phenotype. We hypothesized that the response to neoadjuvant chemotherapy (NAC) would correlate with the change in the proportion of cancer stem cells in tumors before and after initial 2 weeks of NAC treatment.

We obtained breast cancer cells from 9 patients by fine Needle Aspiration (FNA) before and after 2 doses of NAC. Five patients were treated with paclitaxel and 4 with doxorubicin/cyclophosphamide. Six patients had triple negative, 2 ER+, and 1 ER-/Her2+ tumors. All patients had at least T2 stage disease. One patient had a complete pathologic response (RCB 0), 3 minimal residual disease (RCB I), 3 moderate response (RCB II), and 2 extensive residual disease (RCB III).

We quantified the percentage of CD44<sup>high</sup>/CD24<sup>low</sup> breast cancer stem cells (BCSCs) by flow cytometry in FNA samples obtained before and after 2 weeks of NAC. The samples were treated with RBC lysis buffer to remove the erythrocytes. The leucocytes were stained for CD45 and gated out before the CD44<sup>high</sup>/CD24<sup>low</sup> quantification.

In all patients who had RCB 0, I or II there was no significant change in the percentage of BCSC before and 2 weeks into chemotherapy. However 2 patients with RCB III showed 2 and 3 fold increase in the percentage of BCSC.

In summary, the change in the percentage of BCSC upon 2 weeks of NAC correlated with the degree of treatment response in all 9 patients. Assessment of the BCSC burden before and after initial 2 weeks of chemotherapy may be a useful approach for predicting chemotherapy response.

**(4) CREB EXPRESSION IN THE HUMAN DENTATE GYRUS AND ADULT NEUROGENESIS IN THE CONTEXT OF MAJOR DEPRESSIVE DISORDER**

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Selective serotonin reuptake inhibitors (SSRI) are commonly used antidepressants affect serotonergic signal transduction pathways and increase stem cell proliferation in the dentate gyrus (DG) of the human hippocampus (Boldrini et al, 2009, 2012). Research has focused on the role of G-protein-coupled receptors, associated second messenger pathways, and transcription factors on stem cell proliferation, maturation and survival (Blendy, 2006). The cAMP responsive element-binding protein (CREB) is a leucine zipper-containing transcription factor that is involved in numerous intracellular signaling pathways. Membrane receptors initiating these pathways include neurotrophin receptors, glutamate-gated ion channel receptor subunits (GluR1), N-Methyl-D-aspartate (NMDA) receptors, L-type Ca<sup>2+</sup> channels, and G-protein-coupled receptors. Their intracellular signaling cascades phosphorylate CREB at serine 133, activating CREB-mediated gene transcription (Carlezon et al, 2005). CREB plays a critical role in neural plasticity, specifically in cellular and behavioral mouse models of learning and memory (Figurov et al, 1996; Korte et al, 1995; Silva et al, 1998). In animal models CREB seems to be involved in both the mechanism of action of antidepressants and depression itself (Blendy, 2006). Selective serotonin reuptake inhibitors (SSRI) increase CREB activity in the rat hippocampus (Nibuya et al, 1996). In addition, viral-mediated expression of CREB in the rat hippocampus produces an antidepressant-like effect in behavioral models of depression, such as the forced swim test and learned helplessness paradigms (Chen et al, 2001). The findings suggest that down-regulation of CREB could contribute to the pathophysiology of major depressive disorder (MDD), and that its up-regulation could explain the therapeutic efficacy of SSRI. CREB expression levels in relationship to antidepressant treatment and amount of neurogenesis have never been studied in the human brain.

We performed immunohistochemistry and stereology in postmortem adult human brain tissue, from SSRI-treated and untreated subjects with MDD and controls with no psychiatric diagnosis or treatment (n=10 each), to compare cellular expression of CREB in the DG of the hippocampus in these groups and to correlate CREB expression with amounts of stem cells, proliferating cells, neuroblasts and mature neurons in each group.

We found that granule neurons expressing non-phosphorylated CREB are more in untreated MDD compared with SSRI-treated MDD and controls ( $p < .05$ ), while there is no difference in number of glial cells expressing non-phosphorylated CREB between groups. In SSRI-treated MDD, subjects with higher clinical functioning have fewer granule neurons expressing non-phosphorylated CREB.

CREB is involved in stem cell proliferation, maturation, and survival, the non-active form of CREB is more in granule neurons in untreated MDD, and less in SSRI-treated MDD and controls suggesting they might have more active (phosphorylated) CREB sustaining higher levels of neurogenesis.

**(5) MAPK-ACTIVATED VASCULAR NICHE INHIBITS FUNCTIONAL HEMATOPOIESIS AND ENHANCES LEUKEMIC PROGRESSION**Michael C. Gutkin<sup>1</sup>, Michael G. Poulos<sup>1</sup>, Jason M. Butler<sup>1</sup><sup>1</sup>Department of Genetic Medicine, Department of Surgery, and Ansary Stem Cell Institute

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Hematopoietic stem cells (HSCs) are defined by their ability to undergo self-renewal and maintain the capacity to generate mature, multi-lineage hematopoietic cells. These qualities make HSCs clinically valuable in bone marrow (BM) transplantation settings, for many hematological disorders. Despite advances in our understanding of HSC biology, the exact mechanisms regulating the balance between self-renewal and lineage-specific differentiation are still unknown. Within the hematopoietic microenvironment, we have shown that Akt-activated endothelial cells (ECs) are indispensable in supporting HSC self-renewal, whereas MAPK promotes the differentiation of HSCs. Based on this data, we set out to test if endothelial-specific MAPK-activation inhibits the vascular niche from supporting homeostatic hematopoiesis. We generated a mouse model in which MAPK is activated specifically in ECs, demonstrating a defect in phenotypic and functional HSCs. To test if the functional defects in the HSCs were due to the MAPK-activated vascular niche, we isolated BM ECs and observed a decrease in the potential to support the *ex vivo* expansion of functional HSCs. Co-cultured HSCs displayed a decrease in quiescence and an increase in differentiation into granulocytic myeloid cells. Furthermore, MAPK-activated BM ECs are endowed with the capacity to expand more primitive and aggressive acute myeloid leukemia clones, suggesting that the signaling pathways within ECs can drive and dictate the BM microenvironment's role in supporting normal or malignant hematopoiesis. Taken together, our *in vivo* model and EC/HSC co-culture system will allow us to screen for angiocrine factors that support the HSC self-renewal and inhibit the expansion and aggressiveness of hematopoietic malignancies.

**(6) IMMUNOSUPPRESSION FOR STEM CELL-DERIVED TREATMENTS IN NEURODEGENERATIVE DISEASE: IF, WHY, WHAT, AND WHEN?**James Carter<sup>1</sup>, Stefan Irions<sup>2</sup>, Dustin Wakeman<sup>3</sup>, Natalie Hellmers<sup>1</sup>, Yaa Obeng-Aduasare<sup>1</sup>, Elizabeth Calder<sup>2</sup>, Viviane Tabar<sup>2</sup>, Claire Henchcliffe<sup>1</sup>

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Stem cell transplantation therapies hold promise for remediation of damage from degenerative disease and traumatic insult to the central nervous system (CNS). However, there remains a need for guidelines in many aspects of the clinical application of CNS cell transplantation. Among the most important unanswered questions is whether to use peri- and post-transplant immunosuppression. We therefore reviewed previously implemented immunosuppressive regimens, their adverse event profiles, and related clinical outcomes as well as autopsy data from major clinical trials in CNS cell therapy. These included fetal mesencephalic cell

transplant for Parkinson's and Huntington's disease, retinal pigmented epithelial cell transplant for Parkinson's disease, and embryonic stem cell-derived oligodendrocyte progenitors transplant for spinal cord injury, among others. (1) Microglial and inflammatory responses in human recipients of allogeneic cell transplants are well documented although mostly of unclear significance. (2) Clinical trial outcome measures demonstrate graft maintenance in at least some individuals in the absence of immunosuppressive treatment, but there are data arguing in favor of at least short-term immunosuppression. (3) Immunosuppression increases risk to the participant including infection or, rarely, tumor formation, and increases study burden. (4) Theoretical concerns exist based upon suppressing a host immune response that may support neurorestoration. Therefore, while it seems prudent to include immunosuppression in upcoming CNS transplantation trials, it will be important to develop a clear understanding of its therapeutic benefit in order to justify increased burden on study participants and investigative teams already navigating the complexity of a nascent therapy. Use of newer combination therapies, as well as novel imaging modalities to monitor the effect of immunosuppression, is predicted to improve safety and tolerability. However, we argue for a concerted effort to define consistent protocols with respect to immunosuppressive agents, dosage, and timeframe, and to develop and incorporate biomarkers to define immune dysregulation following transplant.

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**(7) iPSC-DERIVED ASTROCYTES: A ROLE FOR THERAPY IN PARKINSON'S DISEASE?**

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Mutations in leucine rich repeat kinase 2 (LRRK2) are associated with sporadic and familial forms of Parkinson's disease (PD), and have been found in about 2% of PD patients. Mutant LRRK2 expression in neurons results in neurite injury that precedes cell death. Functional neurotransmission abnormalities and dystrophic neurite morphology have been reported in transgenic mouse models of mutant LRRK2. While numerous cell biological processes, including autophagy, mitochondrial homeostasis, calcium signaling and microtubule stability, have been studied in LRRK2 mutant neurons, less is known about the possible upstream impact of LRRK2 mutations on astrocytes. As many of the processes that require remediation in the damaged CNS are controlled during normal development by astrocytes, we hypothesized that LRRK2 mutations may also disrupt astrocyte function, thereby contributing to disease onset and progression.

We have previously demonstrated the derivation of functionally distinct astrocyte populations from both rodent and human glial precursors, using distinct astrocyte inducing cytokines. While CNTF-induced astrocytes resembled reactive astrocytes found in the injured CNS, BMP-induction resulted in astrocytes with extensive regeneration promoting properties. Here we investigate the properties of LRRK2-mutant iPSC derived astrocyte populations and have found that neural stem cells

derived from LRRK2-iPS cells can give rise to distinct astrocyte populations. While a deficiency in LRRK2 mutant astrocytes will provide additional clues regarding PD pathology and disease progression, the ability to generate regeneration promoting astrocytes from patients may allow the development of autologous astrocyte therapies.

[Supported by NYSTEM contract C026877]

**(8) TRANSIENT NICHE DERIVED SIGNALS REGULATE SUBVENTRICULAR ZONE TYPE B NEURAL STEM CELL PROPERTIES TO PROMOTE INJURY REPAIR**

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Type B neural stem cells (NSCs) of adult mouse subventricular zone (SVZ) reside in a specialized microenvironment that provides them with various spatiotemporal cues that balance their cycling between quiescence, self-renewal and differentiation states. However, how NSCs shuttle between these states during pathological conditions is poorly understood. Here we show that during the onset of acute subcortical white matter (SCWM) demyelination, NSCs displayed an upregulated symmetric division state generating more NSCs. These NSCs later at the peak of injury exhibit downregulated self-renewal properties and underwent lineage progression to generate regenerative progenitors that initiated SCWM remyelination. Mechanistically, initial alterations in WNT/ $\beta$ -catenin signaling regulate NSC mode of division at the onset of injury (3 days post injury) and these changes are instigated by alterations in expression levels of canonical Wnt3a and non-canonical Wnt5a ligands in SVZ niche. And at the peak of injury (7 days post injury), alterations in SVZ vascular niche perturb Notch signaling in NSCs to drive their lineage progression. Finally, a forced expression of Notch active form in the SVZ during injury rescued the NSC phenotype observed above but also prevented the timely generation of progenitors and as a consequence, the process of remyelination was delayed at a functional level. Our results demonstrate that pathological conditions induce transient changes in SVZ niche that regulate NSC fate to initiate a repair process.

**(9) GENOMIC INSTABILITY LIMITS REPROGRAMMING FOLLOWING NUCLEAR TRANSFER**

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Somatic nuclear transfer (SCNT) enables the rapid conversion of a somatic cell type to an embryonic one within a single cell cycle. Using SCNT, we demonstrate that DNA damage is a limiting factor for successful reprogramming. Transcriptome sequencing reveals that genes involved in DNA damage, such as *Gadd45a* and *Rad51*, are among the most significantly upregulated transcripts in SCNT embryos. Here we show that  $\gamma$ H2AX<sup>+</sup>RPA<sup>+</sup> foci, a marker of DNA damage, become apparent in SCNT embryos following progression through S-phase in the 2<sup>nd</sup> cell cycle, coinciding with the major wave of zygotic gene activation. Notably, SCNT embryos exhibit elevated frequencies of  $\gamma$ H2AX<sup>+</sup>RPA<sup>+</sup> foci relative to parthenotes suggesting that aberrant epigenetic status of the incompletely reprogrammed somatic nuclei may be the cause of DNA damage. *Brca1*, *RPA32* and *Rad51* co-localize with  $\gamma$ H2AX<sup>+</sup> foci, an indication of stalled replication forks. SCNT embryos derived from *Brca1* null oocytes exhibit significant increase in the number of  $\gamma$ H2AX<sup>+</sup>RPA<sup>+</sup> foci. The frequency of DNA damage is dependent on donor cell-type – transferred embryonic nuclei exhibit lower frequency of mitotic segregation errors relative to differentiated cell-types. Our studies show that genetic instability is a major impediment to cellular reprogramming after nuclear transfer, as has previously been observed in iPS generation. Unlike after iPS reprogramming, we can exclude that abnormal transcriptional reprogramming or the limited proliferation potential are cause for DNA damage. These results suggest that genomic stability is intimately tied to cellular identity, and that cellular states outside of a normal physiologically relevant state compromise the integrity of the genome, which is detected by genome surveillance proteins. Therefore, we propose that tumor suppressors (such as *BRCA1*) not only safeguard genomic stability, but are also ‘gatekeepers of cell identity’, suppressing the proliferation of abnormally reprogrammed cells, and perhaps of tumor cells.

## (10) MICRORNA REGULATION OF ADULT NEURAL STEM CELL STATES

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Adult neural stem cells are specialized astrocytes that generate neurons in restricted regions of the mammalian brain. The largest neurogenic region is the subventricular zone, which lines the lateral ventricles. Stem cell astrocytes give rise to new neurons in both homeostatic and regenerative conditions, suggesting that they can potentially be harnessed for regenerating the brain after injury, stroke, or neurodegenerative disease. A key step in recruiting adult neural stem cells for brain repair is to define the molecular pathways regulating their switch from a quiescent to an activated state. MicroRNAs are small non-coding RNAs that simultaneously target hundreds of mRNAs for degradation and translational repression. MicroRNAs have been implicated in stem cell self-renewal and differentiation. However, their role in adult neural stem cell activation is unknown. We performed miRNA profiling of FACS purified quiescent and activated adult neural stem cells to define their miRNA signatures. Bioinformatic analysis identified the miR 17~92 cluster as highly upregulated in activated (actively dividing) stem cells in comparison to their quiescent counterparts. Conditional deletion of the miR 17~92 cluster in FACS

purified neural stem cells in vitro reduced adult neural stem cell activation, proliferation and self-renewal. In addition, mir17~92 deletion led to a selective decrease in neuronal differentiation. Finally, we identify Sphingosine 1 phosphate receptor 1 (s1pr1) as a computationally predicted target of the miR 17~92 cluster. S1pr1 is highly expressed in quiescent neural stem cells. Treatment of quiescent neural stem cells with S1P, the ligand for S1PR1, reduces their activation by 50% and decreases proliferation by 46%. We are currently investigating the functional effect of miR 17~92 deletion on S1PR1 signaling. Together, these data reveal that the miR 17~92 cluster is a key regulator of adult neural stem cell activation from the quiescent state and subsequent proliferation.

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### **(11) IDENTIFICATION OF PUTATIVE ADULT STEM CELL NICHES BY COMPARATIVE EVALUATION OF THE HUMAN AND MOUSE OVARIAN SURFACE AND TUBAL EPITHELIA**

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Ovarian cancer is the most deadly gynecological malignancy. Due to its silent progression nearly 70% of women are diagnosed at advanced, usually incurable stages. The tissue (or tissues) of origin of the most common ovarian cancer, high-grade serous adenocarcinoma, remains insufficiently elucidated. Some studies point to the ovarian surface epithelium (OSE), while others argue for the tubal epithelium (TE) of the uterine (fallopian) tubes. Recently, we have reported the existence of cancer-prone OSE stem cells in the mouse (Flesken-Nikitin et al., *Nature* 495: 241-245, 2013). Such cells mainly reside in the hilum region, the transitional/junctional area between the OSE, mesothelium and TE. To evaluate the relevance of our observations to human biology we have performed a comparative histological evaluation of mouse and human ovaries and uterine (fallopian) tubes. Furthermore, we have successfully established primary cell cultures of human OSE and TE derived from the anatomically defined areas of the ovary and uterine tube. A number of stem cell markers, including ALDH1A, LEF1, AXIN2, active  $\beta$ -CATENIN and KRT5/6 are expressed in the discrete areas of both species. We have also observed distinct morphology and increased proliferative potential of the human hilum OSE, consistent with our earlier mouse studies. Furthermore, ALDEFLUOR positive human hilum OSE express higher levels of stem cell markers KRT5, KLF4, LEF1, and MET, as compared to cells isolated from distal parts of the ovary. Characterization of human TE cells and preparation of human OSE and TE organoids are currently being performed. In sum, our study suggests significant functional similarities between the mouse and human OSE and TE. Further studies

will establish if such similarities are indicative of increased transformation potential of human OSE and/or TE stem cells.

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**(12) BMP MEDIATED CROSS-TALK BETWEEN POLARIZED MACROPHAGES AND HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES**

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Cardiomyocytes (CMs) differentiated from human pluripotent stem cells (hPSC-CMs) represent a promising therapeutic target for clinical applications due to their unique capability of self-renewal and differentiation into cardiovascular lineages. Following cardiac injury, the heart undergoes a dynamic inflammatory response that includes a pro-inflammatory phase followed by a pro-healing phase. These phases are characterized by the presence of distinct subsets of pro-inflammatory macrophages (M1) and pro-healing macrophages (M2a and M2c). Macrophages will inevitably interact with the repair cells, potentially affecting their function and survival. In the present study, we investigate the recruitment of these macrophages by hPSC-CMs, as well as the effect of the macrophages on the cardiac cells.

In order to study the interactions between the hPSC-CMs and inflammatory cells, peripheral blood-derived macrophages were used as a model of cardiac inflammation following an infarct. We quantified the recruitment of each macrophage subtype against hPSC-CM conditioned medium in an inverted Boyden chamber assay. We also cultured hPSC-CMs with macrophage-conditioned medium as well as with macrophages in a transwell system. After analyzing both the inflammatory cells and repair cells, it was found that the subtype of macrophage present was able to affect the gene expression, growth and viability of the cardiac repair cells. We also found a potential involvement of some BMP proteins secreted by hPSC-CMs as well as by macrophages. This is a key preliminary step towards designing engineered cardiac patches that can harness the inflammatory environment in order to improve the survival of hPSC-CMs.

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**(13) 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 frequent heterozygous mutations in Histone H3.3 variant

(*H3FA*) in pediatric glioblastoma including brainstem gliomas and supratentorial gliomas. K27M mutation occurs in the earliest age group and is largely confined to the midline of the brain, mostly pons and thalamus, whereas G34R/V mutation is predominantly found in supratentorial gliomas in older patients, implying different biological entities. However their specific functions are still unclear. Using human embryonic stem (ES) cell system, we show that H3.3K27M expression synergizes with p53 loss and PDGFRA activation in neural progenitor cells (NPCs) derived from human ES cells, resulting in neoplastic transformation. The phenotype, gene expression profile and key histone modifications, as well as *in vivo* behavior of the transformed precursors derived from ES cells are highly similar to those seen in patient tumors. Genome-wide analyses indicate a resetting of the transformed precursors to a developmentally more primitive stem cell state, with evidence of major modifications of histone marks at several master regulator genes. Drug screening assays identified a menin inhibitor as an effective inhibitor of tumor growth *in vitro* and *in vivo*. Our findings prove that K27M mutation is a driver mutation in pediatric glioma and our human ESC-based model is a useful platform to reveal molecular mechanism of gliomagenesis as well as to develop novel therapeutic strategy.

[Supported by NYSTEM contract # C026879]

#### **(14) MODY3 iPSC-DERIVED $\beta$ -CELLS TO MODEL DIABETES DUE TO HNF1A DEFICIENCY**

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MODY3 is a form of Maturity Onset Diabetes of the Young that accounts for 60% of cases of MODY and is caused by mutations of the *HNF1a* gene, leading to impaired  $\beta$ -cell mass and reduced insulin production. HNF1 $\alpha$  is a transcription factor known to play an important role in  $\beta$ -cell development and function but the mechanisms are not well understood. By generating insulin-producing pancreatic  $\beta$ -cells from MODY3-patient's iPSCs, we can characterize the cellular and molecular defects due to HNF1a deficiency and thus get a better understanding of the mechanisms underlying  $\beta$ -cell development, function and survival.

To this purpose, we have generated iPSCs-derived  $\beta$ -cells from patients with heterozygous loss-of-function mutations of the gene *HNF1a*. We also generated *HNF1a* homozygous and heterozygous null mutations in hESCs and hiPSCs by using the genome-editing CRISPR/Cas9 technology. The generation of insulin-producing  $\beta$ -cells from these different cell lines will serve as *in vitro* and *in vivo* model for MODY3. In our *in vitro* studies, we report that iPSCs-derived  $\beta$ -cells from patients with MODY3 have a reduced  $\beta$ -cells generation, insulin-production and glucose response due to *HNF1a* mutations. Transcriptional profiling also showed that expression of genes involved in glycolysis (GCK, GLUT1 and GLUT2) are decreased in MODY3 patient cell lines. Moreover, in agreement with our *in vitro* studies, mice transplanted with iPSCs-derived  $\beta$ -cells from patients with MODY3

have an impaired insulin secretion. By reproducing these results in the isogenic homozygous and heterozygous null mutants, we expect to see similar or more drastic results in terms of  $\beta$ -cell function deficiencies.

**(15) TUMOR MICROENVIRONMENT OF METASTASIS (TMEM) REPRESENTS A STEM CELL NICHE IN BREAST CANCER PATIENTS**

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Cell surface biomarkers CD44, CD24, CD133 and intracellular biomarker ALDH1 have been used to identify breast cancer stem cells (BCSC). BCSC are clinically significant because they are resistant to chemotherapy and they can initiate tumor growth and metastasis in very small numbers. Recent studies suggest that juxtacrine signaling from monocytes and macrophages support BCSC niche. Macrophages represent one of the components of the micro-anatomical sites of hematogenous dissemination of breast cancer cells called Tumor MicroEnvironment of Metastasis (TMEM), which are predictors of metastatic outcome in patients. We hypothesized that TMEM rich tumor microenvironments represent niche for BCSCs, which are prerequisite for systemic dissemination of cancer and metastatic outgrowths.

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

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

Our findings indicate that TMEM-rich microenvironments represent a niche for breast cancer stem cells. Unraveling the mechanisms of TMEM – BCSC interactions may have therapeutic implications for treatment of metastatic disease.

**(16) ENDOTHELIAL CELL-JAGGED2 REGULATES HEMATOPOIETIC STEM AND PROGENITOR CELL MAINTENANCE AND RECONSTITUTION**

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**ABSTRACT:** VE-Cadherin<sup>+</sup>VEGFR2<sup>+</sup> bone marrow endothelial cells (BMECs) regulate hematopoietic stem and progenitor cell (HSPC) homeostasis and reconstitution via secretion of angiocrine factors such as sKitL, SDF-1 and Notch ligand Jagged1, etc. In endothelial cell-specific Jagged1 knockout mice, HSPC self-renewal is compromised yet still present; residual Notch activity still remains in HSPC, arguing for complimentary mechanism of Notch activation. We hypothesize that the endothelial-derived Notch ligand Jagged2 regulate HSPC homeostasis and reconstitution via activation of Notch signaling. To this end, we generated endothelial cell-specific Jag2 knockout mouse models; via *in vitro* coculture experiments and *in vivo* transplantation studies, we have demonstrated that 1) endothelial cell-Jagged2 mRNA is efficiently knocked out from vascular endothelial cells in VE-Cadherin<sup>+</sup> Jag2<sup>fl/fl</sup> (Jag2<sup>ECKO</sup>) mice; 2) Endothelial cell (EC)-Jagged2 is necessary to maintain the normal WBC count and total hematopoietic cell number in the bone marrow; 3) EC-Jagged2 is necessary for HSPC repopulating capacity, as demonstrated by comparative repopulating assays; 4) Notch2 receptor is the main receptor expressed on murine HSPCs, and Hey1 is one of the downstream effectors of EC-Jagged2 induced Notch activity in HSPCs; 5) the role of EC-Jagged2 in maintaining HSPC self-renewal is cell non-autonomous, demonstrating the requirement of a robust vascular niche for normal HSPC function; 6) endothelial cell-Jagged2 is required for hematopoietic recovery and overall mice survival under myelosuppressive conditions such as sub-lethal irradiation and serial 5-fluorouracil injections.

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## (17) A NEW APPROACH TO FOR THE TREATMENT OF PARKINSON'S DISEASE – AN UPDATE

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The lack of cells suitable for transplantation remains a big problem for Parkinson's Disease (PD) therapy. Such cells, if manufactured in defined conditions at high purities would bring relief to those no longer responding to conventional PD therapy. A fundamental characteristic of PD is the progressive and irreversible loss of dopamine-producing neurons. Cell therapy may be a superior treatment when compared to pharmacological or electromechanical therapies. Despite some problems with fetal grafts, cell therapy for PD remains attractive due to the possibility of restoring actual dopamine (DA) neurons capable of integration. Our

discovery enabled the derivation of large numbers of transplantable dopaminergic neurons from human pluripotent stem cells (hPSCs) and opened the door for a truly novel PD therapy. With the help of an award by NYSTEM (contract #C028503), we are able to develop this therapy over the next 4 years to a clinical candidate. Today, we are in year 3 of our award and after our first meeting with the FDA we are now planning and performing pilot studies to inform our pre-IND meeting. We anticipate that we will have completed safety and toxicology testing by the summer of 2017, at which time we will seek IND approval for this new therapy. I will highlight some of product optimizations, namely cell sorting for the cell surface marker CD142 and cell freezing, that we believe will have a large impact on our ability to bring a safe and efficacious therapy to the PD community. Further, I will discuss in detail the challenges we encountered in moving this cell therapy from the lab to the clinic. We believe that sharing our experience with the stem cell community will be of great value to all attendants.

**(18) FGF RECEPTOR SIGNALING DURING THE EMERGENCE OF PLURIPOTENCY IN VIVO**

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Our overarching goal is to determine how pluripotency is established in its native context, within the early mammalian embryo. The pre-implantation period of mammalian early embryonic development is devoted to the specification of two extra-embryonic tissues, the primitive endoderm (PrE) and trophoctoderm (TE), and their spatial segregation from the pluripotent epiblast (EPI), which will give rise to all somatic tissues. We previously demonstrated the requirement of FGF4 for the formation of both EPI and PrE cell lineages. However, it is still unclear how FGF signaling is transduced differently in EPI and PrE progenitors to ensure the generation of balanced numbers of cells for each of these two lineages. To gain further insight, we are want to dissect the unique and overlapping roles of FGFR1 and/or FGFR2 the two receptors expressed during preimplantation development and able to transduce the FGF4 signal.. Our previous single-cell expression profiling studies revealed *Fgfr1* to be expressed in both EPI and PrE progenitors, whereas *Fgfr2* is expressed only in PrE progenitors. Although inactivation of *Fgfr2* alone results in post-implantation lethality, a spectrum of phenotypes observed in *Fgfr1* mutant embryos ranging from pre to post-implantation raise the possibility that FGF signaling might be transduced differently in EPI and PrE through two different receptors and activating distinct downstream effectors. Our data shed light on previously unknown roles for FGF receptors in the establishment and maintenance of pluripotency during early mouse development.

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**(19) PHARMACOLOGICAL REGULATION OF EPIGENETIC MECHANISMS EFFECTS THE REGENERATIVE CAPACITY OF CARDIAC PROGENITOR CELLS**

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A promising cellular source for repairing the diseased heart are endogenous cardiac progenitor cells (CPCs), which contribute to myocyte replacement during normal homeostasis. However, the need to obtain CPCs from heart biopsies, as well as their low numbers within the heart, are impediments in treating severely diseased individuals. As part of efforts to examine pharmacological approaches for optimizing the use of CPCs for myocyte regeneration, we investigated the use of the DNA demethylation reagent 5-azacytidine and the G9a histone methyltransferase inhibitor BIX01294.

5-azacytidine has been widely employed for promoting the myocardial differentiation of both cardiac and noncardiac stem cells. However, the well-characterized activity of 5-azacytidine as a skeletal myogenic inducer has led us to question its value as a tool for cardiac studies. To address this issue, we examined this drug's effect on atrial tissue, which contains cardiac but not skeletal muscle progenitors. 5-azacytidine exposure caused atrial cells to elongate and express the skeletal myogenic transcription factors MyoD and myogenin. 5-azacytidine treatments also promoted the formation of skeletal myocytes from atrial cells, as indicated by dual staining for myogenin and sarcomeric  $\alpha$ -actin.

Our laboratory reported that BIX01294 induces precardiac marker expression by bone marrow-derived stem cells and allowed these cells to undergo myocardial differentiation when subsequently exposed to cardiogenic stimuli. Here, we show that treating CPCs with BIX01294 enhanced their proliferation without changing their molecular profile or compromising their cardiac competency. Moreover, BIX01294 treatments of fully differentiated cardiomyocytes neither changed their cell phenotype nor decreased their contractile activity, which suggests BIX01294, unlike 5-azacytidine, does not possess negative effects that would undermine its use for cardiac repair. Together these data indicate that BIX01294 can serve as an expansion factor for CPCs and may have utility as a reagent that can generate large numbers of native CPCs for treating heart diseases.

## **(20) KERATIN 14 IS A POTENTIAL MARKER OF STEM/PROGENITOR CELLS IN ADULT SALIVARY GLAND**

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Loss of salivary gland function affects millions of people in the U.S. and is caused by various etiologic factors including radiation therapy of head and neck region, auto-immune disease, cytotoxic drugs and aging. Stem cell-based therapy may offer an effective treatment option for these patients; however, such therapies require fundamental knowledge about the identity of salivary gland stem/progenitor cells as well as a clear understanding of the underlying mechanisms of tissue renewal and regeneration. During our first attempt to identify potential stem cells as the slowest cycling cells in mouse submandibular glands, we discovered Keratin14 expressed by a unique cell population with stem/progenitor cell characteristics. Immunofluorescent analysis of sections obtained from developing, adolescent and

adult submandibular glands unveiled subpopulations of K14(+) cells at spatially defined domains in the ductal network. Subsequent genetic labeling and fate mapping of K14(+) cells in adult glands demonstrated that under normal steady-state conditions, these cells self-renew and give rise to differentiated K19(+) ductal cells. In response to severe injury however, K14-expressing progenitors displayed multi-potency by giving rise to both ductal and acinar cells. Our findings strongly indicate that K14 marks a new stem cell population which contributes to homeostasis and regeneration of adult salivary glands.

**(21) ELECTRICAL CONDITIONING ESTABLISHES FUNCTIONAL HUMAN STEM-CELL DERIVED CARDIOMYOCYTES**

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Human stem cell derived cardiomyocytes have unique potential to ameliorate cardiovascular disease. The therapeutic success of this cell type depends critically on their ability to respond to and integrate with the surrounding electromechanical environment. Among the signals present in the myocardium are electric fields, which arise early in development and persist throughout life. Here we show the functional consequences of applying biomimetic electrical signals in cultures of nascent cardiomyocytes differentiated from human ES and iPS cells. Cardiomyocytes were exposed to four electrical stimulation regimes mimicking those found in the human heart: 0.5 Hz (bradycardic), 1 Hz (eucardic), 2 Hz (tachycardic), using unstimulated cells as a control. Exposure to electrical stimulation matured cardiomyocytes, inducing broad changes in cardiac gene expression, protein organization, and sarcomeric ultrastructure, towards a more mature cell phenotype. Uniquely, stem cell derived cardiomyocytes responded to the electric signals by adapting their autonomous beating rate to the rate at which they were stimulated. This adaptive effect was mediated by a) the emergence of a fast-conducting, GJA5+ cell population, and b) KCNH2, a voltage gated potassium channel responsible for repolarization. Blockade of KCNH2 abrogated the differences in induced automaticity and could also inhibit the formation of this adaptive behavior. The rate adaptive effect was robust: persistent over a two-week period and commutative to surrounding unstimulated cardiomyocytes. Conditioned cardiomyocytes were more responsive to physiologic stimuli and more resistant to electrical stress. Together, these data validate electrical conditioning as a method to establish cardiomyocyte function, with implications for arrhythmia, heart development, and stem cell based heart regeneration.

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**(22) CRITICAL ROLE OF ENDOTHELIN RECEPTOR SIGNALING IN FOLLICULAR MELANOCYTE STEM CELLS IN ADULT SKIN**

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Melanocyte stem cells (McSCs) residing in the hair follicle niche are essential for hair pigmentation and have the potential to also regulate epidermal pigmentation. A better understanding of the molecular mechanisms that govern these stem cells holds broad implications in pigmentation disorders including gray hair and vitiligo. In this study, we investigated the role of Endothelin (Edn) signaling in adult McSCs. To elucidate the role of EdnrB signaling in McSCs in vivo, we genetically deleted EdnrB specifically in melanocytes during the hair cycle in adult mice. Loss of EdnrB in melanocytes markedly inhibited McSC proliferation upon anagen induction compared to control mice. In addition, these melanocytes failed to properly express differentiation markers including tyrosinase, which are critical for melanogenesis. These defects were manifested grossly by hair graying in mutant mice. Furthermore, we found that loss of EdnrB led to a reduction of McSCs, suggesting that EdnrB is required for maintenance of McSCs. Conversely, we found that Edn1 overexpression promotes McSC proliferation and differentiation during anagen. Previously, we found that McSCs can exit the follicular niche to generate epidermal melanocytes in response to skin injury or UVB irradiation. In addition to the role of Edn1 during normal homeostasis, we show that Edn1 overexpression in the epidermis promotes the upward migration of McSCs toward the epidermal surface following skin injury. Strikingly, Edn1 overexpression can promote establishment of epidermal melanocytes even in the absence of wounding, which is not observed in control mice. Collectively, our study demonstrates that Edn/EdnrB signaling is a key regulator of McSCs during regeneration of hair melanocytes as well as epidermal melanocytes, revealing a potential novel approach for treating skin pigmentation disorders.

[Supported by NYSTEM contract C026880]

**(23) TARGETED BLVRB GENE KNOCK-OUT IN IPSC CELLS AS MODELS FOR LINEAGE-RESTRICTED HEMATOPOIETIC DIFFERENTIATION**

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Human blood cell counts are tightly controlled within narrow physiological ranges, largely controlled by cytokine-integrated signaling and transcriptional circuits that regulate multi-lineage hematopoietic stem cell specification. Genetic loci influencing blood cell production account for <10% of platelet and red blood cell variability, establishing that the majority of genomic modifier loci remain unelucidated. Human platelet transcriptomic sequencing followed by extended thrombocytosis cohort analyses identified a single loss-of-function *BLVRB* (biliverdin IX $\alpha$  reductase)

mutation causally associated with clonal and non-clonal disorders of enhanced platelet production, and presumably regulating megakaryocyte/erythroid lineage fate *via* oxidation-reduction (redox) functional coupling during the terminal stage of heme degradation. To understand the role of BLVRB in the development of megakaryocytopoiesis and/or erythrocyte development, we introduced a mutation in exon 3 of the *BLVRB* gene in CD34<sup>+</sup>-derived iPSC cells (NCRM1) using a lentivirus/CRISPR construct. Transient expression of single-guide (sg) RNA followed by GFP<sup>+</sup> selection of genetically-modified NCRM1 cells established that high-fidelity deletion strategy was restricted to targeted exon 3 sequences encoding the co-factor/substrate redox functional domain. Four mutant clones were expanded, encompassing 6-, 6-, 17-, or 18-bp deletions resulting in restricted subset(s) of amino acid changes and/or reading frame shifts with or without early stop codons. Immunoblot analysis confirmed attenuated BLVRB expression in three subclones (E2C3, E2E3 and E2G3) compared to wild-type, expression levels suggesting that targeted deletion of the functionally active redox domain could affect protein stability; all subclones harboring BLVRB mutations exhibited disordered proliferative function compared to non-mutated iPSCs, as an initial parameter of cellular endpoint assays. These data establish a reproducible platform for targeted screening of novel gene functions identified in human genetic screens, and when integrated with the human phenotypic screen, implicate the redox coupling of BLVRB in a regulatory function of hematopoietic stem cell lineage fate.

**(24) VASCULAR NICHE DEPENDENT CONVERSION OF ADULT MURINE ENDOTHELIAL CELLS INTO LONG-TERM ENGRAFTABLE HEMATOPOIETIC STEM CELLS**

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Generation of authentic engraftable hematopoietic stem cells (HSCs) from pluripotent stem cells has confronted with hurdles. To circumvent this, we have converted human adult endothelial cells (ECs) into multipotent hematopoietic progenitor cells without transitioning through a pluripotent plight.

To determine whether our conversion strategy of ECs to hematopoietic cells could also generate authentic HSCs, we used murine congenic (CD45.2 into CD45.1) transplant model. To this end, we pioneered an approach to phenocopy the vascular-niche of hemogenic cells, thereby enabling reprogramming of adult murine ECs into engraftable HSCs. Highly purified non-hemogenic adult tissue-specific ECs were transduced with inducible transcription factors FOSB/GFI1/RUNX1/SPI1 (FGRS), and then propagated on serum-free vascular niche monolayers to induce outgrowth of functional and immune-phenotypic HSCs, including their multilineage progenitor cells. Vascular niche produces physiological levels of inductive angiocrine factors, including Notch, BMP and c-Kit pathways that are essential for EC to hematopoietic transition.

These reprogrammed-ECs into HSC (rEC-HSC) from CD45.2 mice acquire colony-forming-cell potential and durably engraft into CD45.1 congenic recipients after primary and secondary transplantation, producing long-term rEC-HSC (LT-HSCs) and myeloid (granulocytic/monocytic, erythroid/megakaryocytic) and lymphoid (T,B and NK cells) progenies.

Notably, while using this approach to reprogram human ECs resulted in formation of scant immature T lymphoid cells (Sandler et al. *Nature* 2014), murine rEC-HSC robustly recapitulate all T and B cells subsets similar to congenic host after 5 months post-transplantation.

Mechanistic studies demonstrate that deployment of angiocrine factors from vascular niche plays a key role in specification of rEC-HSCs. Perturbing Notch and CXCR4 signaling, pathways interfered with specification and expansion of rEC-HSCs. Notably, addition of CXCL12 enhanced conversion efficiency.

Our approach underscores the role of inductive cues from vascular niche in coordinating and sustaining hematopoietic specification and lays a foundation for engineering engraftable autologous true rEC-HSCs and their progenies for treatment of hematological disorders.

**(25) GENERATION OF RETINAL TISSUES FROM HUMAN EMBRYONIC STEM CELLS (hESCS) THROUGH SELF-ASSISTED CELL SORTING**

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Stem cell-based retinal organoid cultures in basic and clinical studies require pure retinal progenitor cells (RPCs) in three dimensions. In retinal cell differentiation from hESCs or hiPSCs in vitro, the cell cultures are frequently a mixture of randomly differentiated cells of many types, and thus the cell differentiation in vitro is in a disorganized manner in contrast to the highly regulated morphogenesis in vivo. Although genetically engineered fluorescence markers are valuable tools for monitoring and isolating specific type of cells, the identification and characterization of the intrinsic progenitor cell properties that can be used for cell isolation are likely to be more relevant to clinical applications. Here we report a method for selecting and isolating hESC-derived RPCs based on their intrinsic properties. The RPCs were self-organized into spheres that are readily detectable under an inverted microscope. Histological and molecular characterizations demonstrated that the spheres highly expressed the RPC markers SIX3, RAX, PAX6 and VSX2. Long-term cultures of the RPCs produced a laminar retinal tissue that highly expressed the cone-specific marker PNA. These findings are expected to have multiple applications in stem cell-based retinal research.

**(26) IMPROVING THE INFORMED CONSENT PROCESS FOR FIRST-IN-HUMAN STEM CELL TRANSPLANT TRIALS FOR PARKINSON'S DISEASE: WHAT ARE THE BARRIERS?**

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Neurorestorative approaches in Parkinson's disease (PD) based upon new stem cell technologies are coming closer. As the first clinical trials of intracerebral stem cell transplantation in PD are imminent, it is critical to consider how best to ensure a genuinely informed consent. We have therefore focused upon possible barriers to

such a goal. We performed a systematic review of the literature using search terms: “informed consent”, “therapeutic misconception”, “Parkinson’s disease”, “bioethics”, “stem cell therapy”, “cell transplant”, and identified the following themes: (1) *Barriers to adequate disclosure of risks and benefits (investigator-dependent)*. In first-in-human trials, risks and benefits are incompletely defined. A substantial literature in fetal tissue transplant in PD is only partially applicable, and the possibility of serious risks remains, including tumor formation, graft rejection, and risks of immunosuppressive agents if administered. (2) *Barriers to understanding clinical research methodology (participant-dependent)*. Research participants may have difficulties understanding concepts integral to clinical research, such as equipoise. There is also strong evidence that the therapeutic misconception influenced participant consent in previous surgical trials in PD. Cognitive dysfunction common in PD may or may not impair understanding of informed consent information, thus complicating evaluation of capacity to consent. (3) *Need for extended consent process (investigator- and participant-dependent)*. The traditional model of a single visit to address informed consent appears to be insufficient to obtain a truly informed consent in upcoming clinical trials that involve complex scientific underpinnings, uncertainty in risks and benefits, and recruitment of participants with a neurodegenerative disorder that commonly affects cognition. Extended informed consent processes to consider include the use of multimedia educational materials, assignment of a “research partner”, and multiple disclosure sessions. However, these interventions will require further studies to determine their effectiveness in enhancing the quality of the informed consent process for first-in-human stem cell transplant trials in PD.

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**(27) CANCER-ASSOCIATED POT1 MUTATIONS LEAD TO TELOMERE DYSFUNCTION AND PROMOTE GENOME INSTABILITY**

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Mutations in protection of telomeres 1 (*POT1*) have emerged repeatedly among top hits identified through whole exome sequencing of patients afflicted by various cancers, including chronic lymphocytic leukemia (CLL), melanoma and glioma. The mutations typically cluster to the OB (oligonucleotide / oligosaccharide binding) folds of *POT1*, which are necessary for the DNA binding activity of the protein. Interestingly, *POT1* mutations appear to be acquired in CLL, but are associated with familial forms of melanoma and glioma. Despite extensive sequencing data, minimal functional analysis has been performed to investigate the *POT1* mutants, and the impact of such mutations on cancer initiation and/or progression remains unknown. By combining genome editing tools with biochemical and cell biological approaches, we show that several cancer-associated *POT1* mutations impair its binding to telomeric ssDNA, triggering an ATR-dependent DNA damage response and replication stress-associated phenotypes. The resulting telomere aberrancies ultimately lead to genomic instability, which augments cellular transformation.

[Supported by NYSTEM contract C026880]

**(28) NF-KB INHIBITION IN ENDOTHELIAL CELLS ENHANCES SELF-RENEWAL AND REGENERATION OF THE HEMATOPOIETIC SYSTEM**

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Hematopoietic stem cells (HSCs) inhabit distinct microenvironments within the adult bone marrow (BM), which govern the delicate balance between HSC quiescence, self-renewal, and differentiation. It has been proposed that quiescent HSCs localize adjacent to BM arteriole endothelial cells (ECs), suggesting that the arteriole vascular niche may be the primary niche maintaining long-term, self-renewing HSCs. Because the BM arteriole vascular niche is composed of tightly-associated perivascular cells, we sought to isolate and examine the capacity of arteriole BM ECs to support the maintenance of *bona fide* HSCs in the absence of confounding perivascular cells. Here, we demonstrate that Akt-activated BM arteriole ECs display the instructive capacity to expand long-term, multi-lineage HSCs *ex vivo*, *in lieu* of complex serum and cytokine supplementation. Based on this observation, we sought to identify effectors downstream of Akt activation in ECs responsible for regulating HSC maintenance *in vivo*. We have found that inhibition of *in vivo* NF-kB signaling in ECs increases the number functional HSCs and accelerates hematopoietic recovery in response to insult, in part, by protecting the vascular niche. Moreover, transplantation of NF-kB-inhibited arteriole BM ECs enhances hematopoietic recovery, protecting mice from pancytopenia-induced death following radiation-induced myeloablation in the absence of a life-saving BM transplantation. Taken together, these data demonstrate that modulating the NF-kB pathway in BM ECs promotes the self-renewal and regenerative capacities of HSCs. Transplantation of properly activated arteriole BM ECs could be used as a therapeutic means to decrease pancytopenias associated with myeloablative treatments to treat a wide array of disease states.

**(29) CYCLIN A2 MEDIATES REPROGRAMMING IN ADULT MAMMALIAN CARDIOMYOCYTES**

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The adult mammalian heart represents an organ with very low abundance of active cycling cells. The annual turnover of cardiomyocytes is about 1% by age of 20, which further shrinks to 0.3% by age of 75 in human. In mammalian heart, cardiomyocytes exit the cell cycle soon after birth. The cell cycle exit coincides with cyclin A2 (*CCNA2*) silencing. In our previous studies, we demonstrated that viral delivery of *CCNA2* induces cardiac regeneration in infarcted hearts of small and large animal models. To explore cell cycle events in these cells, we have optimized culture methods for adult human and mouse cardiomyocytes. After 1 week of

culture, we induced expression of *CCNA2* using adenovirus encoding *CCNA2* cDNA driven by cardiac specific chicken troponinT (cTnT) promoter. Cytokinesis was visualized using time-lapse epifluorescence microscopy after cotransfecting with adenovirus encoding GFP driven by cTnT (cTnT-GFP). Higher events of cytokinesis (~3 fold) were observed in test samples as compared with controls (cTnT-GFP virus only). We then examined the effect of *CCNA2* on dedifferentiation. We detected expression of cardiac precursor marker “Isl” in test samples while it was absent in controls. Immature cardiac markers “non-muscle myosin IIB” and epithelial-mesenchymal transition markers (Vimentin and FSP1) were detected in these cells. We also examined the gene expression of cardiac markers by qPCR and normalized with GAPDH. Gene expressions at 3 weeks of culture compared to day 0 and fold  $\pm$  SEM was determined. We observed decreased expression of adult cardiac markers  $\alpha$ -MHC ( $0.24 \pm 0.12$ ), Ckmt2 ( $0.08 \pm 0.03$ ) and Troponin Tc ( $0.50 \pm 0.02$ ). These observations imply that *CCNA2* mediates dedifferentiation of adult cardiomyocytes *in vitro* to a cardiac progenitor cell phenotype which may re-enter the cell cycle. We are further investigating the potential for these resultant cells to differentiate into functional adult cardiomyocytes *in vitro* and *in vivo*.

### (30) DYNAMICS OF PROLIFERATIVE GERM CELLS DURING EXPANSION AND MAINTENANCE

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In tissues, a pool of stem cells is established during development that maintains the tissue throughout life. A combination of developmental and physiological influences such as suitable niche microenvironment, nutrition and age affect establishment and maintenance of the pool. However, the combined effects of these stimuli on cell cycle regulation remain unclear.

The *C. elegans* germ line provides a simple paradigm for studying the cellular and molecular underpinnings of these influences. The activity of GLP-1 (Notch receptor in germ cells) in response to ligands produced by the niche (distal tip cell, DTC) is required to prevent differentiation and maintain the proliferative fate of germline progenitors. Additionally, during larval stages when the progenitor pool is expanding, the germ line responds to nutrition and nutrient-sensitive pathways like Insulin/IGF and TOR to promote robust cell cycle progression. Once progenitor numbers peak and in the early adulthood (maintenance phase), the progenitors are less sensitive to nutrient cues.

To assess cell cycle dynamics of the progenitor pool, we examined cell cycle parameters at multiple ages, mutants and nutrient conditions. First, we found that germ cells in larvae (expansion phase) cycle faster and have lower average DNA content than adult (maintenance phase). Second, this difference in mitotic indices cannot be attributed to sperm-fated larval cells versus oocyte-fated adult cells. Third, in wild-type adults (but not larvae) the proportion of cells in M/G1/S/G2 phases differs as a function of distance from the DTC. Fourth, under special conditions when expansion occurs in the adult, the dynamics are similar to expansion in larvae. Finally, all these effects vary greatly in cell fate (Notch) versus cell cycle (Insulin) mutants. Taken together, we hypothesize that spatial constraints influence dynamics of cell cycle.

[Supported by NYSTEM contract C026880]

**(31) 3-D CONSTRUCTS—MOLDED VS. PRINTED: THE DIFFERENCES FROM A STEM CELL BASED PERSPECTIVE**

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Additive manufacturing technologies are increasingly being used to replace standard extrusion or molding methods in engineering polymeric biomedical implants, which can be further seeded with stem cells for tissue regeneration. The principal advantage of this new technology is the ability to print directly from a scan and hence produce parts which are an ideal fit for an individual, eliminating much of the sizing and fitting associated with standard manufacturing methods. The question though arises whether devices which may be macroscopically similar, serve identical functions, and are produced from the same material, in fact interact in the same manner with cells and living tissue. Here we show that fundamental differences can exist between 3-D printed and extruded scaffolds which can impact stem cell differentiation and lineage selection. We will show how different manufacturing methods produce features on multiple length scales, and how the differentiation of dental pulp stem cells is affected by both dimensionality and heterogeneity of the surface structures. Furthermore, we will show that surfaces produced by additive manufacturing can be affected differently by various sterilization techniques, which alters cell adhesion and potentially lineage selection.

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**(32) CO-OCCUPANCY OF ENDOTHELIAL GENES BY FLI1 AND SOX17 REPROGRAMS MOUSE AMNIOTIC FLUID CELLS INTO FUNCTIONAL ENDOTHELIUM**

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Endothelial cells (ECs) play essential roles in organ repair and regeneration and therefore can be utilized for regenerative therapies. Several groups, including ours, have generated EC-like cells from pluripotent stem cells and by conversion of differentiated somatic cells. Reprogrammed cells express endothelial markers and

display some EC functions, but the mechanisms through which they can stably adopt the EC identity, and perform EC functions, are unclear. We have been able to reprogram mouse amniotic fluid cells to endothelial cells (RACVECs) by overexpressing the ETS transcription factors, Etv2, Erg and Fli1. Reprogramming does not require transition through a pluripotent state and occurs in cells that initially lack EC markers. In contrast to fibroblast- and pluripotent-derived cells, RACVECs stably express EC markers for weeks in culture and can incorporate into the host vasculature. By analyzing the global transcriptomes and genomic profiles of Fli1 in RACVECs and purified ECs, we have found that Fli1 must co-occupy genomic sites with Sox factors to activate additional EC genes that allow RACVECs to sprout and form tubes *in vitro* and *in vivo*. Specifically, our preliminary data suggest Sox17 allows RACVECs and ECs to perceive extracellular cues and activate EC genes so cells can perform essential EC functions. Our results define a novel interaction between two EC transcription factors and suggest that by using more stringent tests of cell identity, reprogramming can be rationally advanced to yield cells that more faithfully recapitulate the full endothelial program.

[Supported by NYSTEM contract C026878]

### **(33) UNDERSTANDING BETA CELL DYSFUNCTION USING PATIENT INDUCED PLURIPOTENT STEM CELLS**

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Pancreatic beta cell dysfunction can be caused by ER stress, hyperglycemia, inflammation cytokine and genetic mutation in genes encoding beta cell transcription factors. In type 2 diabetes, insulin resistance makes the beta cell produce more insulin, and beta cells gradually lose their function overtime. SUR 1 is a subunit of beta cell ATP-sensitive potassium channel which controls insulin secretion in response to glucose levels. Loss of function mutation in SUR1 gene induces excessive insulin secretion. 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. A stem cell line has been 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. We then modified our beta cell differentiation protocol. We found that addition of EGF induced NKX6.1 expression in PDX1 positive pancreatic progenitors. By the end of differentiation, we were able to obtain up to 50% c-peptide positive cells and half of them were monohormonal and coexpressed beta cell transcription factor PDX1 and NKX6.1. However, no regulation of c-peptide secretion upon glucose stimulation was detected. After transplantation of derived beta like cells in the immunodeficient mice, the grafted cells survived well and the human c-peptide secretion was detected at 2 month after transplantation. The next step is to differentiate the patient stem cell line and its isogenic control into beta cells, and a disease model will be developed to study the beta cell dysfunction caused by excessive production of insulin. This study will add to our understanding

how diabetes progresses, and potentially lead to new ways to prevent and treat the disease.

**(34) METABOLOMIC ANALYSIS FOR THE OPTIMIZATION OF CHONDROCYTE DIFFERENTIATION OF MESENCHYMAL STEM CELLS IN 3D PELLET CULTURE**

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Differentiation of mesenchymal stem cells toward a chondrogenic phenotype has been facilitated through a variety of external stimuli (e.g. mechanical, chemical, electrical). One example of a chemical stimulus, osmotic loading, has been shown to be an effective strategy for growing clinically relevant cartilage grafts from mesenchymal stem cells such as synovium-derived stem cells (SDSCs), leading to increases in bulk mechanical properties and biochemical constituents. Differential metabolite accumulation in varying culture conditions may influence phenotypic development; here we assess the soluble metabolites produced by the fledgling engineered cartilage pellets.

Human bone marrow stem cells ( $1.0 \times 10^6$  cells) were formed into pellets by centrifugation and were cultured for 21 days in their respective media supplemented with L-ascorbic acid and TGF $\beta$ 3. A companion study (Tao et al.) to be reported at this meeting describes complementary proteomics survey results on these pellet cultures as part of a multi-omic approach.

Polar metabolites extracted from engineered cartilage plugs were analyzed by GC-MS (Agilent 7890B/5977A) and identified based on GC retention time and mass spectra as compared to the Agilent Fiehn Library.

These data provide an initial systems-level view of the metabolite profile. Some identified metabolites, were differentially regulated in response to the treatment under hypertonic conditions. For example, preliminary semiquantitative results indicated that two compounds (serine and sorbitol) demonstrated higher abundance in the hypertonic treated samples while some other amino acids (threonine, phenylalanine, and aspartic acid) were more abundant in the isotonic-grown. Sorbitol is known to accumulate in response to osmotic stress in mammalian cells. This observation of accumulation in the hypertonic treatment group serves a positive corroboration of these results.

[Supported by NYSTEM contract C029159]

**(35) PROTEIN PROFILING (HDMS<sup>E</sup>) FOR MONITORING OF CHONDROCYTE DIFFERENTIATION OF MESENCHYMAL STEM CELLS IN 3D PELLET CULTURE IN A MULTI-OMIC APPROACH**

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Mechanical, chemical, or electrical external stimuli can help drive differentiation toward a chondrogenic phenotype. One chemical stimulus, osmotic loading, has been shown to enable an effective strategy for growing clinically relevant cartilage grafts from mesenchymal stem cells such as synovium-derived stem cells (SDSCs),

leading to increases in bulk mechanical properties and biochemical constituents. The proteome of these cultures should reflect phenotypic development.

Protein extraction from developing engineered cartilage pellets is more challenging than most other sample types encountered in proteomics and this preliminary study provided our first analysis of this sample type. In this experiment, proteins were extracted from pellet cultures that had been grown in isotonic (330 mOsM) and hypertonic (400 mOsM) media. Specifically, human bone marrow stem cells were formed into pellets by centrifugation and were cultured for 21 days in their respective media supplemented with L-ascorbic acid and TGF $\beta$ 3.

The most abundant proteins in both low and high osmolality pellets included Fibronectin, Myosin-9, Actin, aortic smooth muscle, and Prelamin-A/C. Fibronectin has a key role in collagen deposition so it is logical that it is present as the most abundant protein in both isotonic and hypertonic treated engineered pellets.

Differential regulation of expression of a number of proteins was detected as a result of hypertonic treatment. For example, triosephosphate isomerase had a ratio of 0.25 for hypertonic/isotonic abundance. Similarly, another glycolytic enzyme, alpha-enolase was downregulated. Versican core protein, an extracellular matrix protein known to disappear developmentally after cartilage development, was downregulated with a ratio of 0.25. Also downregulated were collagen alpha-3(VI) chain and collagen alpha-1(I) chain.

A companion study (Tao et al.) to be reported at this meeting describes complementary metabolomic survey results on these pellet cultures as part of a multi-omic approach.

[Supported by NYSTEM contract C029159]

### **(36) NOVEL ROLE OF PLACENTAL CDX2 CELLS IN CARDIOMYOGENESIS**

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Limited regenerative capacity of the adult mammalian heart is the leading cause of mortality associated with myocardial infarction. Stem-cell based cardiovascular therapies are still inconclusive and an exact cell-type that can be isolated from adult tissues that can generate cardiomyocytes has not yet been identified. Placenta is an ethically compliant and an abundant source of multipotent cells thought to be superior to other adult stem cells in terms of stemness and plasticity. We have previously shown that injury to the maternal heart elicits migration of fetal cells that differentiate into cardiomyocytes *in vivo*. Additionally, we showed that these cells can also differentiate into beating cardiomyocytes *in vitro*. The unique presence of homeodomain protein CDX2 among the migrated cells led us to hypothesize that placental CDX2 could be a novel target that may have cardiogenic potential. In the current study, we confirmed the expression of CDX2 in murine end-gestation placenta using lineage tracing followed by immunostaining with monoclonal anti-CDX2 antibody. Transgenic mouse models allowing a cre-lox recombination strategy were used to label fetal-derived CDX2 cells with GFP. These CDX2-GFP cells were isolated by fluorescence-activated cell sorting (FACS) and studied further. The isolated CDX2 cells showed a round/spherical morphology and subsequently adhered to neonatal cardiomyocyte feeders and displayed various cell morphologies *in vitro*. We further demonstrated that these cells differentiated into

cells that express cardiac markers troponin T and alpha-actinin indicating the cardiomyogenic potential of placental CDX2 cells. The identification of cell types in placenta that can give rise to functional cardiomyocytes is vital to the field of cardiac regeneration as it could potentially allow allogeneic transplantation for cardiac repair. These results suggest that CDX2 cells may represent a specific cell-type that can be isolated from end-gestation placenta which can undergo cardiac differentiation and thus be potentially useful for cardiovascular cell therapy.

[Supported by NYSTEM contract C029565]

**(37) P53 ACTS AS A SWITCH FACTOR TO LICENSE MASTER DIFFERENTIATION GENES EXPRESSION BY TGF-BETA SIGNALS**

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Recent studies have shed light on how the transforming growth factor- $\beta$  (TGF- $\beta$ ) family govern the behaviors of many stem cell populations and interact with master regulators of differentiation. TGF- $\beta$  family member, Nodal, activates mesendodermal differentiation by inducing the expression of master differentiation genes, such as *gooseoid* (*Gsc*). These master regulator genes are often maintained in a quiescent but “poised” state, which may harbor RNA polymerase II at the start site and ready for elongation by appropriate signals. We recently found that nodal-induced Smad4-Smad2/3 and Trim33-Smad2/3 complexes in ESCs can target and activate *Nanog* as well as negative feedback regulators of the nodal pathway in pluripotent ESCs. However, the Smad complexes cannot access *Gsc* and other master differentiation genes in pluripotent ESCs. Smad access to *Gsc* is enabled when ESCs exit pluripotency in LIF-free conditions. But the mechanism remains unknown. An activity should be accumulated as ESCs exit the pluripotency state that enables nodal-driven Smad complexes to target *Gsc* and related genes. Clues as to the identity of this activity came from recent findings that LIF-activated Aurora kinase A (AURKA) inhibits p53 by phosphorylating at two serine sites, and in the absence of LIF, p53 released from this inhibition promotes ESC differentiation. We hypothesize that p53 acts as a determinant of SMAD access to ESC differentiation genes. In support of this hypothesis, we found that p53 and its family members p63 and p73, are required for nodal-driven Smad/Trim33 complexes to bind to and activate *Gsc*. Moreover, the p53 ChIP-seq analysis shows that p53 binds to and upregulates *Wnt8a* and *Wnt3* as ESCs exit from pluripotency. And Tcf3, a downstream mediator of Wnt signaling, directly binds to *Gsc* during EB differentiation. These data supports that p53 induces Wnt expression leading to activation of TCFs, which cooperate with Smad and Trim33 to target mesendoderm differentiation genes.

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**(38) ANALYSIS OF LGR5-POSITIVE CELLS IN MOUSE MAMMARY GLAND**

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Lgr5 is a stem cell marker in multiple adult tissues, and individual Lgr5+ mammary epithelial cells have been reported to be capable of regenerating mouse mammary glands in transplantation assays. Here we investigated the proliferation and differentiation capability of Lgr5+ cells in vitro, and their distribution in mouse mammary gland in vivo. Lgr5+ and Lgr5- cells were sorted from dissociated mouse mammary epithelium by flow cytometry and were cultured in matrigel to evaluate their stem-like properties in vitro. Compared with Lgr5- cells, Lgr5+ cells were more efficient at generating organoids in 3D culture, and the Lgr5+ cell-derived organoids grew to larger size. Moreover, the Lgr5+ cell-derived organoids could be passaged several times and contained cells of both luminal and basal lineages, based on keratin marker expression, and Lgr5+ cells revealed by in situ hybridization. This indicates that Lgr5+ cells have the self-renewal and differentiation capabilities of stem cells in vitro.

The in vivo distribution of Lgr5+ cells was investigated using X-Gal-stained mammary glands from Lgr5-lacZ reporter mice ranging from 5 to 32 weeks old. Lgr5+ cells were found largely in the basal compartment of mammary ducts. In both adolescent and adult mice, we observed a graded distribution of Lgr5+ cells with greater abundance in the nipple-proximal (ventral) ducts than in the lymph-node proximal central region, and fewest Lgr5+ cells in the distal (dorsal) region of the glands. Consistent with this distribution of Lgr5+ cells in vivo, and with the ability of isolated Lgr5+ cells to form organoids in vitro, organoid assays from unsorted mammary epithelial cells prepared from each of the 3 regions (ventral, central, dorsal) revealed a similar gradient of organoid-forming cells with the highest abundance in the nipple-proximal region.

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### **(39) LINEAGE-DEPENDENT FUNCTIONAL ORGANIZATION OF NEOCORTICAL INTERNEURONS**

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GABAergic interneurons provide diverse inhibitions that are essential for the operation of neuronal circuits in the neocortex. However, the mechanisms that control the functional organization of neocortical inhibitory interneurons remain largely unknown. Here we show that the lineage relationship regulates the fine-scale synapse formation and microcircuit assembly of neocortical interneurons. Spatially clustered neocortical interneurons originating from individually labeled progenitors in the medial ganglionic eminence (MGE) and the preoptic area (PoA) preferentially develop electrical, but not chemical, synapses with each other. This lineage-

dependent electrical coupling forms exclusively between the same subtype of interneurons over a range of distances, and promotes action potential generation and synchronous firing between clonally related interneurons. Moreover, this selective coupling facilitates inhibitory chemical synapse formation between clonally related interneurons and the same nearby excitatory neurons. These results suggest a link between the developmental origin of interneurons and their precise functional organization in the mammalian neocortex.

[Supported by NYSTEM contract C026879]

**(40) MODELING THE GENETICS OF HUMAN PANCREATIC DEVELOPMENT AND DISEASE THROUGH GENOME EDITING IN EMBRYONIC STEM CELLS**

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Recent progress in generation of disease-relevant cell types from human pluripotent stem cells (hPSCs) carrying disease associated genetic variants has proven to be highly valuable for studying disease pathophysiology at the cellular level. However, the potential of hPSCs for understanding more complex biological processes such as a multistep developmental process remains uncertain. Here we demonstrate the using of hPSCs for systematic functional validation of 8 pancreatic transcription factors (*PDX1*, *RFX6*, *PTF1A*, *GLIS3*, *MNX1*, *NGN3*, *HES1* and *ARX*) involved in pancreatic development and disease through TALEN and CRISPR/Cas mediated gene editing and hPSC *in vitro* differentiation. By analyzing mutant phenotypes at each differentiation stage, our study was able to define the specific developmental step(s) affected by these mutations, providing the much-needed pathogenic insights currently lacking from human studies. In addition, our study also revealed a number of novel phenotypes, for instance the distinct roles of *RFX6* in regulating the number of pancreatic progenitors and the differentiation into pancreatic endocrine cells at different developmental stages. We have also observed a number of phenotypes that are species-specific, including a divergent role of *NGN3* between mice and humans and a dosage-sensitive requirement of *PDX1* in human pancreatic endocrine development. Thus the hPSC-based genetic model not only complements classic model organisms for studying human development, but also offers the speed and capacity necessary to meet the growing demand for validating disease causality. The systematic probing of transcription factors that regulate pancreatic development also forms the foundation for understanding the transcriptional circuitry involved in human pancreatic development, and provides the information for advancing hPSC-based  $\beta$  cell replacement therapies for treatment of diabetes.

[Supported by NYSTEM contract C026879]

**(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. This new facility should have an important impact on stem cell research, and these new components will serve as an important addition to the statewide stem cell infrastructure.

[Supported by NYSTEM contract C029159]

**(F2) NYSTEM HIGH-THROUGHPUT SCREENING AND CHEMISTRY SHARED FACILITY AT COLUMBIA UNIVERSITY**

Charles Karan<sup>1,7</sup>, Pieter H Bos<sup>4</sup>, Andrea Califano<sup>1,2,7</sup>, Olivier Couronne<sup>1</sup>, Shi-Xian Deng<sup>3</sup>, Xhensila Hyka<sup>1,7</sup>, Hai Li<sup>1,7</sup>, Sergey Pampou<sup>1,7</sup>, Ronald Realubit<sup>1,7</sup>, Alison Rinderspacher<sup>3</sup>, Brent R. Stockwell<sup>4-7</sup>, Arie Zask<sup>4</sup>.

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The NYSTEM High-Throughput Screening and Chemistry Shared Facility is designed to expedite new discoveries in stem cell science and catalyze new translational strategies. The facilities are now fully operational and heavily used, providing stem cell researchers with state-of-the-art tools for mechanistic studies and target discovery, including high-throughput screening, target identification, and medicinal chemistry. An experienced staff leads users through all stages in the process from assay design and development, through data analysis and finally chemical optimization. The screening facility offers access to libraries of small molecules and tools for RNAi experiments. Recently the facility has added multiple cDNA libraries, making these available to our NYSTEM users. The HTS facility has participated in over 44 assays to date. Since 2011 the OCCC has provided 20 research labs with organic synthesis support. Of these projects 18 required synthesis, 3 PK or metabolism studies, and 4 initial assay development. In the CPS

Facility, we have established the capability of designing and synthesizing both chemical probes and therapeutic candidates, using computational and medicinal chemistry. We have been able to start 12 projects. Some of the projects are still ongoing and some were completed successfully. The facility will aid in the prosecution of new stem-cell-related targets and provide new approaches to the treatment of disease. This year, a total of 15 new projects were undertaken in the screening facility and will acknowledge NYSTEM support. For more information on services available from the screening center contact [screening@columbia.edu](mailto:screening@columbia.edu). For information on the chemical probe synthesis facility contact [cps@biology.columbia.edu](mailto:cps@biology.columbia.edu). For more information about the Organic Chemistry Collaborative Center and the services it provides contact [sd184@cumc.columbia.edu](mailto:sd184@cumc.columbia.edu) and/or visit <http://www.columbiamedicine2.org/CPET/r.html>.

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### **(F3) 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. It 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 pathology services offered 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. In the first year of operation, the Stem Cell Pathology Unit has attracted customers from a wide variety of specialties both within and outside of the Cornell University, and continues to evolve to meet the needs of the stem cell research community.

[Supported by NYSTEM contract C024174]

**(F4) THE SKI STEM CELL RESEARCH FACILITY**

Mark J. Tomishima and Isabelle Rivière

The Center for Stem Cell Biology and the Center for Cell Engineering,  
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The SKI Stem Cell Research Facility provides a number of services to the New York State stem cell community including: 1) *training*; 2) *genetic modification* and 3) directed differentiation of human pluripotent stem cells; 4) reprogramming, 5) *shared equipment* and 6) *process development and manufacturing of clinically compatible stem cell products*. Here, we will provide an update to the NYS stem cell community about the ongoing work in our facility. One vignette will describe how the development of our clinical cell product from the manufacturing and analytical point of view has shaped our workflow for the production of other differentiated cell types destined for basic research purposes. We will update the progress on the state of manufacturing in the new cGMP Cell Therapy and Cell Engineering Facility and our efforts to integrate workflows.

[Supported by NYSTEM contract C029153]

**(F5) THE NYU RNAI CORE: AN OPEN ACCESS, HIGH-THROUGHPUT SCREENING RESOURCE FOR FUNCTIONAL GENOMICS**

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Functional genomics and RNAi-based HTS/HCS technologies represent a valuable resource for understanding the biology and regulation of stem cells. The NYU RNAi Core at NYU Langone Medical Center is a state-of-the-art, RNA-interference (RNAi)-based high-throughput/high-content screening platform that supports projects related to stem cell biology, including investigation of the molecular/genetic regulation of embryonic stem cells (ESCs), induced pluripotent cells (iPSCs), and adult stem cells.

Our newly renovated laboratory includes an isolated tissue culture space, climate controlled cold storage room, and dedicated data analysis computers configured with visualization and statistical analysis software packages. Available screening libraries include human and mouse whole genome (with custom updated RefSeq targets) siRNA, and miRNA mimic and inhibitors for reverse genetic screens. Targeted custom libraries will also be available in the near future. Cell-based assays are optimized and developed with the support of Core staff. Our assay instrumentation includes a multimode filter-based plate reader, quad-monochromater, infrared plate reader for In-Cell Westerns, and high content imaging microscopes.

A summary of stem cell related projects will be presented together with our best practices for assay development and optimization, instrumentation and laboratory equipment quality control monitoring, and a comparison of the capabilities of assay instrumentation. We will also describe our newly developed data analysis and visualization workflow which is used to archive and assess screening results and

calculate plate and well based metrics for the selection of potential hits for follow-up screens.

The major goal of the NYU RNAi 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.

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## **PROGRAM AT A GLANCE**

### **THURSDAY, MAY 14, 2015**

1:30 – 2:00	REGISTRATION & POSTER I SET-UP
2:00 – 2:05	OPENING REMARKS
2:05 – 4:05	PLENARY I – NEUROBIOLOGY AND CANCER
4:05 – 4:30	BREAK
4:30 – 6:00	CONSORTIA II & MODERATED Q&A
6:00 – 7:30	POSTER SESSION I & RECEPTION

### **FRIDAY, MAY 15, 2015**

8:30 AM	BREAKFAST & POSTER II SET-UP
9:00 – 11:00	PLENARY II – LINEAGES & DIFFERENTIATION
11:00 – 12:00	POSTER SESSION II
12:00 – 12:30	STEMCELL TECHNOLOGIES PRESENTATION - INTRODUCTION TO INTESTINAL ORGANOID CULTURE
12:00 – 1:00	LUNCH
1:00 – 1:20	PROGRAM UPDATES, POSTER WINNERS ANNOUNCED
1:20 – 2:20	KEYNOTE ADDRESS
2:20 – 2:50	BREAK
2:50 – 4:20	PLENARY III – POTENCY
4:20 – 4:30	CLOSING REMARKS
4:30	ADJOURN