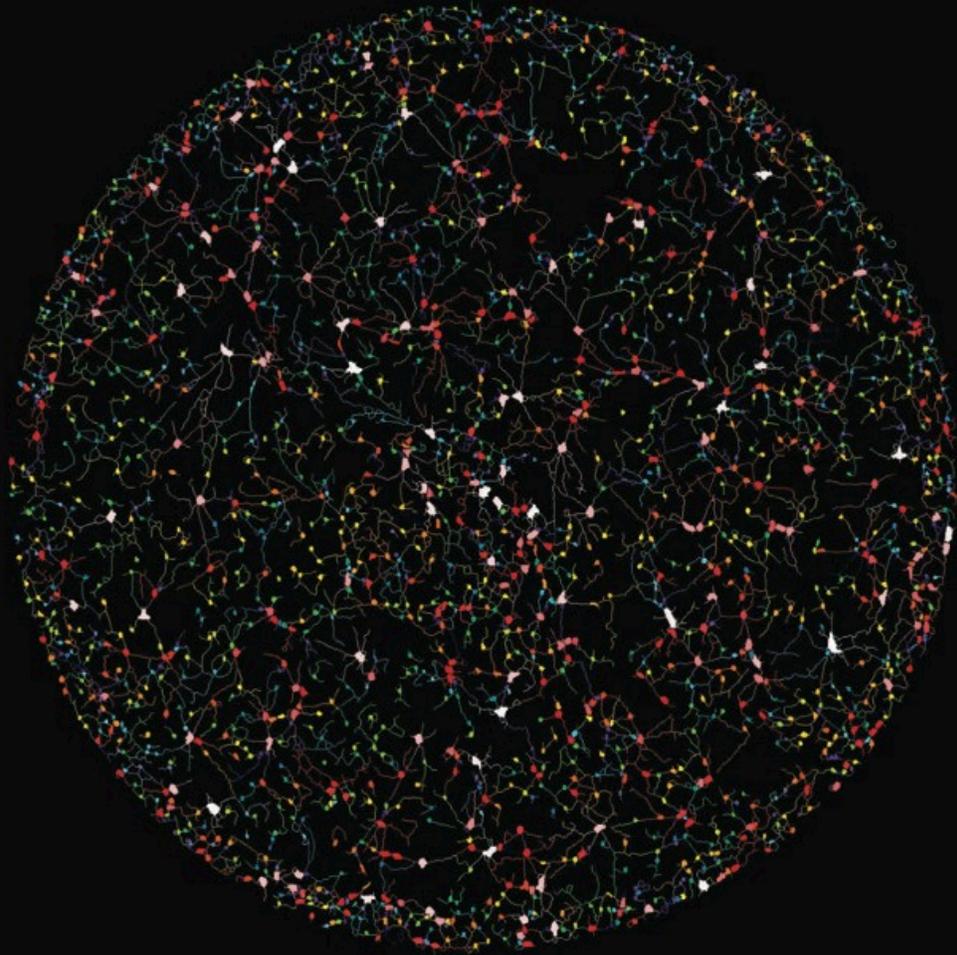


# NYSTEM2014



Gist Croft, Ph.D., The Rockefeller University

May 29 & 30  
The Rockefeller University  
1230 York Avenue, New York, NY



**NYSTEM 2014**  
*Carson Family Auditorium*  
*The Rockefeller University*

**PROGRAM COMMITTEE**

**Emily Bernstein, Ph.D.**, *Icahn School of Medicine at Mt. Sinai*

**Fiona Doetsch, Ph.D.**, *Columbia University Medical Center*

**Paul Frenette, M.D.**, *Albert Einstein College of Medicine*

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

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

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

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Special thanks to

**The Wadsworth Center Photography and Illustrations Unit**

***Survival assay high throughput screening on human neocortical projection***

***neurons differentiated from human ES cells cover photo courtesy of:***

***Gist Croft, the Rockefeller University***

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## GENERAL

### **INFORMATION Sessions**

All sessions will take place in the Carson Family Auditorium.

### **Meals**

Thursday lunch is provided courtesy of Lonza for attendees of the lunchtime presentation.

All others, lunch is on your own – see inside back cover for on campus dining options.

Thursday's reception, sponsored by Stemcell Technologies, will take place in the lobby outside the Carson Family Auditorium.

Friday breakfast will be served in the lobby outside the Carson Family Auditorium.

Friday lunch will be served in the Abby Dining Room and Student and Faculty Club.

### **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. 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 29, 2014**

9:30 AM Registration and Poster Session I Set-Up

10:30 – 10:40 **OPENING REMARKS**  
**Marc Tessier-Lavigne**, *President of the Rockefeller University*

**PLENARY I: Genome Editing & Technology**

10:40 – 12:20 Chair: **Richard Gronostajski**, *University at Buffalo*

10:40 – 10:55 **Patricia E. Bossert**, *Stony Brook University*  
 A STAGING SYSTEM DESCRIBING THE REGENERATION OF A POLYP FROM THE ABORAL PHYSA OF THE ANTHOZOAN CNIDARIAN NEMATOSTELLA VECTENSIS

10:55 – 11:10 **Kacey Ronaldson**, *Columbia University*  
 HUMAN iPS CELL DERIVED CARDIAC MICROTISSUE PLATFORM FOR PREDICTIVE TOXICITY STUDIES

11:10 – 11:25 **Emily Rhodes Lowry**, *Columbia University*  
 EMERGING ROLES OF MOTOR NEURON HYPEREXCITABILITY IN AN iPSC MODEL OF ALS

11:25 – 11:40 **Zhong-Dong Shi**, *Memorial Sloan-Kettering Cancer Center*  
 INVESTIGATING THE ROLE OF GATA6 IN HUMAN ENDODERM DEVELOPMENT BY TALEN AND CRISPR-MEDIATED GENOME ENGINEERING IN HUMAN PLURIPOTENT STEM CELLS

11:40 – 12:00 **Feng Zhang**, *Broad Institute and MIT*  
 DEVELOPMENT AND APPLICATIONS OF CRISPR-CAS9 FOR GENOME EDITING

12:00 – 12:20 **John Schimenti**, *Cornell University*  
 QUALITY CONTROL IN THE GERMLINE AND GENETICS OF HUMAN REPRODUCTION

12:20 – 1:20 **LUNCH**, Sponsored by Lonza for attendees of “Comprehensive Approaches to Stem Cell Research: Stem Cell Growth and Differentiation; Transfection of Embryonic and Adult Stem Cells, and iPS Generation” by **Gregory Alberts**

or ON YOUR OWN: SEE INSIDE BACK COVER FOR OPTIONS

#### 4 | PROGRAM

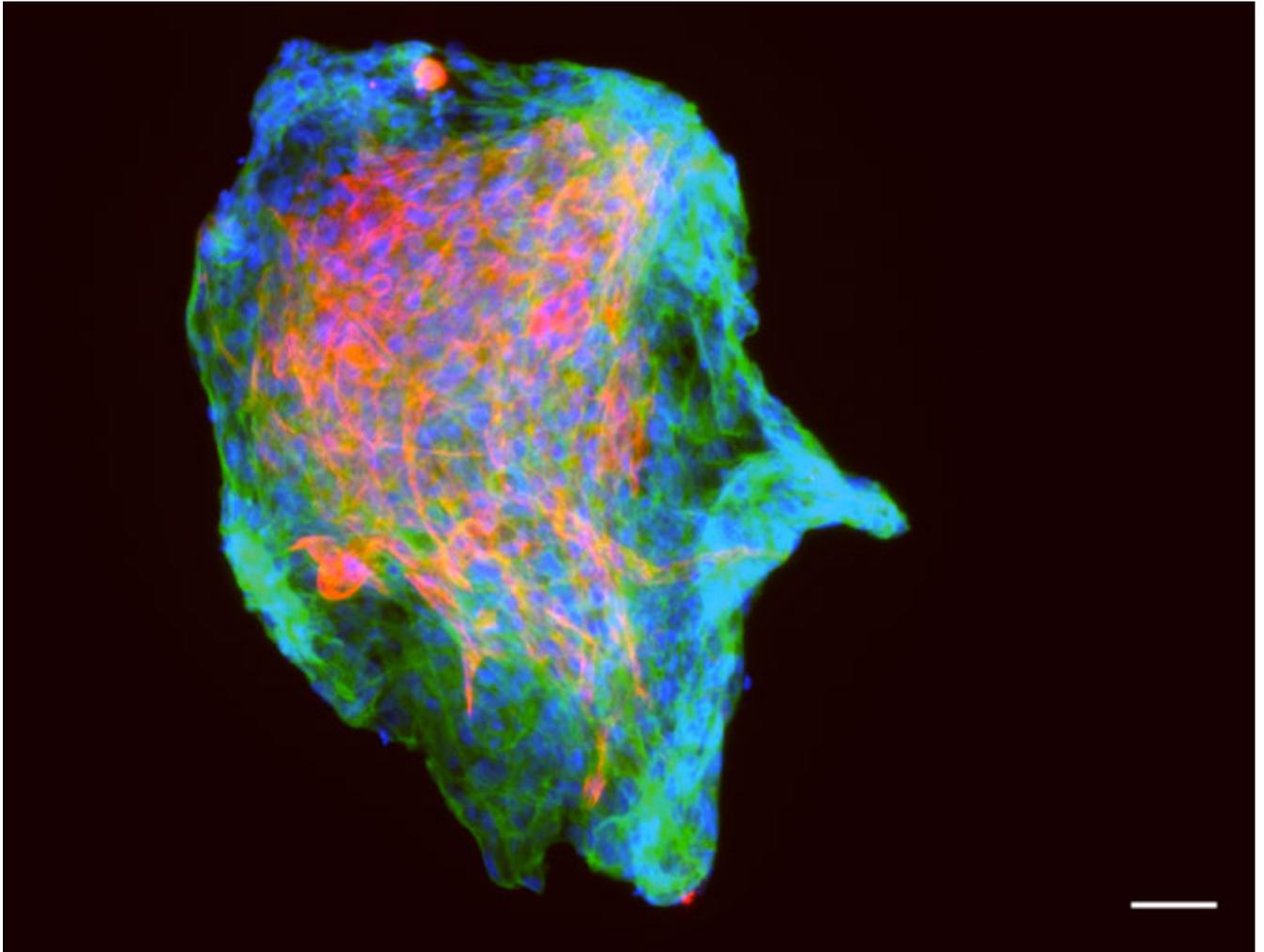
- 1:20 – 1:30     **REMARKS AND INTRODUCTION OF KEYNOTE**  
**Howard Zucker**, *Acting Commissioner, NYS Department of Health*
- 1:30 – 2:30     **KEYNOTE: ASSESSING REPROGRAMMING, DIFFERENTIATION,  
AND CONVERSION WITH NETWORK BIOLOGY**  
**George Q. Daley**, *Children’s Hospital Boston and Harvard Medical  
School*
- 2:30 – 2:45     **TRIBUTE AND PRESENTATION TO LAWRENCE S. STURMAN**
- 2:45 – 3:15     **BREAK AND POSTERS**
- PLENARY II:           Stem Cells in Cancer**
- 3:15 – 4:55     Chair: **Fiona Doetsch**, *Columbia University Medical Center*
- 3:15 – 3:35     **Elaine Fuchs**, *The Rockefeller University*  
SKIN STEM CELLS IN SILENCE, ACTION AND CANCER
- 3:35 – 3:55     **Paul Frenette**, *Albert Einstein College of Medicine*  
HEALTHY AND MALIGNANT HEMATOPOIETIC STEM CELL NICHE
- 3:55 – 4:15     **Viviane Tabar**, *Memorial Sloan-Kettering Cancer Center*  
HUMAN           EMBRYONIC           STEM           CELL-DERIVED  
OLIGODENDROCYTES REMYELINATE THE BRAIN AND RESCUE  
BEHAVIORAL DEFICITS FOLLOWING RADIATION
- 4:15 – 4:35     **Alexander Nikitin**, *Cornell University*  
TRANSITIONAL ZONES: A HOME FOR CANCER-PRONE STEM  
CELL NICHES
- 4:35 – 4:55     **Wenjun Guo**, *Albert Einstein College of Medicine*  
SOX9 IS ESSENTIAL FOR THE MAINTENANCE OF THE KEY CELL  
OF ORIGIN FOR BASAL-LIKE BREAST CANCER
- 5:00 – 6:30     **POSTER SESSION I & RECEPTION**  
Co-sponsored by STEMCELL TECHNOLOGIES

**FRIDAY, MAY 30, 2014**

- 8:30 AM Registration, Breakfast and Poster Session II Set-Up
- 9:00 – 11:00 **SPECIAL SESSION: OVERCOMING BARRIERS TO TRANSLATING BENCH RESEARCH TO THE CLINIC**  
Moderator: **Chris Henderson**, *Columbia University Medical Center*  
Panelists:  
**Michael Basson**, *Nature Medicine*  
**Audrey Chapman**, *University of Connecticut School of Medicine*  
**Pete Coffey**, *UC Santa Barbara and University College London*  
**Joyce Frey-Vasconcells**, *Frey-Vasconcells Consulting; former Deputy Director, Office of Cellular, Tissue, and Gene Therapies, CBER, FDA*
- 11:00 – 12:00 **POSTER SESSION II**
- 12:00 – 1:00 **LUNCH**
- 1:00 – 1:15 **POSTER WINNERS ANNOUNCED**
- PLENARY III: Disease Modeling**
- 1:15 – 3:15 Chair: **Emily Bernstein**, *Icahn School of Medicine at Mount Sinai*
- 1:15 – 1:30 **Ulrich Steidl**, *Albert Einstein College of Medicine*  
 EPIGENETIC REGULATION OF HEMATOPOIETIC STEM CELL FATE BY SPECIAL AT-RICH SEQUENCE BINDING PROTEIN 1 (SATB1)
- 1:30 – 1:45 **Nicole C. Dubois**, *Icahn School of Medicine at Mount Sinai*  
 INVESTIGATING CARDIAC DYSFUNCTION IN DUCHENNE MUSCULAR DYSTROPHY USING HUMAN iPSC-DERIVED ENGINEERED HEART TISSUE
- 1:45 – 2:00 **Cheuk Ka Tong**, *University of California, San Francisco*  
 AXONAL CONTROL OF THE ADULT NEURAL STEM CELL NICHE
- 2:10 – 2:25 **Lúisa Caetano-Davies**, *University of Rochester*  
 RESTORING LOST NEURAL FUNCTION THROUGH MULTIMODAL PRECURSOR DERIVED ASTROCYTES IN A MODEL OF PARKINSON'S DISEASE

6 | PROGRAM

- 2:25 – 2:40     **Donald O. Freytes**, *New York Stem Cell Foundation*  
MACROPHAGE SUBSETS AFFECT THE PHENOTYPE OF  
PLURIPOTENT STEM CELL DERIVED CARDIAC REPAIR CELLS
- 2:40 – 2:55     **Dieter Egli**, *New York Stem Cell Foundation*  
HUMAN OOCYTES REPROGRAM ADULT SOMATIC NUCLEI TO  
DIPLOID PLURIPOTENT STEM CELLS
- 2:55 – 3:15     **Shahin Rafii**, *Weill Cornell Medical College*  
REPROGRAMMING OF HUMAN ENDOTHELIAL CELLS INTO  
ENGRAFTABLE HEMATOPOIETIC MULTILINEAGE PROGENITORS  
BY DEFINED FACTORS AND VASCULAR NICHE INDUCTION
- 3:15             **ADJOURN**



**Cluster of human cardiomyocytes surrounded by supporting cells.**

*Gordana Vunjak-Novakovic, Laboratory for Stem Cells and Tissue Engineering, Columbia University .*

## Keynote Address by George Q. Daley, M.D., Ph.D.

### Assessing Reprogramming, Differentiation, and Conversion with Network Biology

Somatic cell reprogramming, directed differentiation of pluripotent stem cells, and direct conversions between differentiated cell lineages represent powerful approaches to engineering cell identity for applications in research and regenerative medicine. I will describe CellNet, a network biology platform developed to assess the fidelity of cellular engineering and to generate specific hypotheses for improving cell derivations. Analyzing published accounts, we have confirmed that reprogramming to pluripotency is remarkably robust, and found that cells derived via directed differentiation more closely resemble their *in vivo* counterparts than products of direct conversion, as reflected by the establishment of target cell type gene regulatory networks. CellNet analysis, iteratively coupled to experimental validation, provides a rational strategy to enhance cellular engineering.

George Q. Daley, M.D., Ph.D. is the Samuel E. Lux IV Professor of Hematology/Oncology, Director of the Stem Cell Transplantation Program at Children's Hospital Boston, Professor of Biological Chemistry and Molecular Pharmacology and Pediatrics at Harvard Medical School, and an investigator of the Howard Hughes Medical Institute.

Dr. Daley received his bachelor's degree *magna cum laude* from Harvard University (1982), a Ph.D. in biology from MIT (1989), and the M.D. from Harvard Medical School *summa cum laude* (1991). He has served the International Society for Stem Cell Research (ISSCR) as past-President ('07-'08), led the special task forces that produced the ISSCR Guidelines for Stem Cell Research (2006) and Clinical Translation (2008), and is currently the ISSCR Clerk. Dr. Daley has been elected to the Institute of Medicine of the National Academies, American Society for Clinical Investigation, American Association of Physicians, American Pediatric Societies, American Academy of Arts and Sciences, and American Association for the Advancement of Science, and has received the NIH Director's Pioneer Award, the Judson Daland Prize from the American Philosophical Society, the E. Mead Johnson Award from the American Pediatric Society, and the E. Donnall Thomas Prize from the American Society for Hematology. Dr. Daley's research exploits mouse and human disease models to identify mechanisms that underlie cancer and blood disease.

**A STAGING SYSTEM DESCRIBING THE REGENERATION OF A POLYP FROM THE ABORAL PHYSA OF THE ANTHOZOAN CNIDARIAN NEMATOSTELLA VECTENSIS**

Patricia E. Bossert\*, Matthew P. Dunn\* and Gerald H. Thomsen

Department of Biochemistry and Cell Biology, Center for Developmental Genetics, Stony Brook University, Stony Brook, New York

As the sea anemone *Nematostella vectensis* emerges as a model for studying regeneration, new tools will be needed to assess its regenerative processes and describe perturbations resulting from experimental investigation. Chief among these is the need for a universal set of staging criteria to establish morphological landmarks that will provide a common format for discussion among investigators. We have established morphological criteria to describe stages for rapidly assessing regeneration of the aboral end (physa) of *Nematostella*. Using this staging system, we observed rates of regeneration that are temperature independent during wound healing and temperature dependent afterward. Treatment with 25uM lipoic acid delays the progression through wound healing without significantly affecting the subsequent rate of regeneration. Also, while an 11-day starvation before amputation causes only a minimal delay in regeneration, this delay is exacerbated by lipoic acid treatment. Exogenous lipoic acid is a potent antioxidant and modulator of energy metabolism acting at the level of PDH. Taken together, the lipoic acid induced delay during wound healing and synergy between starvation and lipoic acid, point perhaps, to reactive oxygen and/or metabolic flux processes underlying regeneration. Finally, staging the progression of regeneration in amputated *Nematostella* physa based on easily discernible morphological features provides a standard for the field that will provide a reference for the examination of stem cells in the context of *Nematostella* regeneration.

\*Drs. Bossert and Dunn contributed equally to this work.

[Supported by NYSTEM Contract # C028107]

## HUMAN iPS CELL DERIVED CARDIAC MICROTISSUE PLATFORM FOR PREDICTIVE TOXICITY STUDIES

Kacey Ronaldson<sup>1</sup>, Stephen Ma<sup>1</sup>, Timothy Chen<sup>1</sup>, Keith Yeager<sup>1</sup>, Dario Sirabella<sup>1</sup>, Gordana Vunjak-Novakovic<sup>1</sup>

<sup>1</sup>Laboratory for Stem Cells and Tissue Engineering, Department of Biomedical Engineering, Columbia University

Eight out of nine drugs tested in animals fail in clinical studies. Drug responses of cultured human cells are not predictive of whole body physiology. Significant efforts have been invested into developing *in vitro* human models of high biological fidelity for preclinical drug screening and disease modeling. We report the development of a cardiac microtissue component of an integrated heart – liver – vascular drug testing platform. Within our platform, small cardiac organoids are formed by encapsulating iPS-CMs and fibroblasts in a fibrin hydrogel that shrinks around two flexible pillars to form a functionally mature tissue capable of on-line readouts of cardiac health and toxicity. The use of human induced pluripotent stem (hiPS) cells enables the development of personalized treatment regimens previously unattainable. We derived cardiomyocytes from iPS cells by staged molecular induction using RPMI/B27/mTeSR medium supplemented with Ascorbic Acid, CHIR99021, and XAV939. Spontaneous beating is seen 10-12 days after adding CHIR99021, at which point the cells are harvested for microtissue formation. Because the resulting hiPS-CMs are neonatal in phenotype, we investigated the effects of electromechanical conditioning on cell maturation. An improvement in beat frequency and corresponding decrease in peak-to-peak variability and relaxation times were seen as a function of both time and electrical stimulation. Additionally, stimulation enhanced tissue maturation as seen by increased expression of  $\beta$ -MHC, enhanced ultrastructure, a decrease in ET and corresponding increase in MCR. The platform was further validated by demonstrating physiologically relevant responses to drugs, as seen by an increased beat frequency when exposed to  $\beta$ -adrenergic drugs. The platform was also used to investigate models of the inflammatory response. We found that supernatant from M1 macrophage media had the greatest impact on inter-beat variability, while 36 hours of exposure to any kind of macrophage media lead to a statistically significant decrease in beat frequency.

## EMERGING ROLES OF MOTOR NEURON HYPEREXCITABILITY IN AN iPSC MODEL OF ALS

Emily Rhodes Lowry<sup>1-4</sup>, Qinghong Yan<sup>2,5,6</sup>, Damian J. Williams<sup>2,7,8</sup>, Derek H. Oakley<sup>1-4</sup>, Kevin Eggan<sup>9</sup>, Hynek Wichterle<sup>1-4</sup>, Christopher E. Henderson<sup>1-4</sup>.

<sup>1</sup>Project A.L.S./Jenifer Estess Laboratory for Stem Cell Research, <sup>2</sup>Center for Motor Neuron Biology and Disease, <sup>3</sup>Departments of Pathology and Cell Biology, Neurology, and Neuroscience, <sup>4</sup>Columbia Stem Cell Initiative, <sup>5</sup>Department of Systems Biology, <sup>6</sup>Department of Biochemistry and Molecular Biophysics, <sup>7</sup>Columbia Electrophysiology Core, <sup>8</sup>Department of Physiology and Cellular Biophysics, Columbia University Medical Center, New York, NY. <sup>9</sup>Harvard Stem Cell Institute, Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA.

Amyotrophic lateral sclerosis (ALS) is a devastating, uniformly fatal adult-onset neurodegenerative disorder with no clear etiology. Patients experience worsening symptoms of weakness as the motor neurons (MNs) that govern voluntary movement degenerate, and typically die within 3-5 years of diagnosis. Most cases of ALS are sporadic; however, a minority of patients develop a familial form involving a disease-triggering mutation in one of several known genes, including C9ORF72, SOD1, TDP-43, FUS, and ANG. The downstream events that culminate in MN degeneration are largely unknown, but there are early increases in MN excitability that make ALS MNs more likely to respond to incoming stimulation from the excitatory neurotransmitter glutamate. Accordingly, by comparing iPSC-derived MNs from familial ALS patients with isogenic control lines, where the disease-causing mutation has been corrected by TALENS, we have shown that ALS MNs respond more strongly to and are slower to recover from glutamatergic stimulation than controls.

One possibility is that hyperexcitability could make MNs more vulnerable to excitotoxic cell death, in which excessive glutamatergic stimulation causes fatal  $Ca^{2+}$  imbalances within the cell. In several models of ALS, we have uncovered selective down-regulation of RBFOX1, an RNA-binding protein that is known to regulate neuronal excitability. We have also found that mice lacking RBFOX1 showed robust motor impairments that mirror those in ALS mice. Importantly, they also exhibited hyperexcitability in the MNs involved in hindlimb movement. The goal of this project is to evaluate the causal relationship between changes in RBFOX1 expression and changes in MN excitability, and to determine how changes in MN excitability ultimately lead to MN death. To accomplish these goals, we are taking advantage of a broad range of iPSC lines, enabling high-throughput analyses in live human ALS MNs that would otherwise be inaccessible in patients.

[Supported by NYSTEM contract numbers C0236715]

## INVESTIGATING THE ROLE OF GATA6 IN HUMAN ENDODERM DEVELOPMENT BY TALEN AND CRISPR-MEDIATED GENOME ENGINEERING IN HUMAN PLURIPOTENT STEM CELLS

Zhong-Dong Shi<sup>1</sup>, Miguel Crespo<sup>2</sup>, Qing Li<sup>1</sup>, Nipun Verma<sup>1</sup>, Shuibing Chen<sup>2</sup>, Danwei Huangfu<sup>1</sup>

<sup>1</sup>Developmental Biology Program, Sloan Kettering Institute, New York, NY and <sup>2</sup>Department of Surgery, Weill Cornell Medical College

There are two distinct endoderm lineages, the extraembryonic endoderm (ExEn) and the definitive endoderm (DE), in early mammalian development. At the blastocyst stage prior to implantation, the ExEn forms the primitive endoderm epithelial layer that separates the epiblast from the blastocoel cavity. The embryonic DE arises at gastrulation from the epiblast following ingression of cells through the anterior primitive streak. Both endoderm lineages play crucial roles in tissue patterning and organ formation during development. In the mouse, *Gata6* is indispensable for ExEn formation and overexpression of *Gata6* in mESCs induces ExEn differentiation. However, there has been no direct evidence reported demonstrating similar role of *GATA6* in human ExEn differentiation. In addition, whether and how *GATA6* contribute to DE differentiation has not been investigated.

In this study, we investigate regulation of human endoderm specification by both inducible over-expressing *GATA6* and by CRISPR-mediated *GATA6* knockout in hPSCs. Doxycycline-inducible *GATA6* hPSC lines were established by TALEN-mediated gene targeting at the *AAVS1* locus. We demonstrate that expressing *GATA6* can significantly improve DE differentiation (~95% CXCR4+/SOX17+/FOXA2+ cells) in the presence of Activin A, a member of the TGF- $\beta$  superfamily known to promote hPSC differentiation into DE. Importantly, these induced DE cells (iDE) can be further efficiently differentiated into the pancreatic and intestinal lineages *in vitro*. Moreover, in the absence of Activin A treatment, *GATA6* expression promotes ExEn differentiation, evidenced by strong expression of ExEn markers *SOX7*, *LAMB1*, and *PDGFR $\alpha$* , but not DE markers such as *CXCR4*. Next we will test whether *GATA6* is required for ExEn and/or DE differentiation by differentiating *GATA6*-null mutant hESCs that we are currently generating using CRISPR technology. Our findings not only provide insights into early human endoderm development, but may also provide new avenue to efficiently generate ExEn and DE cells for drug screening, tissue engineering and cell replacement therapy.

## **DEVELOPMENT AND APPLICATIONS OF CRISPR-Cas9 FOR GENOME EDITING**

Feng Zhang

Broad Institute and MIT, Cambridge, MA

The Cas9 endonuclease from the microbial adaptive immune system CRISPR can be easily programmed to bind or cleave specific DNA sequence using a short RNA guide. Cas9 is enabling the generation of more realistic disease models and is broadening the number of genetically-tractable organisms that can be used to study a variety of biological processes. The Cas9 nuclease can also be modified to modulate transcription, alter epigenetic states, and track the dynamics of chromatin in living cells. In this presentation we will look at the latest developments and applications of the Cas9 nuclease for understanding the function of the mammalian genome. We will also look at the on-going challenges as well as future prospects of the technology.

**QUALITY CONTROL IN THE GERMLINE AND GENETICS OF HUMAN REPRODUCTION**

John Schimenti, Ewelina Bolcun-Filas, Vera Rinaldi, Priti Singh

Cornell University, Ithaca, NY

Germ cells are the ultimate stem cells. Fertility, offspring health, and species success depends on the production of gametes with intact genomes. In meiosis, quality control checkpoints exist to eliminate gametes with DNA damage or unpaired chromosomes. Exploiting a variety of mouse mutants, we have characterized the DNA damage checkpoint pathway operating during female meiosis. Consistent with the unparalleled sensitivity of germ cells to DNA damage, this pathway uses genetic redundancy to minimize the production of genetically defective oocytes. While this stringent pathway is important for genetic quality control, it also contributes to infertility of cancer patients and individuals with genetic defects in meiosis. We have used genetic manipulations to circumvent the checkpoint control, and are developing strategies to temporarily inhibit the checkpoint pathway to preserve fertility of cancer patients undergoing genotoxic therapies. Additionally, we are embarking on systematic identification of infertility alleles in the human population using mouse modeling via genome editing. In sum, we aim to elucidate the genetics of human infertility, and to understand the mechanisms by which error-free gamete production are maintained.

[Supported by NYSTEM contracts CO24174 and CO26442].

## **STEM CELLS IN SILENCE, ACTION AND CANCER**

Elaine Fuchs

Howard Hughes Medical Institute, The Rockefeller University, New York, NY

Stem cells (SCs) have the ability to self-renew long term and differentiate into one or more tissues. Typically, SCs are used sparingly to replenish cells during normal homeostasis. However, even SCs that are quiescent must be able to respond quickly to injury in order to fuel rapid tissue regeneration. How SCs balance self-renewal and differentiation is of fundamental importance to our understanding of normal tissue maintenance and wound repair. Increasing evidence suggests that the regulatory circuitry governing this balancing act is at the root of some types of tumors both in mice and in humans.

The skin is an excellent model system to understand how SCs function in normal tissue generation and how this process goes awry in cancer. We've identified SC niches within the epidermis, hair follicle, sebaceous glands and sweat glands. We've learned that different niches become activated in response to different types of injury, and that during normal homeostasis, SC behavior is controlled not only through cues received from their microenvironment but also through signals emanating from their differentiating lineages. We've been dissecting how extrinsic signaling to SCs trigger a cascade of transcriptional changes that govern SC activation during tissue development, homeostasis and hair regeneration. Our findings provide new insights into our understanding of the process of SC activation, and in so doing have revealed mechanisms which are also deregulated in a variety of different human cancers. Our goal is to understand how SCs start and stop making tissue, and how this changes in cancer. Our recent discoveries on this topic have led us to the realm of identifying and characterizing cancer SCs (tumor-initiating cells) of squamous cell carcinomas (SCCs) of the skin. We developed a new method to knockdown genes specifically in skin and oral progenitors, enabling us to screen not only the differences between cancer and normal SCs, but also the myriad of gene alterations surfacing from the Human Cancer Sequencing project. Our screens have illuminated new oncogenes and tumor suppressors for SCCs, among the most prevalent and life-threatening cancers world-wide that include cancers of lung, esophagus, breast, cervix, prostate, throat and oral tissues. Our findings are unearthing new targets for cancer therapeutics.

**HEALTHY AND MALIGNANT HEMATOPOIETIC STEM CELL NICHE**

Paul S. Frenette

Ruth L. and David S. Gottesman Institute for Stem Cell and Regenerative Medicine Research, Albert Einstein College of Medicine, New York, USA

The identification of niche cells in the bone marrow has proved to be a challenging undertaking due to the complexity of its cellular constituents, the paucity of specific markers to accurately separate stromal cells, and its poorly accessible location in calcified bone. Several recent studies have indicated that the vasculature of the bone marrow may form major niches for maintaining the delicate balance between self-renewal and differentiation of hematopoietic stem cells (HSCs). Our laboratory has previously found a key role for nerve fibers from the sympathetic nervous system (SNS) in regulating HSC migration. These nerves are closely associated with blood vessels in many tissues. Through investigations to characterize the stromal cell targeted by the SNS, we have identified Nestin<sup>+</sup> mesenchymal stem cells (MSC) as a putative niche cell. Evidence that Nestin<sup>+</sup> MSC are niche cells include the significant histological association of HSC and MSC near blood vessels of the marrow, the enrichment in “niche factor” expression, including CXCL12, SCF, Angiopoietin-1, and VCAM-1 in Nestin<sup>+</sup> cells, and the selective regulation of these factors upon mobilization. While adrenergic signals downregulate the expression of genes that retain HSC in the bone marrow, CD169<sup>+</sup> macrophages have the opposite effect; they secrete one or more soluble factors that promote CXCL12 expression in Nestin<sup>+</sup> cells. Further recent studies have revealed distinct Nestin<sup>+</sup> cell subsets wherein the NG2<sup>+</sup> pericyte-like fraction is associated with arterioles and dormant HSCs whereas the reticular-like LepR<sup>+</sup> subset is associated with arterioles and more proliferative HSCs. The complexity of the niche will be illustrated by recent data suggesting a contribution of a differentiated progeny, the megakaryocyte, in promoting HSC quiescence in a distinct niche via CXCL4-mediated feedback loop. Implications of the bone marrow microenvironment in malignancies such as acute myelogenous leukemia will also be discussed.

## **HUMAN EMBRYONIC STEM CELL-DERIVED OLIGODENDROCYTES REMYELINATE THE BRAIN AND RESCUE BEHAVIORAL DEFICITS FOLLOWING RADIATION**

Viviane Tabar

Memorial Sloan-Kettering Cancer Center

Radiation therapy to the brain is a powerful tool in the management of many cancers, but it is associated with significant and irreversible long term side effects, including cognitive decline and impairment of motor coordination. Depletion of the oligodendrocyte progenitor pool<sup>1</sup> and demyelination are among the major long term pathological findings in irradiated brains in rats and humans<sup>2,3</sup>. These effects are more pronounced in the younger population<sup>4</sup> and highly impact therapeutic options<sup>5</sup>. Here we model a clinical course of whole brain radiation in young rats, which exhibit cognitive and motor balance deficits associated with loss of immature oligodendrocytes and demyelination. We also develop a protocol for the derivation and enrichment of functional oligodendrocyte precursors. Bilateral transplantation in the corpus callosum resulted in widespread migration across the white matter tracts with evidence of repletion of the olig2+/O4+ progenitors and remyelination in the cerebral hemispheres but not in the cerebellum which had no evidence of human cells. This structural repair was associated with significant improvement on cognitive testing, but persistent motor incoordination. The grafted cells maintained full commitment exclusively to the oligodendrocyte lineage without giving rise to other glial or neuronal phenotypes. Additional transplants in the cerebellum resulted in its remyelination and the reversal of the motor coordination deficit, thus demonstrating direct association between regional remyelination and behavioral improvement. There were no teratomas, excessive proliferation or adverse side effects following grafting, suggesting considerable potential for human translation. This is the first report to demonstrate extensive remyelination of the brain and rescue of cognitive and motor deficits following a full course of radiation. It paves the way for implementing brain repair as a means of improving quality of life post radiation or even as a potential tool that allows more effective radiation dosing and possibly a more favorable therapeutic ratio for brain radiation, especially in the young patient.

**TRANSITIONAL ZONES: A HOME FOR CANCER-PRONE STEM CELL NICHES**

Alexander Yu. Nikitin

Department of Biomedical Sciences and Cornell Stem Cell Program, Cornell University, Ithaca, New York, USA

Epithelial transitional zones, also known as junction areas, are defined as regions of a connection between two types of epithelia. Location of adult stem cells in junction areas was demonstrated definitively for the limbus region, a narrow transitional zone between the cornea and bulbar conjunctiva, and for the gastro-esophageal junction. A number of transitional zones, such as squamo-glandular junctions of the uterine cervix, the anus and the stomach, are predisposed to cancer. However, it remains unclear whether transitional zone cancers arise from stem/progenitor cells. Recently, we have identified the hilum region of the mouse ovary, the transitional/junction area between the ovarian surface epithelium (OSE), mesothelium and tubal epithelium, as a previously unrecognized stem cell niche of the OSE. OSE stem/progenitor cells of the hilum region have increased transformation potential, and may be the cell of origin of epithelial ovarian cancer. Our current studies support this possibility, and suggest that susceptibility of other transitional zones to malignant transformation may also be explained by presence of still unknown or insufficiently defined cancer-prone stem cell niches.

[Supported by NYSTEM contract C028125]

## **SOX9 IS ESSENTIAL FOR THE MAINTENANCE OF THE KEY CELL OF ORIGIN FOR BASAL-LIKE BREAST CANCER**

Wenjun Guo, John R. Christin

The Ruth L. and David S. Gottesman Institute for Stem Cell Biology and Regenerative Medicine, Department of Cell Biology, Albert Einstein College of Medicine, New York, NY

Luminal Progenitors have been demonstrated as the cell of origin for BRCA1-mutant basal-like breast cancer in both mouse models of the disease and human patient samples. Despite this, a master regulator responsible for the maintenance of luminal progenitor population has yet to be identified. Recently, we found that Sox9 is capable of reprogramming differentiated mammary epithelia to a luminal progenitor-like fate *in vitro*. This suggests that Sox9 may control the homeostasis of luminal progenitors in the normal mammary gland.

To better understand the distribution of Sox9 in the endogenous gland we used a BAC from the GENSAT project to create a Sox9-eGFP reporter. This revealed a bimodal distribution of expression within the luminal compartment and a single positive peak in the basal compartment. When cells from these reporter animals' luminal population were sorted into Sox9+ and Sox9- luminal fractions only the Sox9+ fraction had the ability to form acini *in vitro* demonstrating that the Sox9+ fraction contains the entire luminal progenitor population and that of Sox9 expression is suppressed in mature luminal cells.

To further investigate Sox9's role in the mammary gland we crossed Sox9-floxed mice with MMTV-iCre mice resulting in a mammary gland specific knockout beginning at three weeks of age. When these animals are examined at eight to twelve weeks of age there is severe depletion in the number of luminal progenitor cells and similar loss of acini forming ability *in vitro*.

In addition to shedding light on normal mammary gland development these findings also have implications on the development of differentiation-based therapy for BRCA1-mutant basal-like breast cancer. Forcing these cancerous cells to differentiate by inhibiting Sox9 expression or activity may be a novel approach to treat this currently difficult to treat type of breast cancer.

[Supported by NYSTEM contract # C028109]

## EPIGENETIC REGULATION OF HEMATOPOIETIC STEM CELL FATE BY SPECIAL AT-RICH SEQUENCE BINDING PROTEIN 1 (*SATB1*)

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Hematopoietic stem cells (HSCs) possess extensive self-renewal capacity and the ability to commit to multilineage differentiation, thereby providing a life-long supply of mature peripheral blood cells. Cell fate decisions of self-renewal or differentiation-commitment are tightly regulated by the interplay of transcription factors in HSCs during tissue maintenance, regeneration and repair. We have uncovered Special AT-rich sequence-binding protein 1 (*Satb1*) as a novel regulator integrating different HSC functions by promoting quiescence and repressing differentiation commitment through promoting symmetric self-renewal divisions of HSCs. We utilized a *Satb1*-deficient murine model system to determine *Satb1*-dependent HSC functions and molecular regulation by performing congenic stem cell transplantation, cell cycle and division analyses, in vivo tracing of HSC commitment, asymmetric division assays, and clonogenic differentiation cultures. Our results show that HSCs lacking *Satb1* display defective self-renewal, are significantly less quiescent and have significantly increased and accelerated differentiation commitment caused by reduced symmetric self-renewal and increased symmetric differentiation divisions. Mechanistically, we found that *Satb1* represses the expression of *c-Myc* and *Numb*, two factors which are known to be involved in HSC activation. The *Numb* and *Myc* loci, which are occupied by *Satb1* in wild type HSCs, showed a significant increase of activating histone marks consistent with derepression in absence of *Satb1*. Global DNA cytosine methylation analysis uncovered that *Satb1* KO HSCs harbor premature differentiation-specific DNA methylation changes which closely resemble the pattern found in differentiation-committed multipotent progenitor cells. Finally, we found that *Satb1* can be retained at key loci during mitosis and mediate their immediate transcriptional activity upon cell division, suggesting a role for *Satb1* in regulating appropriate temporal gene expression during stem cell division.

In summary, our findings uncover *Satb1* as a novel regulator of HSC fate through integrating different HSC functions, molecularly mediated by modulating the epigenetic make up of dividing HSCs.

[Supported by NYSTEM contracts # C024306, C026416, C028116, and C024172]

## **INVESTIGATING CARDIAC DYSFUNCTION IN DUCHENNE MUSCULAR DYSTROPHY USING HUMAN IPSC-DERIVED ENGINEERED HEART TISSUE**

Nicole C. Dubois<sup>1</sup>, Damelys Calderon<sup>1</sup>, Christoph Schaniel<sup>1</sup>, Eric A. Sobie<sup>1</sup>, Kevin Costa<sup>1</sup>

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Duchenne Muscular Dystrophy (DMD) is a genetic lethal disorder, affecting approximately 1 out of 2400 male infants worldwide. For a long time, DMD was considered predominantly a skeletal muscle disease, clinically associated with progressive muscle degeneration and respiratory problems. Cardiac complications in DMD became more prominent only recently as the life of DMD patients could be prolonged with improved therapy for the skeletal and respiratory systems. Cardiac abnormalities in DMD patients consist of arrhythmias and dilated ventricular cardiomyopathy with extensive fibrosis. About 95% of patients with DMD develop cardiac complications by 20 years of age and for 20% of these patients the cardiac impairment is lethal. However, due to the previous focus on skeletal and respiratory defects, the specific mechanism of the cardiac defects of this disease are still substantially understudied.

Several mouse models have been used to study DMD, the most prevalent being the mdx mouse. However, these mice fail to accurately recapitulate the human disease, displaying only a mild phenotype with near to normal life span. While significant insights have been obtained from these studies, the field is currently missing a robust model to conduct molecular, cellular and functional studies of the cardiac defect of DMD in a physiological model of human cells.

To address this problem we have generated a panel of hiPSC lines from DMD patients and non-affected family members. Using directed differentiation approaches we have differentiated the hiPSC lines to obtain cardiovascular cell types (cardiomyocytes, endothelial and fibroblast cells). Using bioengineering approaches we will generate functional cardiac tissues composed of these different cells types. These 3D tissues more accurately reflect the intricate interplay between multiple cells within an organ and thus present an ideal model to investigate the cellular and molecular mechanisms underlying the cardiac defects of this complex and fatal disease in human cells.

**AXONAL CONTROL OF THE ADULT NEURAL STEM CELL NICHE**

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The ventricular-subventricular zone (V-SVZ) harbors the largest population of neural stem cells (NSCs) in the adult mammalian brain. How the brain's mature neuronal circuitry influences the activity of adult NSCs remains largely unknown. We show that serotonergic (5HT) axons originating from a subpopulation of neurons in the raphe form an extensive plexus on most of the ventricular walls. Electron microscopy revealed specialized contacts between 5HT axons and NSCs (B1) or ependymal cells (E1). A transsynaptic viral tracer injected into the raphe also labeled the B1 and E1 cells. Electrophysiology showed that activation of the 5HT receptor 2C in B1 cells induced small inward currents. Intraventricular infusion of 5HT<sub>2C</sub> agonist or antagonist increased or decreased V-SVZ proliferation, respectively. These results indicate that supraependymal 5HT axons directly interact with NSCs to regulate neurogenesis.

## RESTORING LOST NEURAL FUNCTION THROUGH MULTIMODAL PRECURSOR-DERIVED ASTROCYTES IN A MODEL OF PARKINSON'S DISEASE

Luísa Caetano-Davies<sup>1,4</sup>, Chung-Hsuan Shih<sup>1,3</sup>, Jennifer Stripay<sup>2</sup>, Heather Natola<sup>2</sup>, Michelle Lacagnina<sup>1</sup>, Kelly Bisciotti<sup>1</sup>, Nuley Seo<sup>1</sup>, Mark Noble<sup>1,4</sup>, Chris Proschel<sup>1,4</sup>

University of Rochester; <sup>1</sup>Department for Biomedical Genetics, <sup>2</sup>Neuroscience Graduate Program, <sup>3</sup>Department of Pathology, <sup>4</sup>Stem Cell and Regenerative Medicine Institute. 601 Elmwood Avenue, Rochester NY14642, U.S.A.

Both traumatic injuries and degenerative diseases of the central nervous system (CNS) present a multitude of problems that need to be addressed in order to restore normal CNS function. These problems include excitatory neurotoxicity, loss of synaptic connections, disrupted neurite outgrowth, neuroinflammation, and cell death. As many of the processes that require remediation in the damaged CNS are controlled during normal development by astrocytes, we hypothesized that transplantation of the appropriate type of astrocytes into the degenerating CNS will promote recovery by limiting detrimental processes and enhancing tissue regeneration.

In the particular case of Parkinson's disease (PD) besides dopaminergic neuron loss, it also includes other pathological changes, like loss of additional neuronal populations. As a means of addressing multiple pathological changes with a single therapeutically-relevant approach, we employed delayed transplantation of a unique class of astrocytes (called Multimodal Precursor-derived AstroCytes, or MPACs), that are generated *in vitro* by directed differentiation of glial precursors. MPACs produce multiple agents of interest as treatments for PD and other neurodegenerative disorders, including BDNF, GDNF, neurturin and IGF1. MPACs also exhibit increased levels of antioxidant pathway components, including high levels of NADPH and glutathione. Delayed MPAC transplantation into the 6-hydroxydopamine lesioned rat striatum restored tyrosine hydroxylase expression and promoted behavioral recovery. MPAC transplantation also rescued pathological changes not prevented in other studies, such as the rescue of parvalbumin+ GABAergic interneurons. Consistent with expression of the synaptic modulatory proteins thrombospondin-1 and 2 by MPACs, increased expression of the synaptic protein synaptophysin was also observed. Thus, MPACs offer a multimodal support cell therapy that provides multiple benefits without requiring prior genetic manipulation.

Derivation of MPACs from PD-patient derived iPSCs will allow the development of autologous MPAC therapies, but also provides the opportunity to test the role of astrocyte dysfunction in PD-related disease processes.

[Supported by NYSTEM contract # C026877]

**MACROPHAGE SUBSETS AFFECT THE PHENOTYPE OF PLURIPOTENT STEM CELL-DERIVED CARDIAC REPAIR CELLS**

Donald O. Freytes<sup>1</sup>, Isabella Pallotta<sup>1</sup>, Emily Wrona<sup>1</sup>

<sup>1</sup>The New York Stem Cell Foundation Research Institute, New York, NY

Cardiomyocytes (CMs) differentiated from human pluripotent stem cells (hPSCs), represent a promising therapeutic cellular target for clinical applications due to their unique capability for self-renewal and differentiation into all cardiovascular lineages. Following cardiac injury, the heart undergoes a number of dynamic processes, including pro-inflammatory and pro-healing phases. These events are characterized by the presence of a distinct subset of macrophages (pro-inflammatory (M1) vs. pro-healing (M2)). Any cardiac repair cell will inevitably be subjected to this environment with potential effects on the repair cells. In the present study, we investigate multiple culture systems based on hPSC-derived cardiac repair cells and macrophages to recapitulate important cellular events found at the site of myocardial injury to predict the survival and engraftment of the repair cells after transplantation.

In order to investigate the interactions between the hPSC-CMs and inflammatory cells, peripheral blood-derived macrophages were co-cultured with cardiac repair cells in 2D and 3D systems. After analyzing both the inflammatory cells and repair cells, it was found that the subtype of macrophage present was able to dramatically affect the gene expression, growth and viability of the cardiac repair cells. Interestingly, the repair cells, in turn, affected macrophage phenotype, suggesting a complex cross-talk between the cells.

All together, these experiments represent the first step toward using a comprehensive co-culture system to gain new insights into the mutual interactions between cardiac repair cells and inflammatory cells in a human system. This system can potentially serve as a human model to test functional engineered cardiac patches.

[Supported by NYSTEM contract # C026721]

## **HUMAN OOCYTES REPROGRAM ADULT SOMATIC NUCLEI TO DIPLOID PLURIPOTENT STEM CELLS**

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The transfer of somatic cell nuclei into oocytes can give rise to pluripotent stem cells, holding promise for autologous cell replacement therapy. Though reprogramming of somatic cells by nuclear transfer was first demonstrated more than 60 years ago<sup>1</sup>, only recently have human diploid embryonic stem cells been derived after nuclear transfer of fetal and neonatal fibroblasts<sup>2</sup>. Because of the therapeutic potential of developing diploid embryonic stem cell lines from adult cells of normal and diseased human subjects, we have systematically investigated the parameters affecting efficiency and developmental potential in their derivation. We found that improvements to the oocyte activation protocol, including the use of both a kinase and a translation inhibitor, and cell culture in the presence of histone deacetylase inhibitors<sup>2,3</sup> enable development of diploid cells to the blastocyst stage. Developmental efficiency varied significantly between oocyte donors, and was inversely related to the number of days of hormonal stimulation required to reach mature oocytes, while the daily dose of gonadotropin or the total number of MII oocytes retrieved did not affect developmental outcome. Using these modifications to the nuclear transfer protocol, we successfully derived diploid pluripotent stem cell lines from both postnatal and adult somatic cells of a type 1 diabetic subject. We consider these findings a significant step towards the use of reprogrammed cells for cell replacement.

[Supported by NYSTEM contract # C026184]

## **REPROGRAMMING OF HUMAN ENDOTHELIAL CELLS INTO ENGRAFTABLE HEMATOPOIETIC MULTILINEAGE PROGENITORS BY DEFINED FACTORS AND VASCULAR NICHE INDUCTION**

Shahin Rafii, Vladislav M. Sandler, Raphael Lis, Ying Liu, Alon Kedem, Daylon James, Olivier Elemento, Jason M. Butler and Joseph M. Scandura

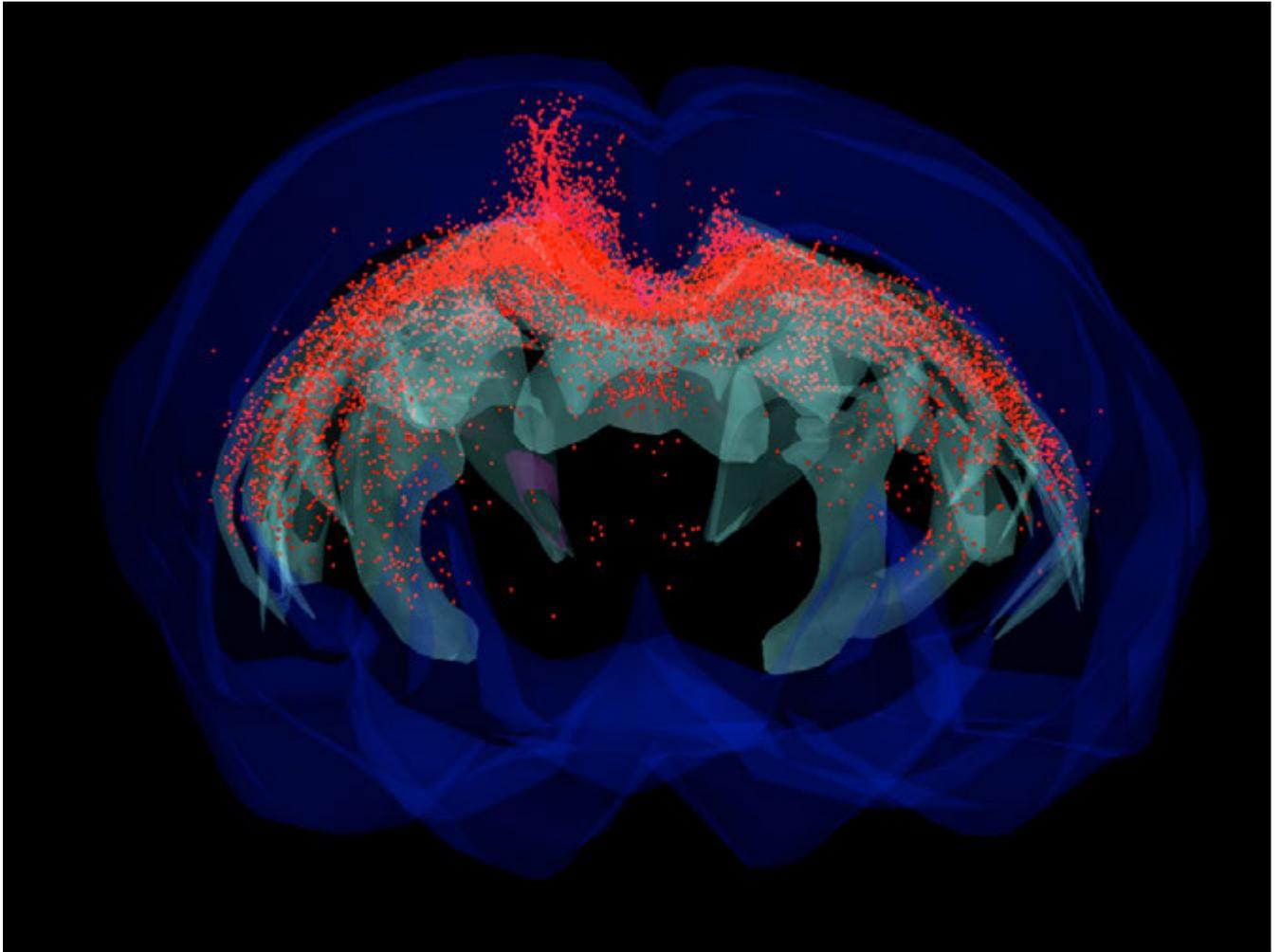
Ansary Stem Cell Institute, Department of Genetic Medicine, and HHMI, Weill Cornell Medical College, New York, NY 10065.

Generating abundant engraftable human hematopoietic cells from autologous tissues promises new therapies for blood diseases. Attempts to differentiate human pluripotent stem cells into hematopoietic cells yields cells with poor engraftment potential. To circumvent this hurdle, we devised a vascular niche model to phenocopy the developmental microenvironment of hemogenic cells thereby enabling direct transcriptional reprogramming of human endothelial cells (ECs) into engraftable hematopoietic cells. In this approach, human umbilical vein ECs (HUVECs) or adult human dermal microvascular ECs (hDMECs) are transduced with transcription factors (TFs), FOSB, GFI1, RUNX1, and SPI1 (FGRS), and then a xenobiotic- and serum-free instructive vascular niche feeder layer (adenoviral E4ORF1-transduced ECs: E4ECs) is used to induce outgrowth of hematopoietic colonies with features of human multipotent progenitor cells (hMPP). We have previously shown that E4EC vascular niche feeder cells promote self-renewal and differentiation of the mouse and human hematopoietic stem and progenitor cells by producing physiological levels of angiocrine growth factors, including Notchligands, BMPs, Kit-ligand, BMPs, and Wnts as well as other pro-hematopoietic growth factors.

These reprogrammed ECs into human MPPs (rEC-hMPPs) contain highly proliferative cells with multilineage colony forming unit (CFU) potential. When transplanted, rEC-hMPPs are capable of long-term multilineage primary and secondary hematopoietic engraftment composed of myeloid (granulocytic/monocytic, erythroid, megakaryocytic) and lymphoid (NK, B) cells resembling self-renewing MPPs. By conditionally expressing FGRS along with vascular niche induction, we optimized generation of rEC-hMPPs with a transcriptional and functional profile similar to repopulating hematopoietic cells. Our approach replicates critical aspects of hematopoietic development and underscores the essential role of vascular niche in orchestrating and sustaining hematopoietic specification and may prove useful for engineering autologous engraftable healthy and long-lasting hematopoietic cells for treatment of inherited and acquired blood disorders.

[Supported by NYSTEM contracts C024180, C026438, C026878, C028117]





**Human oligodendrocyte progenitor cells engraft the adult rat brain.**

*Joana Osorio, Rebecca Bergman, Steve Goldman, Center for Translational Neuromedicine, University of Rochester Medical Center*

## **SPECIAL SESSION: OVERCOMING BARRIERS TO TRANSLATING BENCH RESEARCH TO THE CLINIC**

Moderator: **Chris Henderson**, *Columbia University Medical Center*

Panelists: **Michael Basson**, *Nature Medicine*

**Audrey Chapman**, *University of Connecticut School of Medicine*

**Pete Coffey**, *UC Santa Barbara and University College London*

**Joyce Frey-Vasconcells**, *Frey-Vasconcells Consulting; former Deputy Director, Office of Cellular, Tissue, and Gene Therapies, CBER, FDA*

### **Order of Presentation**

AUDREY CHAPMAN: Ethical Issues in Early Clinical Trials, Including Examples From the First Three Pluripotent Stem Cell Trials

JOYCE FREY-VASCONCELLS: From a Regulatory Standpoint: How to Transition From a Research Environment to the Clinic'

CHRIS HENDERSON: Stem Cells for Drug Discovery and in Vitro Cellular Models for Toxicology/Safety Testing

PETE COFFEY: Real-World Experience of Clinical Trials

MICHAEL BASSON: Questions of Basic Biology that Need to be Addressed for Translation

### **Questions/Issues to be addressed**

1. Which business models are most promising for commercializing cell-based therapies?
2. Are there viable models for insurance reimbursements for stem cell therapies?
3. How can we best approach the challenges of scale-up and manufacturing of cell production for early clinical trials?
4. What regulation/guidelines are needed for stem cell-based therapies, and in particular for pluripotent stem cells?
5. What particular barriers do iPSCs present that other stem cells do not?

**Audrey R. Chapman, PhD**, is Professor of Community Medicine and Healthcare and holds the Healey Memorial Chair in Medical Ethics and Humanities at the University of Connecticut Health Center. Prior to coming to the University of Connecticut in July 2006, she served as the Director of the Science and Human Rights Program at the American Association for the Advancement of Science (AAAS) and the Senior Associate for Ethics for the AAAS Program of Dialogue on Science, Ethics, and Religion. She received a Ph.D. in public law and government from Columbia University and graduate degrees in theology and ethics from New York Theological Seminary and Union Theological Seminary. She is the author, coauthor, or editor of sixteen books and more than 45 peer reviewed articles and reports.

She has worked extensively on ethical and policy issues related to embryonic stem cell research. She serves as the Chair of the University of Connecticut Stem Cell Oversight Committee and is also a member of the State of Connecticut Stem Cell Ethics and Law Working Group. She served as the coauthor a 1999 report *Stem Cell Research and Applications: Monitoring the Frontiers of Biomedical Research* for the American Association for the Advancement of Science and Institute for Civil Society. She has published articles on ethical issues related to stem cell research, patenting, monitoring, and the requirements of early clinical trials on therapeutics derived from pluripotent stem cells in peer reviewed journals including the *American Journal of Bioethics*, *AJOB Neuroscience*, *the Journal of Clinical Research and Bioethics*, and the *Kennedy Institute of Ethics Journal*.

Her recent research on genetic technologies has focused on issues related to genetic research on addiction and noninvasive genetic prenatal diagnosis.

**Joyce L. Frey-Vasconcells, PhD.** Regulatory Expert, Frey-Vasconcells Consulting, LLC is considered one of the foremost regulatory experts regarding cell therapies, combination products, gene therapies, tumor vaccines, and tissues and brings extensive regulatory expertise and experience for this unique group of products. Prior to starting Frey-Vasconcells Consulting, Dr. Frey-Vasconcells served 6 years as a regulatory consultant for Pharmanet working with industry whose mission is to foster product development in the areas of cell therapy, tissues, gene therapy, and tissue engineered products. Prior to joining Pharmanet, she served more than 12 years at the FDA. At FDA, Dr. Frey-Vasconcells was the Deputy Director, Office of Cellular, Tissue, and Gene Therapies (OCTGT) with the Center for Biologics Evaluation and Research (CBER). She was instrumental in developing many of CBER's science and public health policies regarding the regulation of cells, tissues, gene therapies, tumor vaccines, and combination products (tissue engineered products). In 2001, Dr. Frey-Vasconcells was named the Regulatory Expert for Cell Therapies at FDA. She also served on various committees related to combination products, tissue reference group, and HHS committee on tissue engineering to name a few.

Since starting Frey-Vasconcells Consulting, Dr. Frey-Vasconcells has continued working with industry on an individual basis and with organizations whose mission is to foster product development in the areas of cell therapy, tissues, gene therapy, and tissue engineered products. She is clearly considered one of the foremost regulatory experts regarding tissues, cell therapies, combination products, gene therapies, and tumor vaccines and is recognized by various state and country stem cell networks as an expert in the regulation of stem cell therapies. She brings extensive regulatory expertise and experience for this unique group of products.

**Christopher E. Henderson, PhD.** After spending much of his career in France, Dr. Henderson moved in 2005 to Columbia University in New York, where he is Gurewitsch/Vidda Professor of Rehabilitation and Regenerative Medicine, in Pathology and Cell Biology, Neurology and Neuroscience. At Columbia, he co-founded the Center for Motor Neuron Biology and Disease, a new initiative in translational neuroscience that creates a continuum from research on motor neurons through to clinical research on the motor neuron diseases ALS (amyotrophic lateral sclerosis) and SMA (spinal muscular atrophy). Dr. Henderson is also director of the Columbia Stem Cell Initiative, a group of 120 laboratories across the university using stem cells to better understand or treat human disease. More recently he became director of Target ALS, a \$25M privately-funded consortium that aims to stimulate industry investment in ALS research by supporting cutting-edge translational research and nationwide core facilities in academia.



**Pete Coffey, PhD.** Dr. Coffey received his D. Phil at Oxford University and was a member of the faculty at Oxford and later the University of Sheffield as Lecturer and Senior Lecturer. He was formerly Professor and Head of Ocular Biology and Therapeutics at the Institute of Ophthalmology at University College London, where he also served as Chair of Cellular Therapies and Visual Science. Dr. Coffey has received many honors and awards, including the prestigious Estelle Doheny Living Tribute Award in 2009, Retinitis Pigmentosa International's Vision Award in 2009, the CIRM Leadership Award in 2010, and the New York Stem Cell Foundation Roberston Prize in 2011.

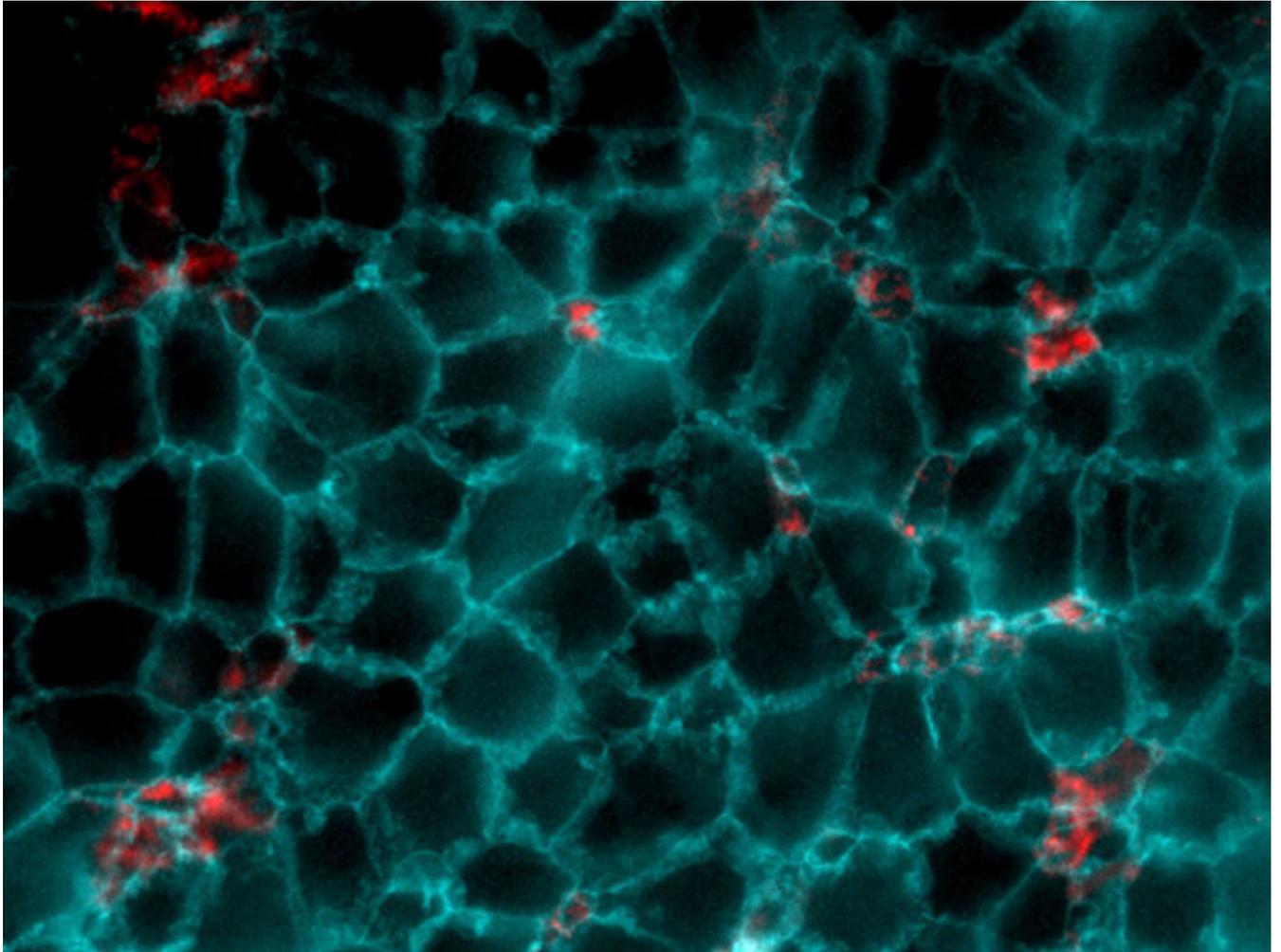


**Michael Basson, PhD.** Michael has been at *Nature Medicine* since 2003 where he is now a Senior Editor, responsible for the selection, peer review and publication of manuscripts on cardiovascular disease and the hematopoietic system. He frequently attends international scientific conferences to represent *Nature Medicine* in his field of expertise.

As a graduate student at the University of California at Berkeley, USA, in Jasper Rine's laboratory, Michael studied the sterol biosynthetic pathway and its rate-limiting enzyme HMG-CoA reductase in baker's yeast. His subsequent postdoctoral work was on developmental genetics with Bob Horvitz at the Massachusetts Institute of Technology, where he helped identify one of the first microRNAs to be discovered, *let-7*.

Michael then worked in the biotech industry, first on developing new methods for the identification of new drug targets by making use of model organisms such as the nematode *C. elegans*. He then studied mechanisms of tumor angiogenesis as part of an effort to identify anti-cancer targets.

# POSTER LIST



**Lateral Surface of the SVZ Whole Mount.**

*Jenny Yue Wang, Neural Stem Cell Institute, Regenerative Research Foundation*

**POSTER ASSIGNMENTS**

- (1) NEUTRAL COMPETITION OF STEM CELLS IS SKEWED BY PROLIFERATIVE CHANGES DOWNSTREAM OF HH AND HPO
- (2) LINEAGE SPECIFIC ASSEMBLY OF PRIMARY CILIA IN THE MOUSE EMBRYO
- (3) DEFINING GLIOBLASTOMA STEM CELL HETEROGENEITY
- (4) CHILD ADVERSITY LONG-TERM EFFECTS ON STEM CELLS AND THEIR PROGENY IN HUMAN HIPPOCAMPUS
- (5) 3<sup>rd</sup> VENTRICLE NEURAL STEM CELL NICHE DYNAMICS FOLLOWING FOCAL ISCHEMIC LESION
- (6) HUMAN VENTRICULAR CARDIAC ORGANOID CHAMBERS AS PRE-CLINICAL MODELS OF THE HUMAN HEART
- (7) EPIGENETIC REPROGRAMMING ENHANCES THE EXPANSION OF CORD BLOOD STEM CELLS
- (8) A METABOLIC SIGNATURE OF COLON CANCER INITIATING CELLS
- (9) PATIENT-SPECIFIC BETA CELLS REVEAL REDUCED INSULIN PRODUCTION AND GLYCOLYSIS DUE TO HNF1A HAPLOINSUFFICIENCY
- (10) EPIGENETIC CHANGES IN HEMATOPOIETIC STEM CELLS FROM HEALTHY INDIVIDUALS ARE PROGNOSTIC FOR ACUTE MYELOID LEUKEMIA
- (11) LOSS-OF-FUNCTION SCREEN FOR BREAST TUMOR INITIATING CELLS REVEALS PRC1 DEPENDENCE
- (12) WNT5A AND WNT11 SIGNAL COOPERATIVELY TO INHIBIT CANONICAL WNT SIGNALING AND PROMOTE CARDIAC PROGENITOR FATE VIA THE CASPASE-DEPENDENT DEGRADATION OF AKT
- (13) ESTABLISHING ASSAYS OF HUMAN STEM CELL-DERIVED NEOCORTICAL PROJECTION NEURON SURVIVAL AND MATURATION
- (14) PLATELETS ACTIVATE PULMONARY CAPILLARY VASCULAR NICHE TO DRIVE LUNG ALVEOLAR REGENERATION
- (15) ELECTRICAL CONDITIONING OF HUMAN EMBRYONIC STEM CELL DERIVED CARDIOMYOCYTES
- (16) PARKIN MUTATIONS REDUCE THE COMPLEXITY OF NEURONAL PROCESSES IN IPSC-DERIVED HUMAN NEURONS

- (17) DEFINING A NOVEL CANCER-PRONE STEM CELL NICHE FOR THE OVARIAN SURFACE EPITHELIUM
- (18) DISSECTING THE ROLE OF THE HISTONE VARIANT MACROH2A DURING BREAST CANCER PROGRESSION
- (19) RAF-1 REGULATES TRANSCRIPTION IN LEUKEMIC STEM CELLS UNDERGOING RA-INDUCED DIFFERENTIATION
- (20) DEVELOPING A HUMAN ES CELL DERIVED DOPAMINE NEURON SOURCE FOR CELL THERAPY IN PARKINSON'S DISEASE - FROM ACADEMIC TO TRANSLATIONAL RESEARCH
- (21) A RAPID AND EFFICIENT 2D/3D NUCLEAR SEGMENTATION METHOD FOR ANALYSIS OF EARLY MOUSE EMBRYO AND STEM CELL IMAGE DATA
- (22) THE DNA METHYLATION INHIBITOR 5-AZACYTIDINE PROMOTES THE TRANSDIFFERENTIATION OF CARDIAC CELLS TO SKELETAL MYOCYTES
- (23) CRITICAL ROLE OF ENDOTHELIN RECEPTOR SIGNALING IN FOLLICULAR MELANOCYTE STEM CELLS IN ADULT SKIN
- (24) GENE THERAPY ON PATIENT-SPECIFIC STEM CELL LINES WITH MEMBRANE FRIZZLED-RELATED PROTEIN DEFECT
- (25) HUMAN CORD BLOOD DERIVED STEM CELLS SIGNIFICANTLY IMPROVE SURVIVAL IN A MURINE MODEL OF RECESSIVE DYSTROPHIC EPIDERMOLYSIS BULLOSA
- (26) USING THE *C. ELEGANS* POSTEMBRYONIC MESODERM AS A MODEL TO DISSECT MECHANISMS UNDERLYING CELLULAR PLASTICITY AND IN VIVO REPROGRAMMING
- (27) GENERATION OF A MOUSE ESC REPORTER LINE TO IDENTIFY VENTRICULAR CARDIAC CONDUCTION SYSTEM CELLS DURING DEVELOPMENT
- (28) DIVERSITY AND UNIFORMITY: DERIVATION AND CHARACTERIZATION OF MINORITY PLURIPOTENT STEM CELL LINES
- (29) DNA DAMAGE RESPONSE AND STEM CELL PROPERTIES OF MURINE METASTATIC TESTICULAR GERM CELL TUMORS
- (30) LINEAGE ALLOCATION IN THE MAMMALIAN EMBRYO: DEFINING A TRANSCRIPTIONAL CIRCUITRY

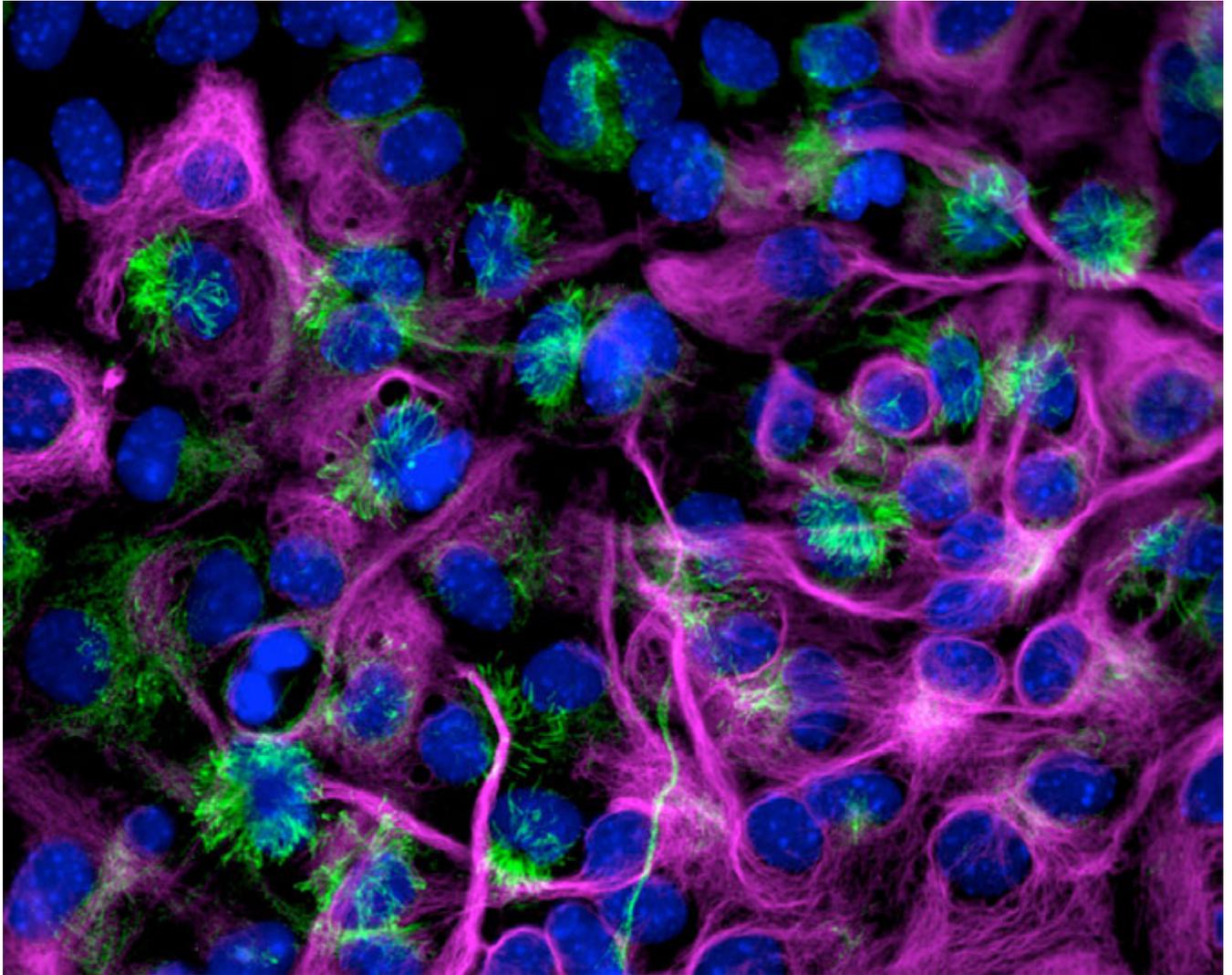
- (31)** TELOMERE DYSFUNCTION AS A POTENTIAL CANCER DRIVER
- (32)** USING SUBSTRATE MECHANICS TO EXPAND CD34+ HSC
- (33)** REGULATED CELL CYCLE DEPENDENT TRANSCRIPTIONAL SILENCING PROMOTES GERMLINE STEM CELL DIFFERENTIATION
- (34)** ROLE OF STRAD IN HIPPOCAMPAL ADULT NEUROGENESIS
- (35)** ELUCIDATING THE ROLE OF S6-KINASE IN CELL FATE SPECIFICATION USING THE *C. ELEGANS* GERM LINE
- (36)** GROWTH FACTOR PRIMING DIFFERENTIALLY MODULATES COMPONENTS OF THE EXTRACELLULAR MATRIX PROTEOME IN CHONDROCYTES AND SYNOVIUM-DERIVED STEM CELLS
- (37)** REPROGRAMMING OF HUMAN ENDOTHELIUM INTO HEMATOPOIETIC MULTI-LINEAGE PROGENITORS BY DEFINED FACTORS AND VASCULAR NICHE INDUCTION
- (38)** SMALL RNAs DERIVED FROM lncRNA *RNase MRP* HAVE GENE-SILENCING ACTIVITY RELEVANT TO HUMAN CARTILAGE-HAIR HYPOPLASIA AND THE U12-DEPENDANT SPLICEOSOME
- (39)** ART-27 REGULATES SPERMATOGONIAL STEM CELL DIFFERENTIATION AND SERTOLI CELL FUNCTIONS IN THE MOUSE TESTIS
- (40)** GATA6 LEVELS MODULATE PRIMITIVE ENDODERM CELL FATE CHOICE AND TIMING IN THE MOUSE BLASTOCYST
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- (42)** A HIGH-THROUGHPUT EXTRACELLULAR MATRIX PLATFORM IDENTIFIES EXTRACELLULAR MATRIX-CELL INTERACTIONS THAT MAINTAIN PLURIPOTENCY
- (43)** SPATIOTEMPORAL CONTROL OF CANCER STEM CELLS BY VERSATILE MICRORNA MECHANISMS
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- (45)** DENTAL PULP CELL DIFFERENTIATION AND DEDIFFERENTIATION IS REGULATED BY MECHANICS OF POLYBUTADIENE FILMS

- (46) CHARACTERIZATION OF STROMAL DERIVED FACTOR -1 MEDIATED CHEMOTAXIS OF TRANSPLANTABLE RETINAL PROGENITOR AND PHOTORECEPTOR PRECURSOR CELLS
- (47) SYNCHRONIZED AND HIGHLY EFFICIENT REPROGRAMMING IN A CELL-TYPE DEPENDENT MANNER
- (48) ASYMMETRICALLY MODIFIED NUCLEOSOMES AND THEIR ROLE IN THE ESTABLISHMENT OF BIVALENT DOMAINS IN EMBRYONIC STEM CELLS
- (49) A “BTB-POZ” KEY TO GROUND-STATE PLURIPOTENCY DURING SOMATIC CELL REPROGRAMMING
- (50) Brd4 INTERACTS WITH Oct4 AND CONTROLS PLURIPOTENCY AND X-CHROMOSOME INACTIVATION
- (51) VALIDATION OF GWAS ALLELES WITH AMD PATIENT-SPECIFIC STEM CELL LINES
- (52) HETEROGENEITY OF STEM CELLS IN AMNIOTIC FLUID
- (53) APPLICATION OF GRAPHENE BASED POLYMER NANOCOMPOSITE SUBSTRATE TO INDUCE ADULT DENTAL PULP STEM CELL (DPSC) DIFFERENTIATION
- (54) STUDYING PANCREATIC DEVELOPMENT AND DIABETES IN A DISH THROUGH EFFICIENT GENETIC MANIPULATION OF HUMAN EMBRYONIC STEM CELLS

### **FACILITIES POSTERS**

- (F1) RENSSELAER CENTER FOR STEM CELL RESEARCH
- (F2) DEVELOPMENT OF cGMP-COMPLIANT STEM CELL CULTURE PROCESSES AT THE UPSTATE STEM CELL cGMP FACILITY
- (F3) NYSTEM HIGH-THROUGHPUT SCREENING AND CHEMISTRY SHARED FACILITY AT COLUMBIA UNIVERSITY
- (F4) THE NYU RNAI CORE: AN OPEN ACCESS, HIGH-THROUGHPUT SCREENING RESOURCE FOR FUNCTIONAL GENOMICS
- (F5) PLURIPOTENT STEM CELL PRODUCTION AND CHARACTERIZATION OF STEM CELLS DERIVATIVES IN THE HELMSLEY STEM CELL CORE FACILITY AT COLUMBIA

# POSTER ABSTRACTS



## **A Protected Provenance.**

*Freyja K. McClenahan, Holly Colognato, Department of Pharmacology,  
Stony Brook University*

**(1) NEUTRAL COMPETITION OF STEM CELLS IS SKEWED BY PROLIFERATIVE CHANGES DOWNSTREAM OF HH AND HPO**Marc Amoyel<sup>1\*</sup>, Benjamin D. Simons<sup>3,4,5</sup> and Erika A. Bach<sup>1,2\*</sup>

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Neutral competition, an emerging feature of stem cell homeostasis, posits that individual stem cells can be lost and replaced by their neighbors stochastically, resulting in chance dominance of a clone at the niche. A single stem cell with an oncogenic mutation could bias this neutral process and clonally spread the mutation throughout the stem cell pool. The *Drosophila* testis provides an ideal system for testing this model. The niche supports two stem cell populations which compete for niche occupancy. Here we show that cyst stem cells (CySCs) conform to the paradigm of neutral competition and that clonal deregulation of either the Hedgehog (Hh) or Hippo (Hpo) pathway allows a single CySC to colonize the niche. We find that the driving force behind such behavior is accelerated proliferation. Our results demonstrate that a single stem cell colonizes its niche through oncogenic mutation by co-opting an underlying homeostatic process.

[Supported by NYSTEM contract #C028132]

**(2) LINEAGE SPECIFIC ASSEMBLY OF PRIMARY CILIA IN THE MOUSE EMBRYO**Fiona K. Bangs<sup>1</sup> and Kathryn V. Anderson<sup>1</sup>

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Primary cilia are required to respond to specific intercellular signals essential for embryonic development and adult tissue homeostasis in mammals. Many cells in vertebrates have a single primary cilium, but the tissue distribution of cilia has not been described systematically. To define when and where primary cilia appear in the mouse embryo, we used transgenic mice that express the cilium-specific protein ARL13b fused to mCherry as well as the basal body marker Centrin2-GFP. As previously described centrosomes (which can serve as templates for cilia) first appear at the 16-32 cell stage, but cilia are not detectable until after implantation. When cilia appear, they are distributed in a lineage-specific pattern: primary cilia are detected in the epiblast at the time of cavitation and are present on all epiblast cells and the derivatives of the epiblast (definitive endoderm, mesoderm, ectoderm). In contrast, extraembryonic cells of both the visceral endoderm and trophectoderm have centrosomes but no cilia. Stem cell lines derived from embryonic lineages recapitulate the pattern of ciliogenesis: all non-dividing epiblast stem cells are ciliated, whereas extraembryonic endoderm stem cells (XEN cells) and trophectoderm stem cells (TS) cells are not ciliated. The mechanisms that have been described to regulate ciliogenesis during the cell cycle do not appear to be responsible for the lack of cilia in extraembryonic cells. Instead, evidence suggests that the cilia disassembly pathway is constitutively active in these cells.

**(3) DEFINING GLIOBLASTOMA STEM CELL HETEROGENEITY**

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 Departments of <sup>1</sup>Neurosurgery, <sup>3</sup>Pathology and <sup>4</sup>Radiology, <sup>2</sup>Skirball Institute, <sup>5</sup>Genome Technology Center, <sup>6</sup>Brain Tumor Center, NYU School of Medicine; <sup>7</sup>Division of Medical Biotechnology, Paul-Ehrlich-Institut, Langen, Germany

Glioblastoma Multiforme (GBM) is a deadly brain malignancy. Glioblastoma stem cells (GSCs) have the ability to self-renew and differentiate into tumor lineages. GSCs are highly tumorigenic and resistant to current chemoradiotherapy. Although functional criteria defining GSCs are well established, molecular characteristics of GSCs and universal markers identifying GSCs are yet to be discovered.

CD133+ GSCs were shown to have higher tumorigenicity compared to CD133- cells. However, CD133- cells can form tumors and some GBM tumors do not have a CD133+ population. Therefore, CD133 is not a universal GSC marker. The Notch signaling pathway is also essential to GSC self-renewal. Blockage of Notch signaling leads to depletion of GSCs, including CD133+ GSCs and reduced tumorigenicity. Surprisingly, using primary human GBM samples that express a fluorescent protein upon activation of Notch signaling, we observed only partial overlap between CD133+ GSCs and cells with activated Notch cascade (Notch+), suggesting that it may serve as a marker for CD133- GSCs. To test this, we have isolated CD133+/Notch-, CD133+/Notch+, CD133-/Notch+ and CD133-/Notch- populations and characterized them. Our data suggest that CD133+/Notch-, CD133+/Notch+ and CD133-/Notch+ cells have equivalent tumorsphere forming ability and we are testing their *in vivo* tumorigenicity. Notch+/CD133- populations can generate both Notch+ and Notch- cells, whereas Notch- cells do not give rise to Notch+ cells over time. CD133+/Notch- and CD133-/Notch+ have different transcriptional signatures. These results raise the possibility that these cells have different roles in maintaining heterogeneity and establishing the cellular hierarchy within these tumors and have different responses to therapies.

To understand how they interact, we have established a lineage tracing system where we can selectively target CD133+ (Anliker *et al.* 2010) and Notch+ GSCs. This combinatorial system will help us understand the cellular hierarchy shaped by distinct GSC populations and how they contribute to tumorigenesis and response to chemoradiotherapy.

[Supported by NYSTEM contract # C026880]

**(4) CHILD ADVERSITY LONG-TERM EFFECTS ON STEM CELLS AND THEIR PROGENY IN HUMAN HIPPOCAMPUS**

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Childhood adversity exposure (CAE), including abuse, neglect and separation, enhances the risk for psychiatric and internal medicine illnesses. In animals, stress increases proliferation and impairs neuronal differentiation of neural progenitor cells (NPCs) in the dentate gyrus (DG). Long-term cellular effects of CAE have not been studied in the human brain.

We compared numbers of NPCs, mitotic cells and granule cells (GCs) in the DG, performing immunohistochemistry and unbiased stereology in tissue sections throughout the whole hippocampus in subjects without psychiatric diagnosis (C) and with major depressive disorder (MDD), with (MDD\*CAE, C\*CAE) or without (MDD\*noCAE, C\*noCAE) CAE before 16 years of age.

NPCs were more in C\*CAE vs. C\*noCAE ( $p=.003$ ), MDD\*CAE ( $p=.001$ ) and MDD\*noCAE ( $p=.001$ ) in anterior DG, and in C\*CAE vs. C\*noCAE ( $p=.003$ ), MDD\*CAE ( $p=.021$ ) and MDD\*noCAE ( $p=.005$ ) in mid DG. Anterior DG GCs were more in C\*CAE vs. MDD\*CAE ( $p=.001$ ) and MDD\*noCAE ( $p<.001$ ) and in C\*noCAE vs. MDD\*noCAE ( $p=.013$ ). Mid DG GCs were fewer in MDD\*noCAE vs. C\*CAE ( $p=.003$ ) and C\*noCAE ( $p=.033$ ). NPCs and GNs did not differ between groups in posterior DG and between MDD\*CAE and MDD\*noCAE in any subregion. There were no between-group differences in mitotic cell number at the time of death.

In the DG region controlling emotional responses, C\*CAE had more NPCs and GNs vs. C\*noCAE and both MDD groups, suggesting the resilient human phenotype (C\*CAE) relies on higher neuroplasticity responses to stress. Fewer GCs in MDD\*noCAE vs. C\*CAE and C\*noCAE suggests psychopathology has a negative effect on cellular plasticity. No differences in cell numbers between MDD\*CAE and MDD\*noCAE suggest a deficit in neuroplastic responses to stress in subjects prone to develop psychopathology. Future studies dissecting the mechanisms of stress effect on cellular viability, may lead to new treatments to prevent the onset of psychiatric diseases in subjects exposed to extreme life events.

**(5) 3<sup>rd</sup> VENTRICLE NEURAL STEM CELL NICHE DYNAMICS FOLLOWING FOCAL ISCHEMIC LESION**

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It is now well established that the adult mammalian brain maintains neural stem cell reserves in two discrete regions, the dentate gyrus (DG) of the hippocampal formation, and in the sub-ventricular zone (SVZ) lying along the lateral ventricle wall. In recent years the 3<sup>rd</sup> ventricle lying proximal to the hypothalamus has also been shown to house limited-potential progenitors, most likely either  $\alpha$  or  $\beta$  tanycytes (a radial glia-like cell population), which slowly differentiate in response to leptin and insulin-growth factor (IGF) signalling – finally adding neuronal and glial cells (NG2+) to the arcuate and paraventricular nuclei. The proliferative zone of the 3<sup>rd</sup> ventricle also extends into thalamic territory, though whether adult neurogenesis occurs in these dorsal reaches of the 3<sup>rd</sup> ventricle remains an open question. We studied the response of 3<sup>rd</sup> ventricle neural stem cells (NSCs) to focal thalamic lacunar ischemia in wild-type (WT) C57Bl6/J mice as well a mouse model with a global knock-out of the high mobility group B2 (HMGB2) gene - loss of which leads to heightened neurogenesis in the adult SVZ NSC niche. We have previously established a role for this chromatin protein (HMGB2) in modulation of embryonic neurogenesis via shotgun proteomics, and in the adult HMGB2 knock-out mouse SVZ. It is critically important to characterize and potentially “boost” endogenous cell replacement by NSCs following ischemic injury in thalamic and hypothalamic territories, potentially through signalling of chromatin/transcription factors such as HMGB2, which could lead to insights into conditions such as Dejerine-Roussy syndrome (thalamic pain syndrome) as well as heat shock/exhaustion (hypothalamic in origin) – as both are caused by ischemic damage to those particular brain regions.

[Supported by: AHA Predoctoral Fellowship 12PRE12060489 to RB]

**(6) HUMAN VENTRICULAR CARDIAC ORGANOID CHAMBERS AS PRE-CLINICAL MODELS OF THE HUMAN HEART**

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Despite extensive animal testing, new drugs often exhibit unexpected cardiotoxicity in patients. 3D model tissues engineered from human stem cells could improve the efficacy of preclinical screening. This study aims to demonstrate the first functional engineered human (h) ventricular (v) cardiac organoid chambers (hvCOC) that yield essential measures of global heart function (pressure, volume, electrical conduction).

To create hvCOC, ten million human ventricular cardiomyocytes, obtained from directed specification of embryonic stem cells (hESC), are mixed with type-I collagen and Matrigel. 1.2 mL of the solution is transferred into the spherical gap between an inner silicone balloon and an outer agarose cup. As the tissue compacts, the agarose is removed after 24 hours and the balloon removed by day 10-11. The resulting chamber displays a hollow spheroidal geometry with diastolic volume approximately 135  $\mu$ L (diameter  $\sim$ 6.5 mm).

hvCOCs beat spontaneously at 0.2-0.8 Hz, with 1:1 capture up to 3.0 Hz. Ejection fraction decreased from  $4.3 \pm 0.1\%$  (mean $\pm$ SD of multiple beats) at 0.8 Hz to  $3.1 \pm 0.4\%$  at 2 Hz, indicating negative frequency dependence. An intact Frank-Starling mechanism was evident from a 1.8-fold increase in developed pressure as luminal pressure was increased by 1.1 mmH<sub>2</sub>O. Optical mapping revealed that as pacing frequency increased from 0.5 to 3.0 Hz, chamber surface conduction velocity (CV) decreased from  $8.35 \pm 0.49$  cm/s to  $0.45 \pm 0.05$  cm/s and action potential duration at 50% of maximum decreased from  $215 \pm 14$  ms to  $95 \pm 28$  ms (mean $\pm$ SD of multiple sites). A dose-dependent chronotropic response to isoproterenol caused spontaneous beating to increase from 0.24 Hz (baseline) to 0.48 Hz (10 nM) and 0.67 Hz (100 nM), with a concomitant decrease in CV.

Thus, despite sub-physiologic performance, hESC-derived hvCOCs replicate several key features of natural cardiac electromechanical pump function, providing a novel platform for developing next-generation models of the human heart for enhanced *in vitro* preclinical screening applications.

**(7) EPIGENETIC REPROGRAMMING ENHANCES THE EXPANSION OF CORD BLOOD STEM CELLS**

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Cord blood (CB) cells that express CD34 have extensive hematopoietic capacity and rapidly divide *ex vivo* in the presence of cytokine combinations; however, many of these CB-CD34+ cells lose their marrow repopulating potential. To overcome this decline in function, we treated dividing CB-CD34+ cells *ex vivo* under serum-free culture conditions. Treatment of CB-CD34+ cells with the histone deacetylase inhibitor valproic acid (VPA), following an initial 16 hour cytokine priming, increased the number of pluripotent cells (CD34+CD90+) generated; however,

the degree of expansion was substantially greater in the presence of both VPA and cytokines for a full 7 days. We have previously identified that VPA treatment of CB-CD34+ cells generate a distinct stem cell phenotype including CD34+CD90+CD184+CD49f+CD45RA-ALDH+CD117+SOX2+OCT4+NANOG+ZIC3+.

A hierarchy of HSCs has been reported previously such as rapid SCID repopulating cells (R-SRC) are detected in NSG mice 2-4 weeks after their transplantation and are CD34+CD90-CD49f-, intermediate SRC(IT-SRC) repopulate after 12-14 weeks and are CD34+CD90+CD49f- and long-term SRC (LT-SRC) repopulate >20 weeks and are CD34+CD90+CD49+. We demonstrated that VPA expanded HSC grafts contained far greater numbers of these three subpopulations of CD34+CD90-CD49f-(R-SRC:  $6.1 \times 10^7 \pm 2.1 \times 10^7$  vs  $1.5 \times 10^6 \pm 3.8 \times 10^5$ /CB collection;  $p=0.008$ ), CD34+CD90+CD49f-(IT-SRC:  $1.4 \times 10^8 \pm 5.8 \times 10^7$  vs  $1.3 \times 10^5 \pm 2.2 \times 10^4$ /CB collection;  $p=0.05$ ) and CD34+CD90+CD49f+(LT-SRC:  $8.7 \times 10^8 \pm 4.9 \times 10^8$  vs  $8.7 \times 10^4 \pm 7.0 \times 10^4$ /CB collection;  $p<0.05$ ) as compared to primary CB.

Limiting dilution analysis revealed that VPA treatment resulted in an increase in the number of SRC as compared to primary CB-CD34+ cells (36 fold;  $p \leq 0.002$ ) as well as cells cultured under control conditions (299 fold;  $p \leq 0.002$ )( $n=111$ ). Furthermore, VPA treated CD34+ cells established multilineage engraftment in primary, secondary and tertiary NSG mice with no evidence of hematological malignancies. However, teratomas were observed exclusively in mice injected with ES cells but not VPA expanded CD34+ cells.

These data indicate that epigenetic reprogramming of CB-CD34+ cells by VPA generated far greater numbers of functional stem cells that can be used as a graft for transplantation.

[Supported by NYSTEM contract # C026431]

**(8) A METABOLIC SIGNATURE OF COLON CANCER INITIATING CELLS**

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Colon cancer initiating cells (CCICs) are more tumorigenic and metastatic than the majority of colorectal cancer (CRC) cells. CCICs have also been associated with stem cell-like properties. However, it has remained largely unclear whether CCICs isolated from different CRC tumors indeed share common mechanisms that account for their phenotype, or alternatively they are completely different cells that were categorized simply by their tumorigenic capacity. To address this question, we first analyzed 5 GEO microarray datasets that measured the transcriptomes of CD133+ versus CD133- CRC cells. The transcriptome analysis in these 5 datasets shows that 11,048 genes are differentially expressed in CD133+ CCICs and these differential genes enrich in 282 pathways. Among the 282 enriched pathways, metabolic pathways scored highest gene hit rates – 50 curated metabolic pathways involve 614 differentially regulated metabolic genes, which suggested that CD133+ cells consistently reprogram certain metabolic functions differentially from CD133- cells. Unbiased metabolomics by high-resolution qExactive liquid chromatography–mass spectrometer (LC-MS) further corroborated the metabolic signature of CD133+ CCICs, which involve the glycolysis, TCA cycle, and cysteine/methionine metabolism pathways. The metabolite substrates, such as  $\alpha$ -ketoglutarate and s-adenosylmethionine, involved in epigenetic regulations are highly altered, suggesting a potential epigenetic link. RNA-seq, metabolic flux analysis (MFA), and functional assays are currently being performed to

further establish such links. The involved metabolic enzymes and metabolites may provide biomarkers for CRC diagnosis and prognosis. New CRC treatments may also target them to suppress CCICs in the tumor population to reduce the risk of relapse and metastasis.

[Supported by the Cornell Stem Cell Program, which is funded through multiple NYSTEM awards]

**(9) PATIENT-SPECIFIC BETA CELLS REVEAL REDUCED INSULIN PRODUCTION AND GLYCOLYSIS DUE TO HNF1A HAPLOINSUFFICIENCY**

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Transcription factors control beta cell differentiation, replication and function. The majority of instances of congenital forms of diabetes are caused by haploinsufficiency of transcription factors (e.g. HNF1A, HNF4A, HNF1B, PDX1). These genes, for instance HNF1A and HNF4A, have been linked to type 2 diabetes in genome wide association study. Systematically characterizing cellular and molecular defects in pancreatic beta cells deficient of these transcription factors will shed light on the mechanisms that underlie beta cell development, function and survival, and hence point to molecules implicated in the development and progression of diabetes. We generated stem cells from diabetic subjects with heterozygous loss-of-function mutations of the gene, *HNF1A* (MODY3) which accounts for ~50% of cases of MODY. MODY3 patients have fasting and postprandial hyperglycemia, renal glycosuria, and vascular complications and require insulin treatment. Retinopathy and coronary heart disease also occur in MODY3 patients. But little is known about deterioration of pancreatic beta cells during disease progression, especially at cellular and molecular level. Using stem cell-derived patient-specific beta cells, we found that cells with HNF1A mutations have reduced insulin-production and glucose response. We also found decreased beta cell generation due to HNF1A mutations. Global transcriptional analysis indicates that expression of genes involved in glycolysis are decreased in MODY3 cells. We also found declined insulin secretion in MODY3 cell after long term culture in vitro (in a novel 3D system) or in vivo (in mice). In summary, our results reveal molecular and cellular alterations caused by mutations in HNF1A in pancreatic beta cells.

**(10) EPIGENETIC CHANGES IN HEMATOPOIETIC STEM CELLS FROM HEALTHY INDIVIDUALS ARE PROGNOSTIC FOR ACUTE MYELOID LEUKEMIA**

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Hematopoietic stem cell (HSC) differentiation encompasses long-term repopulating HSC (LTHSC), short-term HSC (STHSC), common myeloid progenitors (CMP), megakaryocyte-erythroid progenitors (MEP) and granulocyte-monocyte progenitors (GMP). A locus-specific map of DNA methylation in human HSC from healthy individuals had not been assembled thus far. To analyze DNA methylation and corresponding gene expression changes in LTHSC, STHSC, CMP and MEP we used the nano-HELP assay, enabling analysis of minute numbers of FACS-sorted cells (~2000). During transitions from LTHSC to STHSC and CMP to MEP, changes in methylation were balanced. Strikingly, a significant decrease in DNA cytosine methylation was observed during HSC commitment from STHSC to CMP stage with 95% of significantly altered loci being demethylated. We identified a 561-loci-classifier that was able to distinguish between the stages of HSC commitment and differentiation. Interestingly, correlation between methylation and gene expression could be found only on a locus-specific level but not on a genome wide scale.

We hypothesized that changes in DNA cytosine methylation during physiologic differentiation of HSC might be prognostic for acute myeloid leukemia (AML). We analyzed published DNA methylation profiles from patients with AML in conjunction with survival data. The 561-loci human HSC commitment-associated classifier was able to distinguish between groups with superior and inferior overall survival in three independent cohorts of AML patients. This relationship between molecular changes and overall survival could not be demonstrated for gene expression classifiers that had been derived in an identical fashion. Taken together, commitment of healthy human HSC is accompanied by significant demethylation at the STHSC to CMP transition. A 561-loci epigenetic classifier can distinguish stages of commitment and differentiation of human healthy HSC and is prognostic for AML. Further studies will elucidate the utility of the epigenetic classifier as a predictive tool and the mechanisms underlying these phenomena.

[Supported by NYSTEM grants C024306 and C024172]

**(11) LOSS-OF-FUNCTION SCREEN FOR BREAST TUMOR INITIATING CELLS REVEALS PRC1 DEPENDENCE**

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Breast tumor-initiating cells (BTICs) are a subset of cells within breast tumors that possess both self-renewal and differentiation potential. Gene expression and functional studies have demonstrated a correlation between BTICs and high-grade tumors, poor patient prognosis, and therapy resistance; however, the mechanisms underlying BTIC regulation remains poorly understood. Because epigenetic alterations play a key role in breast cancer progression and in stem cell regulation, we hypothesized that epigenetic factors mediate BTIC function. Using MMTV-Myc tumor-derived mammospheres (MS), which we showed to be enriched in BTICs by *in vivo* tumorigenicity assays and gene expression studies, we performed a pooled shRNA

library screen coupled to next generation sequencing (NGS). Using a library of ~450 shRNAs targeting 60 epigenetic regulators, we screened for chromatin factors critical for BTIC function. We identified multiple members of the Polycomb Repressive Complex 1 (PRC1), with shRNAs targeting PRC1 members significantly depleted in MS cells, but not in control bulk cells. We have validated that knockdown of PRC1 members reduces MS formation, invasion and BTIC markers in MMTV-Myc cells and human MDAMB-231Luc cells *in vitro*. In addition, the Polycomb ortholog Cbx8 is required for tumorigenesis *in vivo*. RNA-Seq analysis in Cbx8 knockdown vs. control cells revealed a highly significant Notch signaling node. Our data suggests a novel role for Cbx8 in positively regulating Notch signaling in BTICs. ChIP experiment is currently underway to decipher the mechanisms by which Cbx8 regulates Notch signaling and BTIC formation. In accordance with our screen, The Cancer Genome Atlas (TCGA) reports several members of PRC1 to be overexpressed in breast tumors, and we are further exploring the clinical relevance of PRC1 members in breast cancer patient samples.

**(12) WNT5A AND WNT11 SIGNAL COOPERATIVELY TO INHIBIT CANONICAL WNT SIGNALING AND PROMOTE CARDIAC PROGENITOR FATE VIA THE CASPASE-DEPENDENT DEGRADATION OF AKT**

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Wnt proteins, ligands that regulate cell behavior via a canonical signaling pathway that induces b-catenin dependent transcription, promote the expansion of the second heart field (SHF) progenitors that give-rise to the majority of cardiomyocytes. However, activating b-catenin also causes loss of the SHF, highlighting the need to precisely control b-catenin. We recently reported that two divergent Wnt ligands, Wnt5a and Wnt11, cooperatively restrain b-catenin from disrupting SHF development. Yet while these data indicate that Wnt5a/Wnt11 are needed to control b-catenin in the SHF, the mechanism these ligands use to inhibit b-catenin are unclear. A previous study showed that Wnt11 inhibited b-catenin and promoted cardiomyocyte maturation via Caspase proteases. Consistent with these data, recombinant Wnt5a and Wnt11 proteins are sufficient to increase Caspase activity in ES cells and staining for activated Caspase-3 is reduced in Wnt5a/Wnt11 double mutant hearts. Furthermore, treating ES cells with Caspase inhibitor blocks the ability of Wnt5a/Wnt11 to promote SHF gene expression *in vitro* while injecting pregnant dams with Caspase inhibitor reduces mRNA for cardiac progenitor markers in gestating embryos. Caspase inhibitors also block the loss of b-catenin protein and reporter activity caused by Wnt5a/Wnt11 treatment. Interestingly, we have found that Wnt5a/Wnt11 induce the Caspase-dependent degradation of AKT and treating differentiating ES cells with AKT inhibitor increases the levels of SHF markers to levels similar to those seen after Wnt5a/Wnt11 treatment. Together, these indicate suggest that Wnt5a/Wnt11 inhibit the canonical Wnt pathway and promote SHF development via the Caspase-dependent degradation of AKT.

**(13) ESTABLISHING ASSAYS OF HUMAN STEM CELL-DERIVED NEOCORTICAL PROJECTION NEURON SURVIVAL AND MATURATION**

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The neocortex is the most evolutionarily distinct part of the brain, and the substrate for sensory processing and motor planning, as well as higher cognitive functions. Neurodegenerative diseases and injuries affecting the neocortex, like Alzheimer's and stroke, have devastating impacts on neocortical functions and quality of life as well as major social costs, but few or no effective therapies. Human stem cells can now be differentiated to different categories of neocortical projection neurons including many affected in disease or injury *in vivo*. This opens the door to a new generation of cell-based models to study degeneration in these specific human cells, and thus new hope for the identification treatments. We describe the development of assays, including high throughput image-based screens, to study the survival and function of human stem cell-derived neocortical projection neuron subtypes. We hope these assays will identify new pathways and drug targets in neocortical degeneration. [Supported by NYSTEM contract # C028128 and Rockefeller Private Funds]

**(14) PLATELETS ACTIVATE PULMONARY CAPILLARY VASCULAR NICHE TO DRIVE LUNG ALVEOLAR REGENERATION**

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The lung alveoli regenerate after surgical removal of the left lung lobe by pneumonectomy (PNX) (Ding et al, *Cell*, 2011). Nevertheless, how the alveolar regeneration process is initiated remains to be defined. Here, we show that activated platelets trigger lung alveolar regeneration by supplying paracrine stromal-derived factor1 (SDF1). After PNX, platelets stimulate SDF1 receptor CXCR4 on pulmonary capillary endothelial cells (PCECs) to deploy membrane-type metalloproteinase MMP14 (Ding et al, *Nature*, 2010; Ding et al, *Nature*, 2014). Membrane MMP14 expressed by vascular niche subsequently liberates heparin-binding epidermal growth factor (HB-EGF) to elicit propagation of type 2 alveolar epithelial progenitor cells (AEC2s), provoking regenerative alveolarization. In both thrombopoietin knockout (*Thpo*<sup>-/-</sup>) mice lacking circulating platelets and platelet-depleted thrombocytopenic mice, lung alveologenesis after PNX was diminished. Conditional deletion of *Sdf1* in platelets (*Sdf1*<sup>ΔPLT/ΔPLT</sup>) using platelet-specific *platelet factor 4-Cre* similarly inhibited lung alveolar regeneration. Notably, intravascular infusion of *Sdf1*<sup>+/+</sup> but not *Sdf1*-deficient (*Sdf1*<sup>Δ/Δ</sup>) platelets rescued the defective lung regeneration in pneumonectomized *Thpo*<sup>-/-</sup> mice. Consequently, in mice with inducible endothelial cell-specific ablation of *Cxcr4* (*Cxcr4*<sup>ΔEC/ΔEC</sup>), alveolar regeneration were impaired, and this disrupted lung regeneration in *Cxcr4*<sup>ΔEC/ΔEC</sup> mice could not be rescued by either thrombopoietin injection or platelet infusion. Therefore, we have identified a paradigm of lung regeneration in which recruited platelets release paracrine SDF1 to initiate alveolarization after PNX, extending beyond their traditional contribution as hemostatic thrombocytes. Therapeutically, modulating lung regeneration by platelet infusion could enable new regenerative therapies for lung disease.

[Supported by NYSTEM contract numbers C024180, C026438, C026878 and C028117]

**(15) ELECTRICAL CONDITIONING OF HUMAN EMBRYONIC STEM CELL DERIVED CARDIOMYOCYTES**

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Differentiated human cardiomyocytes, from either embryonic or induced pluripotent stem cells have great potential to ameliorate heart disease through therapeutic engraftment into the diseased heart. One of the key concerns is the electromechanical mismatch between the host and therapeutic cells. Most of the native heart development occurs in the presence of electrical signals. We previously showed that electrical field stimulation improves conductive and contractile properties of neonatal rat cardiomyocytes, but the conditioning of human embryonic stem cell derived cardiomyocytes (hESC-CM) is only beginning to be explored. The addition of this native, biophysical signal may be a method to guide the contractile function and development of cardiomyocytes.

We found that electrical field stimulation for seven days engendered development of cardiomyocytes on multiple levels, including improved contractile protein assembly, myofibrillar ultrastructure, and expression of cardiac genes. This led to functional changes in strain generation, calcium handling, and electrophysiology. Uniquely, the frequency of electrical stimulation can condition hESC-CMs, programming the autonomous beating frequency of the cardiomyocytes over a physiologic range (30-120 BPM), an effect which was persistent for two weeks following the end of stimulation. We identified the molecular basis for the observed changes: KCNH2 (hERG), a voltage gated potassium channel responsible for cell repolarization. Blockade of this channel abolished the differential beating frequencies, and washout of the blocker led to recovery of differential beating frequencies. This demonstrates the potential for cardiomyocytes to adapt and remember induced autonomous beating rates. Overall, electrical conditioning of immature hESC-CMs markedly improved the contractile protein assembly and enabled programming of autonomous beating frequency, creating a form of cardiac cell memory.

[Supported by NYSTEM contract # C026449]

**(16) PARKIN MUTATIONS REDUCE THE COMPLEXITY OF NEURONAL PROCESSES IN IPSC-DERIVED HUMAN NEURONS**

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Parkinson's disease (PD) is characterized by the degeneration of nigral dopaminergic (DA) neurons and non-DA neurons in many parts of the brain. Mutations of parkin, an E3 ubiquitin ligase that strongly binds to microtubules, are the most frequent cause of recessively inherited Parkinson's disease. The lack of robust PD phenotype in parkin knockout mice suggests a unique vulnerability of human neurons to parkin mutations. Here, we show that the complexity of neuronal processes as measured by total neurite length, number of terminals, number of branch points and Sholl analysis, was greatly reduced in induced pluripotent stem cell (iPSC)-derived TH<sup>+</sup> or TH<sup>-</sup> neurons from PD patients with parkin mutations. Consistent with these, microtubule stability was significantly decreased by parkin mutations in iPSC-derived neurons. Overexpression of parkin, but not its PD-linked mutant nor GFP, restored the complexity of neuronal processes and the stability of microtubules. The results suggest that parkin maintains the morphological complexity of human neurons by stabilizing microtubules.

[Supported by NYSTEM contracts C028129 and C026714, National Basic Research Program grant of China 2011CB504100, VA Merit Award C026714 and NIH grant NS061856]

**(17) DEFINING A NOVEL CANCER-PRONE STEM CELL NICHE FOR THE OVARIAN SURFACE EPITHELIUM**

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Ovarian cancer is the fifth-leading cause of cancer death among women in the United States. The shortage of definitive information about the cell of origin of the most common and aggressive type of ovarian cancer, high-grade serous adenocarcinoma (HGSA), significantly complicates understanding the pathogenesis of this malignancy. Utilizing stem cell markers ALDH1, CD133, CK6b, LEF1 and LGR5 we have identified a population of slow cycling ovarian surface epithelium stem cells (OSE-SCs), which mainly reside in the hilum region, the transitional/junction area between the OSE, mesothelium and tubal epithelium, of the mouse ovary (Flesken-Nikitin et al., *Nature* 495: 241–245, 2013). Hilum OSE-SC exhibit higher proliferative potential and extended self-renewal ability in serial sphere generation assays in cell culture as characteristic for adult stem cell. OSE-SC proliferate during estrous cycle and contribute to OSE regeneration in long-term lineage tracing experiments in vivo. Deletion of tumor suppressor genes *p53* and *Rb* results in increased proliferation and reduced senescence of OSE-SC, while orthotopic transplantation of these cells leads to formation of HGSA, consistent with frequent alterations in *p53* and *RB* pathways in this type of human cancer. To test the relevance of our observations to human biology we have performed a comparative pathological evaluation of mouse and human ovaries and uterine (fallopian) tubes. A number of stem cell markers, including CD44, ALDH1A, LEF1, active  $\beta$ -catenin and CK5/6 are expressed in anatomically defined areas of the ovarian surface and tubal epithelia of both species. These markers are also expressed in some cells of mouse and human HGSA. In summary, our work identifies a novel stem cell niche for the OSE in the mouse, shows that genetic alterations of niche OSE-SC lead to HGSA and lays the groundwork for identification and characterization of cancer-prone stem cell niches in human oophoro-salpingeal epithelium.

[Supported by NYSTEM contract # C028125]

**(18) DISSECTING THE ROLE OF THE HISTONE VARIANT MACROH2A DURING BREAST CANCER PROGRESSION**

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Histone variants are emerging players in the regulation of normal developmental processes and in the progression of tumorigenesis. Our lab has recently shown that the histone variant macroH2A is deposited in chromatin during embryonic stem cell differentiation and normal development, creating an epigenetic barrier to somatic cell reprogramming towards pluripotency, together with the histone mark H3K27me3. Moreover, we have also shown that macroH2A creates a barrier for melanoma progression by regulating CDK8. We therefore hypothesize that macroH2A may act as global repressor that is evicted from chromatin during cancer progression, akin to reprogramming.

Here we report that the loss of macroH2A correlates with invasive stages of breast cancer. We also identified the loss of macroH2A in highly invasive triple negative breast cancer cell lines. Genome wide profiling of macroH2A and H3K27me3 in normal mammary epithelial cells (HMEC), luminal (MCF7) and basal (MDA-MB-231L) cancer cells shows that there is a consistent loss of the macroH2A/H3K27me3 repressive domains in metastatic cell lines, allowing for the activation of oncogenic pathways. Interestingly, re-expression of macroH2A2 in MDA-MB-231L affects cell proliferation and mammosphere formation, which indicates that macroH2A expression may be depleting tumor initiating cells, or breast cancer stem cells. On the other hand, down-regulation of macroH2A in MCF7 cells increases a cancer stem cell-like population. Our data suggests that depletion of macroH2A may be important for the dedifferentiation of cancer cells, rendering these cells more aggressive. Currently, we are investigating the molecular mechanisms by which macroH2A acts as a repressor, and the actual pathways that are involved in the maintenance of a cancer stem cell population devoid of macroH2A.

**(19) RAF-1 REGULATES TRANSCRIPTION IN LEUKEMIC STEM CELLS UNDERGOING RA-INDUCED DIFFERENTIATION**

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All *trans*-Retinoic acid (RA) is an embryonic morphogen that can regulate self-renewal versus differentiation of tumor stem cells. It is extremely effective for treating a small subset of cancers; for instance, when patients with acute promyelocytic leukemia (APL) undergo RA treatment, the leukemic cells terminally differentiate into mature myeloid cells. HL-60 is a highly characterized model leukemic stem cell line originating from a patient with a related leukemia, acute myeloblastic leukemia that is responsive to RA. In contrast to the classical transient MAPK signaling along a Raf/Mek/Erk axis leading to the induction of mitosis, RA treatment in HL-60 cells causes a durable, hyperactive MAPK signaling that drives myeloid differentiation. Raf-1 is a well-studied vital serine/threonine kinase involved in MAPK signaling, among other pathways. At 48 hours after RA treatment, about half of the Raf-1 in the cell moves to the nucleus, thus indicating a unique pathway for RA-mediated signal transduction that has the opposite effect of the classical, mitogenic pathway. The hypothesis driving this study was that the novel localization of the Raf-1 kinase to the nucleus effects transcriptional changes that contribute to the RA-induced differentiation of myeloblastic leukemia cells. Preliminary data indicate that pS621 Raf-1 associates with several transcription factors, most notably NFATc3, in the nucleus

of HL-60 cells undergoing RA-induced differentiation. Raf-1 effects on transcriptional activity were characterized through ChIP-seq analysis identifying its association with certain DNA sequences and target genes, including MLL3, NSMCE2, and ZNF407; furthermore, the mRNA transcript levels of these genes in normal RA-induced differentiation were compared to untreated cells and cells blocked from differentiation by treatment with the MAPK pathway inhibitor PD98059. These studies illustrate a novel paradigm for nuclear Raf-1 function, and reveal more knowledge about the novel Raf-1 mediated genomic control of differentiation in leukemic stem cells.

[Supported by NYSTEM contract #C026718 (shared equipment); and NIH RO1 RO1 [CA033505](#) and R01 [CA152870](#)]

**(20) DEVELOPING A HUMAN ES CELL DERIVED DOPAMINE NEURON SOURCE FOR CELL THERAPY IN PARKINSON'S DISEASE - FROM ACADEMIC TO TRANSLATIONAL RESEARCH**

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Parkinson's Disease (PD) is the second most common neurodegenerative disorder, affecting ~5 million patients worldwide. A fundamental characteristic of PD is the progressive and irreversible loss of dopamine-producing neurons in the midbrain, resulting in disabling motor dysfunction. Multiple therapies have been developed, but none routinely replaces the lost cells. Yet, despite the challenges met in fetal grafting trials, cell therapy remains attractive due to the possibility of restoring actual dopamine (DA) neurons function and integration.

We recently made a major discovery that enables the derivation of nearly unlimited numbers of authentic midbrain DA (mDA) neurons from human embryonic stem cells (ESCs). Based on these findings our team secured a 4-year contract with NYSTEM to file an 'investigational new drug (IND)' application for PD. This discovery has the potential to become the first-in-human clinical trial for PD using hESC-derived neurons. We entered the early stages of this project and are presenting our progress towards FDA approval for this novel PD therapy. In the presentation we will use a dopaminergic neuron PD therapy as an outline to highlight the unique challenges one faces when moving a promising finding from the bench to the clinic. For our group, this meant assessing the relative risks of hESCs vs. induced pluripotent stem cells (iPSCs). We next had to decide on the ideal media formulation for the expansion of hESCs and are now adapting the differentiation process to be compatible with current good manufacturing practices (cGMP). In parallel, work to set-up a suitable, physical laboratory for on-site manufacturing of the clinical product has begun. The second large class of experiments is designed to address the safety, toxicology and efficacy of the differentiated cells. Lastly, we have a team of clinicians that define the target patient population, the surgical procedure and the immunosuppression regimen.

[Supported by NYSTEM Contract # C028503]

**(21) A RAPID AND EFFICIENT 2D/3D NUCLEAR SEGMENTATION METHOD FOR ANALYSIS OF EARLY MOUSE EMBRYO AND STEM CELL IMAGE DATA**

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A developmental process can only be studied within a correct temporal and spatial context. Therefore, cell fate specification and differentiation in developmental biology is principally investigated via microscope imaging. In particular, the generation of nuclear-localized fluorescent reporters, such as human histone H2B-green fluorescent protein (GFP) fusion proteins, enables 3D time-lapse (i.e. 4D) live imaging at single-cell resolution. However, to understand the mechanisms of cell fate choice and its behavior, one needs to perform a quantitative analysis on the image data. Although there are several commercially available software tools for such analysis, the complex morphology of embryonic stem (ES) cells in culture or within pre-implantation embryos impedes an efficient quantitative study, which is necessary to decipher the mechanisms controlling pluripotency or differentiation *in vitro* as well as *in vivo*. To address this need, we developed MINS (modular interactive nuclear segmentation) as a MATLAB/ C++-based segmentation tool tailored for counting cells and fluorescent intensity measurements in 2D and 3D image data of early mouse embryos as well as ES cells grown in culture. In this study, we applied MINS to manually analyze 2D/3D/4D image data and confirmed the accuracy and efficiency of MINS in the quantitative analysis.

**(22) THE DNA METHYLATION INHIBITOR 5-AZACYTIDINE PROMOTES THE TRANSDIFFERENTIATION OF CARDIAC CELLS TO SKELETAL MYOCYTES**

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Cardiovascular disease (CVD) remains the most prevalent disorder in United States causing 25% of deaths among the US population each year. The high incidence and prevalence of CVD indicates that novel strategies are needed to improve human healthcare. Recently, it has been established that the heart contains endogenous cardiac committed cells that have a regenerative potential. Many groups have reported the use of the DNA demethylation reagent 5-azacytidine for promoting the cardiac differentiation of endogenous cardiac progenitor cells and stem cells from noncardiac tissues (such as the bone marrow). However, 5-azacytidine also has been widely used to stimulate skeletal myogenesis. So to determine whether 5-azacytidine promotes cardiac differentiation exclusively without committing to other lineages, we cultured adult atrial mouse tissue in the presence of 5-azacytidine. Although atrial cultures contain cardiac but not skeletal muscle progenitors, it was observed that 5-azacytidine was able to induce the expression of the skeletal myogenic transcription factors MyoD and myogenin. In addition, 5-azacytidine treatments caused morphological changes in the atrial cultures, which now contained cells with more elongated cell shapes. In contrast, treatments with the G9a histone methyltransferase inhibitor BIX01294, which we have shown previously to enhance the cardiac potential of bone marrow cells, did not promote phenotypic changes in the atrial cultures. When atrial tissue pretreated with 5-azacytidine was subsequently incubated in low

serum, skeletal muscle differentiation media, cells that expressed both myogenin and sarcomeric  $\alpha$ -actin were observed throughout the cultures. Atrial tissue cultured in low serum media without pre-exposure to 5-azacytidine did not exhibit myogenin-positive cells. These data demonstrate that 5-azacytidine promotes the transdifferentiation of cardiac cells to skeletal myocytes, and suggests that this pharmacological reagent has a compromised efficacy as a cardiac differentiation factor.

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

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Melanocyte stem cells (McSCs) reside in the hair follicle bulge where they 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. Previous studies suggested that Edn promotes melanocyte proliferation in vitro. In addition, we previously reported that at the onset of anagen phase, Edn1 is upregulated in follicular epithelial cells surrounding McSCs that express endothelin receptor B (EdnrB). 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. This resulted in decreased numbers of melanocytes in anagen hair follicles. 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. Conversely, transgenic overexpression of Edn1 throughout the basal layer of the epidermis promoted McSC proliferation and differentiation during anagen phase. Finally, we show that Edn1 overexpression in the epidermis induces the upward migration of McSCs toward epidermal surface. This migration of melanocytes was augmented following skin injury or UVB irradiation. 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]

**(24) GENE THERAPY ON PATIENT-SPECIFIC STEM CELL LINES WITH MEMBRANE FRIZZLED-RELATED PROTEIN DEFECT**

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Membrane frizzled-related protein (*MFRP*) is a newly identified gene that can cause autosomal recessive retinitis pigmentosa (RP). *MFRP* encodes a retinal pigmented epithelium (RPE)-

specific membrane receptor of unknown function. This study focus on modeling MFRP-caused RP using patient-specific induced pluripotent stem cells (iPSCs), applying gene therapy to correct the cellular phenotype *in vitro* and discovering potential functional role of MFRP in RPE cells. iPSCs had been created by Yamanaka factors from two patients who carried mutation on *MFRP* gene and wild-type donor. Patient-specific iPSCs differentiated into RPE fate and AAV8-*MFRP* applied on patient-specific iPS-RPE cells to convert the cellular phenotype. Immunocytochemistry, transmission electron microscopy (TEM) imaging and measurement of transepithelial resistance (TER) were used to study and confirm the correction of cellular phenotype. AAV8-*CTRP5* applied on human autopsy RPE to study the relationship between MFRP and its binding partner CTRP5. Antibodies to MFRP, CTRP5,  $\beta$ -actin, CRALBP and GAPDH were used to probe extracts from iPS-derived RPE, autopsy RPE to measure the expression level.

Patient-specific MFRP deficient iPS-RPE cells presented morphological and functional phenotype, including disorganized actin stress fibers, increased numbers of focal adhesions, loss of apical microvilli and significantly decreased TER.  $\beta$ -actin expression was higher in MFRP deficient iPS-RPE compared with wild-type. Application of AAV-*MFRP* rescued actin disorganization phenotype, recovered apical microvilli and increased TER in MFRP deficient iPS-RPE. In both *MFRP* deficient iPS-RPE and CTRP5 over-expressed RPE, CTRP5 expression opposed MFRP expression. Over-expression of CTRP5 in wild-type RPE cells phenocopy MFRP deficient RPE cells. A favorable response to gene therapy in patient-specific cell lines suggested that this form of retina degeneration caused by *MFRP* mutations is a potential target for interventional trials. MFRP and its dicistronic partner, CTRP5, exist in an antagonistic relationship to regulate actin organization in RPE cells.

**(25) HUMAN CORD BLOOD DERIVED STEM CELLS SIGNIFICANTLY IMPROVE SURVIVAL IN A MURINE MODEL OF RECESSIVE DYSTROPHIC EPIDERMOLYSIS BULLOSA**

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Recessive dystrophic epidermolysis bullosa (RDEB) is a severe skin blistering disease caused by mutations in COL7A1-encoding type VII collagen (C7). Currently, there is no curative therapy available for patients with RDEB. Our previous studies demonstrated that human umbilical cord blood (HUCB) derived unrestricted somatic stem cells (USSCs) express C7 and facilitate wound healing in a murine wounding model. In this study, intrahepatic injection of USSCs significantly improved the blistering phenotype and enhanced the life span in the C7 null (*Col7a1*<sup>-/-</sup>) mouse. The injected USSCs were demonstrated to migrate to the sites of blistering and engraft in the recipients' skin. We further demonstrated expression of C7 at the basement membrane zone and normalization of previously disorganized integrin  $\alpha 6$  distribution, consistent with an overall histological improvement in the epidermal-dermal adherence following USSC treatment. We also demonstrated that USSCs treatment induced an infiltration of macrophages with a regenerative "M2" phenotype. Our data suggest that HUCB- derived USSCs improved the RDEB phenotype through multiple mechanisms and may represent a novel source of stem cells for treatment of patients with RDEB.

**(26) USING THE *C. ELEGANS* POSTEMBRYONIC MESODERM AS A MODEL TO DISSECT MECHANISMS UNDERLYING CELLULAR PLASTICITY AND IN VIVO REPROGRAMMING**

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The reprogramming of cell identity has tremendous potential for cell replacement therapies. Realizing this potential requires that we understand the molecular mechanisms underlying how different cell fates are specified during normal development, and how and when certain somatic cells are capable of changing their identity. The *C. elegans* postembryonic mesoderm lineage, the M lineage, provides an attractive model for these studies at single cell resolution. The M lineage is derived from a single pluripotent precursor cell, which proliferates and differentiates into 32 cells of 6 different types. These include 14 striated body wall muscles, 16 non-striated egg-laying muscles of four different types, and 2 macrophage-like non-muscle coelomocytes (CCs). We have previously found that two transcription factors, a type F forkhead transcription factor LET-381 and a SIX homeodomain transcription factor CEH-34 function in a feed-forward manner to specify the non-muscle coelomocyte cells (CCs). We further showed that LET-381/FoxF and CEH-34/SIX are sufficient to reprogram other cells into CCs in a context-dependent manner. To uncover the molecular basis of this context-dependency, we have been using both forward and reverse genetic approaches to identify factors positively required for specifying M-derived CCs, and factors that prevent other cells from being reprogrammed to CCs. To date, both forward and reverse genetic screens have led to the identification of KIN-1, the protein kinase A (PKA) catalytic subunit, and several unknown factors defined by mutations, being required for the proper specification of M-derived CCs. We are currently in the process of identifying the unknown factors and determining how they and KIN-1 function in CC specification, and what their relationship is with LET-381/FoxF and CEH-34/SIX. We are also in the process of screening of factors that prevent ectopically expressed LET-381 and CEH-34 from reprogramming other cells to CCs.

[Supported by NYSTEM contract #C028110]

**(27) GENERATION OF A MOUSE ESC REPORTER LINE TO IDENTIFY VENTRICULAR CARDIAC CONDUCTION SYSTEM CELLS DURING DEVELOPMENT**Karen Maass<sup>1</sup>, Jia Lu<sup>1</sup>, Fiona See<sup>1</sup>, Guoxin Kang<sup>1</sup>, Camila Delgado<sup>1</sup>, and Glenn I. Fishman<sup>1</sup><sup>1</sup>NYU School of Medicine, Division of Cardiology

We have previously demonstrated that contactin2 (Cntn2) protein is enriched in Purkinje cells of the adult cardiac conduction system (CCS). Here we present a mouse embryonic stem cell (mESC) reporter line to identify Purkinje-like cardiomyocytes in vitro.

Methods and Results: Dual reporter Purkinje cell mESC were generated from Cntn2-eGFP blastocysts, transduced with  $\alpha$ MHC-mCherry lentivirus. The mESC expressed markers of pluripotency (Oct3/4; Sox2, Nanog) and differentiated into all three germ layers (Nestin,  $\alpha$ -fetoprotein, Brachyury). Cultures differentiated under serum-free, cardiogenic conditions were enriched in cardiomyocytes (n=5), consisting of single positive  $\alpha$ MHC-mCherry [57.76 $\pm$ 3.7%] and double positive  $\alpha$ MHC-mCherry/Cntn2-eGFP cells [1.9 $\pm$ 0.9%]. Compared with eGFP negative cardiomyocytes (n=15), double positive cells (n=11) revealed electrophysiological characteristics consistent with Purkinje-like derivatives, including increased peak sodium currents (180.3 $\pm$ 20.5 pA/pF vs. -118.0 $\pm$ 21.2pA/pF), faster upstroke velocities (133.7 $\pm$ 12.3 V/s vs. 60.0 $\pm$ 13.2V/s) and elongated action potential duration (APD90=170.2 $\pm$ 17.5ms vs. APD90=120.6 $\pm$ 17.3ms). Calcium imaging demonstrated chronotrop regulation and spontaneous Ca<sup>2+</sup> oscillations in mCherry+eGFP+ cells. Immunofluorescence analysis demonstrated endogenous expression of Purkinje cell markers, including Cntn2, Cx40 and Troponin-T, in

double positive cells. Quantitative real-time PCR analysis of double positive cells verified cardiomyocyte-specific transcript expression [Mlc2v:  $3.8 \times 10^6$ -fold ( $p < 0.05$ ); Nkx2.5: 178-fold ( $p < 0.05$ ) compared to double negative cells]. Moreover, double positive cells expressed significantly elevated levels of CCS-specific transcripts compared to mCherry-single positive cells [Cntn2: 31-fold ( $p < 0.001$ ); Cx40: 878-fold ( $p < 0.01$ ); Cav3.1: 11-fold ( $p < 0.05$ ); HCN4: 7-fold ( $p < 0.001$ )].

Conclusion: The dual reporter Purkinje cell mESC reporter line permits identification and enrichment of ventricular CCS derivatives. Ongoing experiments study effects of small molecules and miRs on Purkinje cell fate.

[Supported by NYSTEM contract numbers C024327 and C028115]

**(28) DIVERSITY AND UNIFORMITY: DERIVATION AND CHARACTERIZATION OF MINORITY PLURIPOTENT STEM CELL LINES**

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We have derived new human pluripotent stem cell lines from African American, Hispanic and Asian origin to increase the diversity of lines available as an important resource for basic and translational research. These karyotype normal low passage, xenofree derived and propagated lines are being analyzed for transcriptome, miRNA and epigenetic histone modifications using the SUNY Buffalo Stem Cells Sequencing/Epigenomic Analysis Facility and its Next Gen Sequencing Core. For differentiation analysis using embryoid bodies (EBs) we have developed several high throughput platforms for EB formation and differentiation. These nanotechnology-based platforms utilize computer aided design (CAD) software and photolithography with spin-coated hydrogel polymer to generate microarrays, microsieves and patterning devices. These new tools provide highly uniform EB precursors for lineage differentiation and organoid formation, eliminating variations in size and shape due to fusion events. Although other methods such as hanging drop or spin cultures have been used by others for generating EB uniformity, they do not offer tight control of EB size nor allow tracking of EB formation in transparent stable and stationary templates. We find that the size of human EBs is critical to their ability to uniformly respond to differentiation cues, affecting timing and efficiency. By further combining EB microsieves and pre-templating with a post-patterning step we are able to ensure uniform settling of EBs, an additional parameter that can affect statistical analysis, thus providing a powerful set of tools to evaluate and optimize differentiation. The internal core structure of EBs varies dependent on their size and as revealed by confocal microscopy and serial microtome sectioning. The new lines bring ethnic diversity into pluripotent stem cell studies along with new tools for uniformity and high throughput analysis and are expected to expand and facilitate stem cell research in New York and beyond.

[This work is supported by NYSTEM contract # C026186]

**(29) DNA DAMAGE RESPONSE AND STEM CELL PROPERTIES OF MURINE METASTATIC TESTICULAR GERM CELL TUMORS**

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Testicular germ cell tumors (TGCTs) are the most common malignancies of young men and are unusual in being highly responsive to conventional chemotherapy, even after metastasis.

Because these tumors contain cells that express pluripotency markers such as OCT4, NANOG, and SOX2, we hypothesize that they also share other stem cell features, including a unique DNA damage response (DDR) which may explain their exquisite chemosensitivity. To investigate how stem cell properties and DDR mechanisms contribute to the treatment sensitivity of TGCTs, we generated a novel mouse model featuring conditional inactivation of the *Pten* tumor suppressor and activation of the *Kras* oncogene. *Cre/loxP* recombination was used to target these oncogenic events specifically to pre-meiotic spermatogonia via a *Stra8-Cre* transgene that is expressed in postnatal spermatogonia and sporadically in a limited number of earlier spermatogonial progenitors. By four weeks of age, 75% of mice with both oncogenic manipulations (*Pten/Kras* mice) had developed teratocarcinoma, a mixed germ cell neoplasm containing both teratoma and embryonal carcinoma, the latter having well-established stem cell characteristics. Consistent with these histopathological features, the *Pten/Kras*-induced TGCTs contained clusters of cells expressing OCT4, which is used as a diagnostic marker for human embryonal carcinoma. Interestingly, early neoplasms in this model were nearly devoid of the DNA break marker  $\gamma$ -H2AX, suggestive of distinct DDR properties relative to solid cancers originating from somatic tissues. Moreover, the OCT4-positive cells were chemosensitive and selectively depleted from primary TGCTs of cisplatin-treated *Pten/Kras* mice, findings that contrast with the chemoresistance typically observed for cancer stem cells. This may help explain the chemosensitivity and high response rate of TGCTs. Continued analysis of this model holds great promise for elucidating how stem cell properties of germ cell-derived cancers impact DDR activity during tumor development and therapy, with important implications for the prevention and treatment of a variety of cancers.

[Supported by NYSTEM contract #C026421]

**(30) LINEAGE ALLOCATION IN THE MAMMALIAN EMBRYO: DEFINING A TRANSCRIPTIONAL CIRCUITRY**

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Regenerative medicine holds great promise for replacing or regenerating damaged tissues or organs. Embryonic stem cells (ESC) and induced pluripotent stem cells (IPS) have the potential to differentiate into all the cell types of an adult and thus have great potential for replacement cells in disease. A major quest in regenerative medicine is elucidating the mechanisms directing cellular differentiation and de-differentiation (epigenetic reprogramming). A common transcriptional circuitry orchestrates these cellular decisions in human and mouse systems. Here we investigate the transcriptional circuitry underlying a fundamental binary choice cells need to make: namely, the decision to attain pluripotency versus one to differentiate. Understanding the mechanisms of cell lineage specification is imperative to utilize the unlimited potential of these stem cells. The knowledge of how lineage allocation takes place can serve as a reference to unravel general mechanisms of similar processes during development as well as mechanisms of cell transformation and cancer.

**(31) TELOMERE DYSFUNCTION AS A POTENTIAL CANCER DRIVER**

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Malignancies of lymphocytes are an incurable, heterogeneous group of diseases affecting thousands of people every year. They include chronic lymphocytic leukemia (CLL), the most

common form of adult leukemia in the Western world, as well as a heterogeneous group of malignant T-cell lymphomas collectively called cutaneous T-cell lymphoma (CTCL). Whole exome sequencing of CLL and CTCL patients has been used in recent studies to uncover the genes involved in the pathogenesis of these afflictions. Somatic mutations in *protection of telomeres 1* (POT1) emerged repeatedly among the top hits found in these reports. POT1 is a subunit of the shelterin complex, which is necessary to prevent unwanted recognition and repair of the chromosome ends as dsDNA breaks. Strikingly, the mutations in POT1 appear to be early events in the clonal evolution of CLL. In the present work we test the ability of the previously reported POT1 mutations to initiate or promote cancerous transformation, uncovering the mechanisms involved in the process. Preliminary data indicate POT1 mutations affect its protective function at telomeres, leading to replication stress-associated phenotypes. Replication stress manifests as partially replicated DNA that triggers harmful DNA repair activities causing genomic instability, a hallmark of cancer. Thus, mutations in POT1 may favor the development of cancer in the lymphatic system by inducing genomic instability. Using an array of *in vivo* and *in vitro* experiments, we are currently distinguishing between POT1 mutations as potentially sufficient ('initiators') or necessary ('promoters') for cancerous transformation. In parallel, we are testing the interaction of the POT1 mutants with factors necessary for proper replication at telomeres, deciphering the mechanism of the phenotypes triggered by the mutations. Understanding the functional impact and transformation potential of the POT1 mutations could provide valuable knowledge for diagnostic and treatment of patients that harbor such mutations.

[Supported by NYSTEM contract # C026880]

**(32) USING SUBSTRATE MECHANICS TO EXPAND CD34+ HSC**

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Hematopoietic stem cells (HSCs) offer therapeutic alternatives for a variety of diseases. However their clinical application is currently limited by the ability to expand these cells without inducing their differentiation. To address this deficiency we utilized two potential effectors of cell behavior and evaluated the impact of Notch signaling together with substrate mechanics. Notch signaling was evaluated using transductants harboring the cytoplasmic domain of Notch and substrate mechanics were evaluated using transglutaminase-crosslinked gelatin hydrogels with moduli varying from 2000Pa to 7000Pa. HSC proliferation and maintenance of stemness (CD34+, CD38-) were evaluated five days post-plating. The presence of Notch alone did not significantly affect proliferation or maintenance of stemness. Similarly, although cell modulus conformed to hydrogel modulus, varying modulus alone did not significantly alter proliferation or maintenance of stemness. However, in Notch-expressing cells grown on the softest hydrogel there was a five-fold increase in cell number and a three-fold increase in the percentage of CD34+, CD38- stem cells. In addition, optical imaging indicated that the cells on the hydrogels were coordinated with a high degree of order. As the highest order was observed on the softest matrix, the data indicate that HSCs sense surface deformation and migrate accordingly. Cell modulus was also influenced by cell density such that the cell modulus was reduced by clustering of cells. This latter observation suggests that formation of the cluster may also contribute to preserving stemness. These results show conclusively that HSCs interact with the substrates, where the mechanics of the substrate determine cellular self-organization, and ultimately differentiation and proliferation.

**(33) REGULATED CELL CYCLE DEPENDENT TRANSCRIPTIONAL SILENCING PROMOTES GERMLINE STEM CELL DIFFERENTIATION**

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Loss of stem cell differentiation has implications in aging and in diseases such as cancer. Thus, it is critical to identify factors that mediate stem cell self-renewal and differentiation. The germ line is the ultimate stem cell as it is both totipotent and immortal. Hence, paradigms established in the germ line can be extrapolated to other stem cell systems. *Drosophila* germline stem cells (GSCs) are a great model system to understand differentiation as its germline is well characterized. During *Drosophila melanogaster* embryogenesis, *polar granule component (pgc)*, a global transcriptional silencer, represses somatic transcription in primordial germ cells to promote germ line fate. Surprisingly, we have discovered that Pgc is also expressed during oogenesis. We found that Pgc is expressed in the stem cell daughter, cystoblast (CB), for a part of its life cycle suggesting that it may be expressed in a cell cycle dependent manner. To determine in which stage of the cell cycle Pgc is being expressed, we used different cell cycle markers simultaneously with a reporter for Pgc and found that majority of the CBs expressed Pgc in the early G2 phase, prior to differentiation. Additionally, we have shown that in loss of *pgc*, cell cycle is disrupted and majority of the CBs accumulate in the G1/S-phase indicating the role of Pgc in regulating cell cycle during differentiation. In addition, qPCR analysis showed Cyclin B, a regulator of G2 phase, was down-regulated in *pgc* mutants, and reduction of Cyclin B was sufficient to cause a delay in CB differentiation. Therefore, our results favor the idea that transcriptional silencing is needed to “clear” residual stem cell program, such as altered cell cycle in the GSC daughter, prior to differentiation.

**(34) ROLE OF STRAD IN HIPPOCAMPAL ADULT NEUROGENESIS**

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Neurogenesis in the adult dentate gyrus is a coordinated process that involves proliferation and fate specification of neural progenitors, followed by migration, development, maturation and synaptic integration of newborn neurons. Past effort has focused on characterization of adult SGZ stem cell niche, factors affecting stem cell proliferation, dendritic maturation, integration of newborn neurons, and their function. However, the molecular/cellular mechanisms that regulate early events in the development of adult-born dentate granule cells (DGCs) remain largely unknown.

Neurogenesis in the adult hippocampus has demonstrated effects on facilitating learning/memory and can be modulated by environmental and pathological factors, including exercise, stress and antidepressant therapy. Furthermore, adult neurogenesis is altered in, and contributes to pathological conditions such as epilepsy and mental disorders. Thus, understanding the early cellular/molecular mechanisms of adult newborn neuron development may provide clues into these neuropathologies and guide the development of new therapeutics.

Adult-born DGCs show a typical development from a quiescent stem cell to a mature DGC that within four weeks integrates into the existing circuitry. Here, we focus on a critical developmental stage characterized by a series of exquisitely orchestrated dynamic changes that include tangential to radial shift of the soma orientation and establishment of apical/basal polarity that specifies a single apical dendrite oriented to the molecular layer and a single basal axon entering the hilus. The hallmark of this developmental stage is extensive remodeling of the

basal domain that specifies single axon from multiple dendrite-like thick and axon-like thin basal processes. In our studies we identify crucial regulators of these cellular events in developing DGCs. Here we describe the role of the evolutionarily conserved kinase LKB1 and its cofactor STRAD, distinctive determinants for neuronal polarization and axon initiation in the developing embryonic cortex *in vivo*, as regulators of these early events in adult-born DGC development.

All materials generated will be made available to the broader research community for nonprofit use.

**(35) ELUCIDATING THE ROLE OF S6-KINASE IN CELL FATE SPECIFICATION USING THE C. ELEGANS GERM LINE**

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Proper balance of the proliferation and differentiation of stem/progenitor cells is crucial for normal development and tissue homeostasis. We are using development of the *C. elegans* germ line as a model system to study this balance.

Our lab has previously established that the *C. elegans* orthologs of the Target of Rapamycin (TOR) pathway: TOR, p-70-S6-Kinase (S6K) and eukaryotic initiation factor-4E (eIF4E) promote the accumulation of germline progenitors during larval development. Loss of S6K affects both cell cycle and cell fate. Notch signaling is a major regulator of germ cell fate: it promotes proliferation and/or inhibits differentiation of the progenitors. We found that loss of S6K both enhances reduction-of-function Notch (*Notch(rf)*) and suppresses gain-of-function Notch mutant phenotypes. Here, we demonstrate that, similar to its role in promoting progenitor accumulation, S6K acts germline autonomously and requires phosphorylation by TOR to regulate cell fate. However, unlike *Notch(rf) S6K(null)* mutant, *Notch(rf) eIF4E(null)* double mutants retain a progenitor pool, albeit smaller than either single mutant. Similar results are seen when *Notch(rf)* animals are subject to germline-restricted TOR RNAi. These results are consistent with the canonical role of the TOR pathway regulating cell cycle but suggest that S6K has an additional novel role in preventing differentiation in conjunction with Notch.

To determine the molecular mechanism of how S6K prevents differentiation, we tested other known enhancers of *Notch(rf)*: Cyclin-E and MAPK (Fox et al. 2011, Lee et al. 2007). To investigate if these act in a simple linear fashion with S6K to regulate cell fate, we used germline-restricted RNAi to deplete them in *Notch(rf) S6K(null)* mutants. Our preliminary data suggest that neither Cyclin-E nor MAPK acts linearly with S6K to regulate cell fate. Furthermore, we are taking both an unbiased forward genetic and biochemical approaches to identify the molecular interactors involved in S6K-mediated cell fate regulation.

[Supported by NYSTEM Contract # C026880]

**(36) GROWTH FACTOR PRIMING DIFFERENTIALLY MODULATES COMPONENTS OF THE EXTRACELLULAR MATRIX PROTEOME IN CHONDROCYTES AND SYNOVIUM-DERIVED STEM CELLS**

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The destruction of articular cartilage leads to osteoarthritis, affecting 27 million Americans. Adult articular cartilage has a poor healing capacity, which has led to intense research toward development of cell-based therapies for cartilage repair. Recent investigations have shown that chondrocytes and synovium-derived stem cells (SDSCs) are promising cell sources for cartilage repair. Cell passaging and priming with chemical or physical factors are often necessary steps in cell-based strategies for regenerative medicine. The objective of this study was to use comparative proteomics to investigate the impact of growth factor priming on 2D canine chondrocyte and SDSC cultures by identifying differentially regulated cartilage proteins. We hypothesized that primed cells in 2D culture would differentially express some extracellular matrix (ECM) proteins associated with cartilage.

Cartilage and synovium were harvested from adult canine knee joints (euthanized for other purposes). Chondrocytes and SDSCs were isolated and expanded in 2D culture with a cocktail of growth factors for priming (TGF- $\beta$ 1, PDGF-BB, and FGF-2) and compared to unprimed controls. At P2, primed and unprimed chondrocytes and SDSCs were harvested for proteomics. Label-free shotgun proteomic profiling (LC/MS/MS) was performed using data-independent scanning (MS<sup>E</sup>) with traveling wave ion mobility (TWIMS) on a Synapt-G2 mass spectrometer. Analysis focused on 1,766 proteins identified with high confidence (represented by >2 peptides). Overall, the priming effect was greater for SDSCs compared to chondrocytes. In particular, growth factor priming modulated the proteins associated with the ECM in 2D cultures of chondrocytes and SDSCs, inducing a partial dedifferentiation of chondrocytes (most proteins associated with cartilage were down-regulated in primed chondrocytes) and a partial differentiation of SDSCs (some collagen-related proteins were up-regulated in primed SDSCs). In conclusion, MS<sup>E</sup>-TWIMS protein profiling is demonstrated as a technique that could potentially be used to identify predictors of cells' utility in cartilage tissue engineering protocols.

[Supported by NYSTEM Contract # C02361]

**(37) REPROGRAMMING OF HUMAN ENDOTHELIUM INTO HEMATOPOIETIC MULTI-LINEAGE PROGENITORS BY DEFINED FACTORS AND VASCULAR NICHE INDUCTION**

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Efficient generation of transplantable hematopoietic stem cells and progenitors (HSPC) from autologous, non-hematopoietic tissues promises new therapies for hematologic diseases.

Differentiation of human pluripotent cells currently yields limited numbers of poorly engraftable HSPCs. We have developed a method of direct reprogramming of human fetal and adult endothelial cells (ECs) into hematopoietic multi-lineage progenitors (rEC-HMLPs). We identified a set of transcription factors (TFs), FOSB, GF11, RUNX1, AND SPI1 (FGRS), differentially expressed by human umbilical cord HSPCs and ECs. Transduction with FGRS TFs was sufficient to reprogram both fetal human umbilical vein ECs (HUVECs) and adult human dermal microvascular ECs (hDMECs) into engraftable rEC-HMLPs.

Since HSPCs and ECs reside in close proximity during their development, we co-cultured cells transduced with the FGRS TFs, with ECs that composed a vascular niche feeder layer in a xenobiotic- and serum-free environment. The resulting rEC-HMLPs had robust proliferative and multi-lineage colony forming units (CFU) potential, including granulocytic/monocytic, megakaryocytic, erythroid and lymphoid lineages *in vitro* and *in vivo*. A subset of engrafted rEC-HMLPs had the immunophenotypic and transcriptional profile of primary human multi-lineage progenitors, which transcriptionally phenocopied freshly isolated Lin<sup>-</sup>CD34<sup>+</sup> cord blood hematopoietic progenitors (CBHPs). When transplanted, hDMEC-derived rEC-HMLPs engrafted immunodeficient mice for at least 12 weeks in primary and 23 weeks in secondary recipients. By conditionally expressing the FGRS TFs, we further optimized differentiation of ECs into rEC-HMLPs manifesting features of self-renewing multi-potent progenitor populations (MPPs) with increased capacity to differentiate into lymphoid lineages *in vitro*. This multi-stage reprogramming approach replicates critical aspects of hematopoietic development and advances our understanding of the hierarchy of TFs and microenvironmental cues that orchestrate hematopoietic specification. This approach may prove useful for engineering autologous engraftable hematopoietic cells for regenerative medicine.

[Supported Contract # C026878]

**(38) SMALL RNAs DERIVED FROM lncRNA *RNase MRP* HAVE GENE-SILENCING ACTIVITY RELEVANT TO HUMAN CARTILAGE-HAIR HYPOPLASIA AND THE U12-DEPENDANT SPLICEOSOME**

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Point mutations in the *RNase MRP* (RMRP) gene causes the autosomal recessive disorder, human cartilage-hair hypoplasia (CHH). Abnormal function of stem cells can account for major phenotypes of CHH and understanding the role of RMRP in their biology, will likely be fundamental to understanding the disease.

We have found that *RMRP* is the source of two short (~20 nt) RNAs designated RMRP-S1 and RMRP-S2, which function as miRNAs. Several disease-causing mutations map to RMRP-S1 and -S2 and the expression of these RNAs are significantly reduced in several cell lines derived from CHH patients. Tests of gene regulatory activity of RMRP-S1 and -S2 identified over 900 genes that were significantly regulated many of which function in skeletal development, hair development and hematopoietic cell differentiation. Furthermore genes associated with alternative RNA splicing, cell proliferation and differentiation were also highly targeted.

We have since been working on further elucidating the origins and roles of RMRP S1 and S2. We had already established that RMRP S1 and S2 were regulated by Dicer, now we show preliminary data that indicates RMRP S1 and S2 expression is also regulated by Drosha. Drosha is involved in processing primary miRNA transcripts into precursor miRNAs therefore this contributes further evidence to the miRNA-like nature of the lncRNA-derived small RNAs.

We have reported that multiple snRNAs in the U12 spliceosome are down-regulated by RMRP-S1 and S2 [15]. Regulation of snRNA components of the splicing machinery by small RNA species has not previously been reported. Preliminary results show that RMRP S1 and S2 over-expression results in a vast upregulation of U12-dependant splicing.

This work has highlighted a connection between RMRP and Microcephalic Osteodysplastic Primordial Dwarfism type 1 (MOPD1), a severe disorder characterized by extreme multiple organ abnormalities, dwarfism and early morbidity. The disease is caused by point mutations in the snRNA, U4atac, an essential component of the U12-dependent spliceosome [12-14]. Our continuing work focuses on the effect of RMRP S1 and S2 on the U12 spliceosome which will help to draw a functional connection between CHH and MOPD1 and understand the connection between lncRNA-derived small RNAs on snRNAs in the splicing machinery.

**(39) ART-27 REGULATES SPERMATOGONIAL STEM CELL DIFFERENTIATION AND SERTOLI CELL FUNCTIONS IN THE MOUSE TESTIS**

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ART-27 is an X-linked gene that is part of an Androgen Receptor (AR) transcriptional repressor complex in prostate cancer cells. Because of its previously defined role testosterone-mediated signaling, we hypothesized that ART-27 deletion would affect AR signaling in Sertoli cells. In Sertoli cell-specific ART-27 knockout testes, we found loss of round spermatids and elongating sperm in approximately 50% of tubules, with mature sperm in the epididymus. Serum FSH levels were elevated along with increased Inhibin alpha expression in ART-27 deleted mice, consistent with germ cell loss. ART-27 was also shown to interact with URI, an RNA Polymerase subunit (RPB5)-interacting protein. URI deletion results in DNA damage and germ cell loss in *C. elegans* and *Drosophila*, and our lab has shown that ART-27 knockdown reduces URI protein stability. We hypothesized that germ cell-specific ART-27 deletion using Vasa-Cre would reduce URI protein stability and result in germ cell loss. Indeed, germ cell-specific deletion of ART-27 in males leads to infertility and germ cell loss as early as 6 days post-partum (dpp), with complete germ cell absence by 11dpp. This suggests that ART-27 deletion affects spermatogonial stem cell self-renewal. We will further probe the cause of these phenotypes by examining changes in AR target gene expression in ART-27 null Sertoli cells and self-renewal pathways in germ cells.

**(40) GATA6 LEVELS MODULATE PRIMITIVE ENDODERM CELL FATE CHOICE AND TIMING IN THE MOUSE BLASTOCYST**

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Two sequential binary cell fate decisions lead to the formation of the three lineages of the pre-implantation mouse blastocyst. In the first, trophectoderm and inner cell mass (ICM) are specified; in the second, ICM cells adopt a pluripotent epiblast (EPI) or primitive endoderm (PrE) fate. While EPI-biased cells express pluripotency-associated factors such as NANOG and SOX2, the transcription factor GATA6 is the earliest expressed factor specific to PrE-biased cells.

NANOG and GATA6 are thought to be at the center of the gene regulatory network directing EPI and PrE fate, however, their precise and possibly antagonistic roles within the cells of the ICM, as lineage biases emerge, remains an open question. Both genes are initially co-expressed in all cells, but go on to become exclusive to EPI (Nanog) and PrE (Gata6) by around the mid blastocyst stage. This process of lineage restriction is mediated by FGF signaling. EPI-biased cells express FGF4 and activate the RTK/MEK pathway to induce neighboring cells to adopt a PrE fate.

The relationship between FGF/RTK signaling and the segregation of NANOG and GATA6 to EPI- and PrE-biased cells respectively is still unclear. To investigate the mechanistic regulation of this process, we applied an unbiased, quantitative, single-cell resolution image analysis pipeline to analyze embryos lacking or exhibiting reduced levels of GATA6. We identify *Gata6* as the first single transcription factor mutation exhibiting a complete absence of PrE, and demonstrate that GATA6 levels regulate the timing and speed of lineage commitment within the ICM. Furthermore, we show that FGF signaling mediated repression of NANOG is GATA6-dependent, and propose a model where interactions between NANOG, GATA6 and the FGF/ERK pathway determine ICM cell fate. This study provides a framework for quantitative analyses of mammalian embryos, and sheds light on the lineage decision that gives rise to the pluripotent state.

**(41) ROLE OF AZACYTIDINE IN DIFFERENTIATION OF HEMATOPOIETIC STEM CELLS (HSCS) IN HORSES WITH COMMON VARIABLE IMMUNODEFICIENCY (CVID)**

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CVID in horses shares numerous similarities in clinical disease and patient care with CVID in humans, a late-onset condition that impairs B cell development in the bone marrow (BM). B cell development from HSCs is highly regulated by BM extracellular signals, transcription factors (TFs) and epigenetic modifications. Our current transcriptome and quantitative RT-PCR data obtained from BM of CVID-affected and control healthy horses revealed that B cell production is halted at the pre-pro and pro-B cell developmental stage. The expression of the TFs E2A and PAX5, as well as the B cell marker CD19 is significantly lower in CVID-affected horses in comparison to healthy horses. Furthermore, methylation studies by bisulfite PCR showed that the equine PAX5 and CD19 genes in BM of CVID horses are differentially methylated in comparison to healthy control horses. Since epigenetic changes are reversible with inhibitors of DNA methyltransferase, we attempt to rescue B cell lymphopoiesis from BM-derived HSCs of CVID-affected horses using the demethylation agent azacytidine (5-aza-2'-deoxycytidine). Initially, we enriched HSCs from frozen BM aspirates and demonstrated the presence of CD34<sup>+</sup> cells in CVID-affected horses using immunocytofluorescence microscopy (ICFM). We then cultured the CD34<sup>+</sup> cells in the presence of BM-derived stromal cells for 18 days in alpha-MEM and cytokine cocktail. In parallel, another set of cultures was treated with azacytidine (IC50=7.9 µM) on days 8, 9 and 10 of culture. While a low percentage of CD19<sup>+</sup> cells was found in untreated control cultures using ICFM, the same was not measured in treated and untreated cultures from CVID-affected horses. We are currently testing a serum-free culture system with a commercial hematopoietic expansion medium and serum-deprived stromal cells to evaluate how treatment of HSCs with azacytidine can repair B cell differentiation in CVID HSCs.

**(42) A HIGH-THROUGHPUT EXTRACELLULAR MATRIX PLATFORM IDENTIFIES EXTRACELLULAR MATRIX-CELL INTERACTIONS THAT MAINTAIN PLURIPOTENCY**

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Pluripotent stem cells are unique in their ability to self-renew and give rise to all germ-layer derived cell lineages, consequently making them attractive targets for a wide range of research and clinical applications. Pluripotent stem cells interact with their surrounding microenvironment which regulate the maintenance of self-renewal and differentiation both in vivo and in vitro. The pluripotent state is subsequently influenced by a variety of intrinsic and extrinsic signals, but the precise signaling cross-talk that maintains pluripotency remains unclear. Prior work has shown that certain extracellular soluble factors such as basic fibroblast growth factor perturb the maintenance or loss of pluripotency. In vivo and in vitro, cells are contacted by signal-inducing extracellular matrix (ECM), and yet the impact of ECM on the pluripotent signal cascade is largely unstudied. Studies targeting integrins, a family of cell surface receptors that mediate ECM interactions, have implicated integrin-ECM interactions as regulators of pluripotency and cell survival. However, in addition to adhesion, integrins transmit information about the mechanical and chemical state of the extracellular environment, therefore an unbiased evaluation of cell-ECM interactions are necessary to interrogate the potential interactions. Here, we describe an unbiased approach to uncover pluripotency-maintaining ECM combinations, and examine their signaling characteristics using an ECM microarray platform containing 768 unique pairwise ECM molecule combinations. We identify three ECM combinations that support long-term human iPSC/ESC self-renewal and compare pairs of supportive or non-supportive ECM molecules to directly examine their influence on stem cell fate decisions. We reveal that specific ECM combinations induce different SMAD and AKT signaling patterns, and provide a direct link from extrinsic-derived signals to the pluripotency network.

**(43) SPATIOTEMPORAL CONTROL OF CANCER STEM CELLS BY VERSATILE MICRORNA MECHANISMS**

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We discovered that colon cancer stem cells (CCSC) undergo both symmetric and asymmetric division, which balance differentiation versus self-renewal in the tumor population [1]. This decision is determined by the microRNA miR-34a, whose spatial segregation generates a bimodal Notch response that determines cell fate outcomes. This bimodal response is caused by kinetic mutual sequestration between miR-34a and Notch mRNA.

However, three questions remain. First, what is the relationship between miR-34a and the canonical cell fate determinant Numb, which also targets Notch to regulate cell fate symmetry? Second, what is the upstream regulator of miR-34a that causes it to be asymmetrically segregated? And third, does miR-34a generate bimodal responses from all of its target genes? Here, we report a mechanism in which miR-34a targets Numb to generate a bimodal Numb response. Furthermore, miR-34a and Numb form an incoherent feed forward loop (IFFL), which

synergistically enhance Notch and cell fate bimodality by orders of magnitude. Dynamically, the IFFL exhibits adaptive behavior to offset interference from other miR-34a target genes, hence buffering cell fate outcomes from fluctuations in miR-34a levels.

Next, we report a novel, p53-independent mechanism that regulates miR-34a distribution during cell division, this upstream complex can asymmetrically localize to one of the daughter cells and inactivate miR-34a, ensuring self-renewal.

Third, we performed a systematic study of miR-34a regulation kinetics on its many target genes. Quantitative single-cell analysis revealed that miR-34a generates bimodal responses from a small subset of genes that are involved in cell fate determination, but regulating the majority of genes (e.g., metabolic and growth genes) in a graded, continuous manner. These data suggest that a microRNA can regulate its many target genes in a context-dependent manner based on their cellular functions.

[Supported by Cornell Stem Cell Program]

**(44) MODELING HEPATITIS B VIRUS INFECTION IN IPS-DERIVED HEPATOCYTES SUGGESTS A RESTRICTIVE ROLE FOR THE INNATE IMMUNE RESPONSE**

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Worldwide, hepatitis B virus (HBV) infection is the most common viral hepatitis having infected over two billion people and chronically infecting more than 400 million, putting them at increased risk to develop cirrhosis and hepatocellular carcinoma. HBV research has been hampered by the virus's narrow host range and cellular tropism for hepatocytes, which have led to a paucity of robust and reliable infectious systems for HBV. As a consequence, major components of the viral entry process and viral life cycle — including the establishment and persistence of a nuclear cccDNA pool — and many aspects of virus-host interactions have been poorly understood. In this study, we report that iPS-derived hepatocyte-like cells (iHep) support the HBV viral life cycle, and demonstrate the utility of this system for the study of virus-host interactions. We demonstrate that during iHep generation, distinct stages of differentiation are well defined and characterized by the sequential emergence of various hepatocyte-related factors known to be critical for HBV gene expression and replication. By using a reporter for HBV transcription, we show that the virus becomes transcriptionally active at around day 18 of differentiation, the time when its receptor, the bile acid pump NTCP, also starts to be expressed. Interestingly, although a productive infection with establishment of cccDNA is achieved at day 20 of differentiation, it is largely dependent on inhibition of the JAK-STAT pathway, a major component of the innate immune response. Furthermore, HBV infection is accompanied by induction of various interferon-stimulated antiviral genes, including viperin. This argues for the importance of the innate immune response in establishment and maintenance of HBV infection and exemplifies the utility of the iHEPs system for further identification of host targets for future, more effective, anti-HBV drugs.

**(45) DENTAL PULP CELL DIFFERENTIATION AND DEDIFFERENTIATION IS REGULATED BY MECHANICS OF POLYBUTADIENE FILMS**

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Dental pulp stem cells (DPSCs) will differentiate into odontoblasts, osteoblasts, adipocytes or neurons dependent on media compositions. The mechanics and topography of materials can also direct their differentiation, but the challenge lies in designing materials where each property can be varied independently. Here we show how the polyolephin polymer, polybutadiene (PB), forms an ideal substrate to isolate the effects of mechanics, topography, and chemistry. Polybutadiene is a biocompatible rubber analogue of the dental composite, Gutta Percha. It can be synthesized in monodisperse form ( $M_s=205K$ ,  $M_w/M_n=1.49$ ,  $T_g=-95C$ ) to produce substrates whose mechanical properties follow an inverse power law dependence on film thickness. We show that DPSC on PB continually adjust their modulus,  $E_c$ , to obey the same power law as that of the substrate modulus,  $G$ , such that  $E_c=0.5G$ , for all film thicknesses. Furthermore, a critical value,  $G_0$ , exists such that for  $G>G_0$ , DPSC differentiate without need of soluble factors such as dexamethasone. These cells promote crystalline hydroxylapatite deposits identified by SEM, EDX, and glancing incidence X-ray diffraction and express osteocalcin detected immunohistochemically throughout a multilayered sample, even for cells *not* in direct contact with the substrate. DPSC promotion of hydroxylapatite ceased when surface contacts were broken and cells were transferred to PB with  $G<G_0$ . However, upon return to PB surfaces with  $G>G_0$ , cells rapidly and robustly resumed mineralization consistent with a memory of prior differentiation. E-beam lithography was then used to produce multiscale patterned Si supports, which when coated with PB, produced mechanical patterns with  $G>G_0$  within a softer matrix having  $G<G_0$ . Although microscaled patterns resulted in bimodal distributions of cell moduli, biomineralization was suppressed throughout. Surprisingly, cells sensing nanoscale mechanical heterogeneity, promoted robust biomineralization, indicating the importance of the length scale of mechanical heterogeneity.

**(46) CHARACTERIZATION OF STROMAL DERIVED FACTOR -1 MEDIATED CHEMOTAXIS OF TRANSPLANTABLE RETINAL PROGENITOR AND PHOTORECEPTOR PRECURSOR CELLS**

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A growing number of studies are evaluating retinal progenitor cell (RPC) and photoreceptor precursor (PPC) transplantation as an approach to repair retinal degeneration and restore visual function. To advance cell-replacement strategies for a practical retinal therapy, it is important to define the molecular and biochemical mechanisms guiding migration of transplantable cell populations. We have analyzed RPC and PPC CXCR4 receptor expression and evaluated whether exposure to stromal derived factor-1 (SDF-1) influenced their motogenic activity *in vitro*. Expression of CXCR4 receptors on RPCs and PPCs was observed via immunocytochemical staining and validated by Western blot results. Boyden chamber assays were used to initially screen for motogenic effects of SDF on multi-RPCs and PPCs and revealed a concentration-dependent chemotactic response of cells to the ligand at an optimal

concentration of 100ng/ml. Upon exposure to steady state gradients of the ligand in engineered microfluidic chambers, RPCs exhibited significant increases in their center of mass and maximum distances traveled towards the source of SDF. We also ascertained the biochemical signaling requisite for ligand-induced RPC motility by using CXCR4 inhibitor, AMD 3100, to block the SDF receptor and this resulted in a significant decrease in retinal progenitor cell movement in Boyden assays. These results define SDF as a potential chemotactic ligand for RPCs and PPCs. Further studies will characterize receptor expression dynamics and downstream signaling pathways. Data from this work may be applied to defining the molecular mechanisms of transplantable cell migration and to improved efficiency in stem cell therapies for repair of neural retina.

[This work was supported by a National Institute of General Medical Sciences (NIGMS) GM096935 grant (SR) and National Science Foundation (NSF) CBET 0939511(MV)]

**(47) SYNCHRONIZED AND HIGHLY EFFICIENT REPROGRAMMING IN A CELL-TYPE DEPENDENT MANNER**

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Reprogramming of fibroblasts into iPS cells occurs at low frequencies (0.1-1% of input cells) and typically after a considerable lag phase (> 12 days). Researchers in the field have proposed that reprogramming is a stochastic process in which several epigenetic roadblocks have to be overcome. Inhibition of signaling pathways such as TGF $\beta$  and the use of Vitamin C, an antioxidant and cofactor of chromatin modifying enzymes, have been used to overcome epigenetic barriers and enhance the efficiency of reprogramming. Recently it was shown that cells over-expressing the Yamanaya factors and genetically lacking MBD3, a core member of the NuRD repressor complex, gave rise to iPSCs in a deterministic fashion arguing against reprogramming being inherently stochastic.

To assess whether OKSM factors can influence deterministic reprogramming without any genetic manipulation, we have conducted a screen with known small molecules that enhance reprogramming with the goal of finding chemical combinations that synergistically increase the synchronization and efficiency of iPS cell generation. This led to the discovery of a combination of three small molecules (3c) that trigger highly efficient (>95%) and rapid reprogramming (3 days) of mouse fibroblasts and hepatoblasts and deterministic reprogramming of blood progenitor cells. The proof-of-principle that deterministic reprogramming can be achieved without genetic manipulation will greatly facilitate reprogramming experiments and offers a novel approach for the studying of the molecular mechanism leading to the acquisition of pluripotency.

[Supported by NYSTEM contract #C026880]

**(48) ASYMMETRICALLY MODIFIED NUCLEOSOMES AND THEIR ROLE IN THE ESTABLISHMENT OF BIVALENT DOMAINS IN EMBRYONIC STEM CELLS**

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Histone posttranslational modifications (PTMs) are key to the regulation of gene expression. Mononucleosomes contain two copies of each core histone. Despite extensive research on histone PTMs, it remained unknown whether both copies carry identical PTMs *in vivo*. The symmetry state of modifications on sister histones bears directly on their potential inheritance, readout by effector proteins, and the range of mark combinations achievable at a given locus. Bivalent domains contain the activating mark histone H3 lysine 4 trimethylation (H3K4me3) and the repressive mark H3K27me3. Both their nucleosomal conformation and mechanism of establishment remain largely elusive. To assess histone PTM symmetry, we devised an approach based on modification-specific antibodies and quantitative mass spectrometry (MS). We show that chromatin consists of both symmetric and asymmetric nucleosome populations *in vivo*. For both H3K27me2/3 and H4K20me1, we observed a combination of symmetric and asymmetric nucleosomes for ES cells, HeLa cells, and MEFs, suggesting that asymmetry might be a general feature of nucleosomes. To explore its implications, we analyzed co-occurrence of histone marks and obtained evidence for bivalent nucleosomes carrying both H3K4me3 and H3K27me3. Interestingly, PRC2-mediated methylation of H3K27 was inhibited when nucleosomes contain H3K4me3 or H3K36me3 in symmetrical, but not asymmetrical fashion. In line with these findings, MS analysis revealed that H3K27me2/3 and H3K4me3/H3K36me3 asymmetrically reside on opposite tails in bivalent nucleosomes *in vivo*. These findings uncover a potential mechanism for the incorporation of bivalent features into nucleosomes and show how asymmetry might diversify functional nucleosome states. To further clarify the establishment of bivalency and the interplay of the marks involved, we are analyzing how symmetric and asymmetric presence of H3K27me3 affects activity of the H3K4me3 methyltransferases of the SET1/MLL families. Moreover, the readout of bivalent nucleosomes is currently being analyzed in an unbiased fashion using asymmetric nucleosome templates. Recent data will be presented.

[Supported by NYSTEM contract # C026880]

**(49) A "BTB-POZ" KEY TO GROUND-STATE PLURIPOTENCY DURING SOMATIC CELL REPROGRAMMING**

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Nac1 (Nucleus Accumbens-1) belongs to the Bric-a-brac Tramtrac Broad complex/Pox virus and Zinc finger (BTB/POZ) family of transcription factors. It is ubiquitously expressed and originally identified in the nucleus accumbens of rat brain, as a cocaine-inducible gene. Nac1 is also enriched in mouse embryonic stem cells (mESCs), interacts directly with Nanog, and shares many target genes with several stem cells pluripotency factors. Here we show that Nac1 null mESCs can be maintained indefinitely and are pluripotent, as demonstrated by *in vitro* and

in vivo assays. However, loss of Nac1 skews differentiation of mESCs toward the trophectoderm lineage. On the other hand, somatic cell reprogramming using Nac1-deficient mouse embryonic fibroblasts (MEFs) demonstrates that Nac1 is required for efficient generation of high quality iPSC colonies. We found that reprogrammed cells from Nac1-deficient MEFs were arrested at a distinct pluripotency state, and can be rescued to ground state pluripotency by re-introduction of Nac1 transgene, but not other core pluripotency genes such as Nanog and Esrrb. Gene expression profiling during rescue experiments lead us to the identification a major Nac1-regulated pathway that directly controls efficient reprogramming and ground state pluripotency.

[Supported by NYSTEM contract # C028103]

**(50) Brd4 INTERACTS WITH Oct4 AND CONTROLS PLURIPOTENCY AND X-CHROMOSOME INACTIVATION**

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Embryonic stem cell (ESC) pluripotency is controlled by defined transcription factors. During cellular differentiation ESCs undergo a global epigenetic reprogramming. Female ESCs exemplify this process as one of the two X-chromosomes is globally silenced during X-chromosome inactivation (XCI) to balance the X-linked gene disparity with XY males. The pluripotent factor, Oct4 lies at the top of the XCI hierarchy, and regulates XCI by triggering X-chromosome pairing and counting. Here we show that the epigenetic reader, Brd4, a member of the BET protein subfamily interacts with Oct4. Brd4 enriched at many sites where Oct4 binds. Inhibition or depletion of Brd4 alters cell fate away from pluripotency and disrupts proper XCI. It is well known that Brd4 recruits p-TEFb to gene promoters. P-TEFb phosphorylates RNA polymerase II resulting in productive mRNA transcriptional elongation. Consistent with this, we also found that Brd4 recruits p-TEFb to many Oct4 targets in ESCs. Inhibition of p-TEFb activity with the small-molecule flavopiridol dramatically represses the transcription of many Oct4 gene targets. Taken together, we ascribe an important role for Brd4 in maintaining ESC fate and XCI status.

**(51) VALIDATION OF GWAS ALLELES WITH AMD PATIENT-SPECIFIC STEM CELL LINES**

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While the past decade has seen great progress in mapping loci for common diseases, studying how these risk alleles lead to pathology remains a challenge. Age-related macular degeneration (AMD) affects nine million older Americans, and is characterized by loss of the retinal pigment epithelium (RPE). Although the closely linked genome-wide association studies (GWAS) ARMS2/HTRA1 genes, located at the chromosome 10q26 locus, are strongly associated with the risk of AMD, their downstream targets are unknown. Low population frequencies of risk alleles in tissue banks make it impractical to study their function in cells derived from autopsied tissue. Moreover, autopsy eyes from end-stage AMD patients, where age-related RPE atrophy and fibrosis are already present, cannot be used to determine how abnormal ARMS2/HTRA1 expression can initiate RPE pathology. Instead, induced pluripotent stem (iPS) cell-derived RPE from patients provides us with earlier stage AMD patient-specific cells and allows us to analyze the underlying mechanisms at this critical time point. An unbiased proteome screen of A2E-aged patient-specific iPS-derived RPE cell lines identified SOD2-mediated antioxidative defense in the genetic allele's susceptibility of AMD. The AMD-associated risk haplotype (T-in/del-A) impairs the ability of the RPE to defend against aging-related oxidative stress. SOD2 defense is impaired in RPE homozygous for the risk haplotype (T-in/del-A; T-in/del-A), while the effect was less pronounced in RPE homozygous for the protective haplotype (G-Wt-G; G-Wt-G). ARMS2/HTRA1 risk alleles decrease SOD2 defense, making RPE more susceptible to oxidative damage and thereby contributing to AMD pathogenesis.

[Supported by NYSTEM Contract # C02361]

**(52) HETEROGENEITY OF STEM CELLS IN AMNIOTIC FLUID**

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Amniotic fluid cells from second trimester amniocentesis (hAFSC) have been found to be a source of multipotent stem cells which might overcome the limitations of expansion, histocompatibility, tumorigenesis and ethical issues associated with the use of human embryonic cells. Previous work by others demonstrated pluripotency and growth patterns in c-Kit selected cells. We sought to perform a more comprehensive investigation of their pluripotency and the culture characteristics and distribution of stem cell markers in c-Kit selected cells compared to c-Kit negative cells. Using MACS & FACS we found less than 5% of hAFSC were c-Kit positive. However when cultured, between 15-90% of the c-Kit negative cells expressed CD90, SSEA4 or TRA-1-60, in varying amounts. There was persistence of stem cell markers including expression of SSEA4, TRA-1-60, CD90 in vitro through multiple passages and subpopulations in a high percentage of cells. There was increased Oct4, Nanog and Sox2 mRNA expression in cells derived from 15-17 gestation week amniotic fluid samples versus longer gestational ages. Double and triple labeled cell populations were identified by MACS. 5.5% of c-Kit negative cells were triple positive for SSEA4, TRA-1-60 and CD90 expression. This may be a more efficient method than c-kit selection of hAFSC because stem cell markers expression was equal to or exceeded by the c-kit negative cells in our results. Differentiation of amniotic fluid cells was successfully induced for neural, bone and cartilage lineages using specific induction media as demonstrated by morphologic staining and fluorescent histochemistry. The occurrence of triple-labeled cell populations poses the intriguing possibility of cells with a closer resemblance to embryonic stem cells. Our results confirm that hAFSC maintain pluripotency markers in culture over enough passages to provide sufficient numbers of cells for clinical use. Current studies with serum-free media offer therapeutic promise.

**(53) APPLICATION OF GRAPHENE BASED POLYMER NANOCOMPOSITE SUBSTRATE TO INDUCE ADULT DENTAL PULP STEM CELL (DPSC) DIFFERENTIATION**

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Graphene is a new material, which has much potential for biomedical applications. It has unique properties such as high electrical and thermal conductivity, mechanical elasticity, and has been shown to induce differentiation of mesenchymal stem cells. Polyisoprene (PI) is a major component of Gutta Percha, the material used for obturating root canals. Recently, we have shown that this material may enhance dental pulp cell differentiation, which opens up the opportunity for its use in regenerative dental therapy. Currently, in order to achieve the appropriate mechanical and anti-bacterial properties for use in root obturation, PI is compounded with zinc oxide nanoparticles. As these particles are cytotoxic when not encased in PI, we sought to a replacement material more appropriate for regenerative therapies. Here we show that graphene may be an appropriate substitute. It is biocompatible, non-toxic, and provides the desired mechanical and anti-bacterial response. For these experiments 50% graphene and PI suspensions were spun cast from toluene solutions onto Si substrates. Dental pulp cells were plated on the films and incubated in for 28-days in growth media with and without dexamethasone as an inducer of differentiation. Biomineralization and cell differentiation were compared to cells plated on 120 nm PI films and tissue culture plastic controls. In the absence of dexamethasone, no biomineralization was observed on the PI film or on tissue culture plastic. However, copious amounts were observed on the graphene containing PI films. All samples biomineralized in the presence of dexamethasone. Expression of the differentiation marker osteocalcin by immunohistochemistry followed the biomineralization results, suggesting that graphene promoted both differentiation and biomineralization. In addition, although not cytotoxic to human dental pulp cells, graphene containing PI films were found to be cytotoxic to *Escherichia coli* as determined using dead/live staining visualized by confocal microscopy.

[Supported in part by NSF-Inspire]

**(54) STUDYING PANCREATIC DEVELOPMENT AND DIABETES IN A DISH THROUGH EFFICIENT GENETIC MANIPULATION OF HUMAN EMBRYONIC STEM CELLS**

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Recent advances in sequencing technology and genome-wide association studies have led to rapid discoveries of disease-associated genes. For instance, studies have identified novel genes associated with neonatal diabetes, which may also play a role in type II diabetes. However, functional analysis of these genes remains a major challenge.

Human embryonic stem cells (hESCs) provide a great system to recapitulate the complexity of human development *in vitro*. Genetic manipulations of hESCs allow us to determine the functional significance of disease-associated genes for human pancreatic development. By combining the TALEN and CRISPR/Cas9 system, we have developed two rapid and highly efficient genetic manipulation tools in hESCs: iEXPRESS (TALEN) that allows inducible gene

expression for gain-of-function studies, and iCRISPR (CRISPR/Cas9) that enables us to generate gene knockouts for loss-of-function studies.

Using iCRISPR, we have generated hESC knockout mutants for 6 neonatal diabetes-associate genes, *MNX1*, *PDX1*, *PTF1A*, *NGN3*, *RFX6* and *GLIS3*, to investigate the effect of these mutations on pancreatic development with a focus on the specification of insulin-expressing  $\beta$  cells. We observed complete loss of endocrine differentiation in *NGN3* (*NGN3*<sup>-/-</sup>) mutants. Using iEXPRESS, we found that ectopic overexpression of *NGN3* increased differentiation into endocrine cells by approximately 10-fold. Interestingly, ectopic overexpression of *NGN3* also initiated migration of endocrine cells to form islet-like clusters, a process that mimics islet morphogenesis during pancreatic development. Our studies also demonstrated key roles of *PDX1* and *RFX6* in the specification of  $\beta$  cells. A decrease in differentiation into insulin producing  $\beta$  cells was observed in both *PDX1* (*PDX1*<sup>-/-</sup>) and *RFX6* (*RFX6*<sup>-/-</sup>) knockout mutants.

Our results demonstrate the feasibility and potential of genetic studies in a dish. Through efficient and precise genetic manipulation of hESCs, we aim to dissect both conserved and human-specific mechanisms of pancreatic development and diabetes pathogenesis.

[Supported by NYSTEM contract # C026879]

**(F1) RENSSELAER CENTER FOR STEM CELL RESEARCH**

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The Rensselaer Center for Stem Cell Research (RCSCR) provides research support and training in stem cell culture, imaging and analysis. The state-of-the-art laboratory encompasses equipment for standard cell culture and hypoxia studies, as well as liquid handling, time lapse and high-content microscopy. Use of the facility is available to all researchers, especially New York State investigators. Since its establishment in 2012, the RCSCR has supported numerous pilot studies, educational events and internships, and as well as support for a diverse array of stem cell research projects. Research within the stem cell facility is enhanced by direct collaboration with several neighboring Rensselaer research cores, including advanced microscopy, bioimaging, cell & molecular biology, proteomics, nuclear magnetic resonance and animal research facilities.

[Supported by NYSTEM Contract # C026717]

**(F2) DEVELOPMENT OF cGMP-COMPLIANT STEM CELL CULTURE PROCESSES AT THE UPSTATE STEM CELL cGMP FACILITY**

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The University of Rochester Medical Center (URMC) received a grant from the Empire State Stem Cell Board in 2011 for the construction of a facility enabling the production of human stem cell therapies suitable for testing in human clinical trials. The Upstate Stem Cell cGMP Facility (USCGF) became operational in late 2012 and provides ~3600 ft<sup>2</sup> of manufacturing and testing space specifically designed for accelerating “first in man” early phase clinical studies.

The USCGF is a multi-use cGMP facility with capabilities for: the manufacturing of materials for preclinical efficacy and safety/toxicology studies; development of clinical-scale manufacturing processes; development and validation of analytical test methods for clinical trial materials; and cGMP manufacturing of clinical-grade materials for early phase trials. In addition, the USCGF has established a cGMP-compliant Quality Management System consisting of an organizational structure, responsibilities, procedures, processes, and resources integrated to ensure product safety for use in human clinical studies.

The key to establishing a cGMP-compliant manufacturing process is to develop a documented reproducible process using qualified materials and establishing appropriate specifications for product release. This presentation highlights the design characteristics, operations and capabilities of the USCGF, as well as the progress made thus far on the development of cGMP clinical-scale processes to produce materials for two current NYSTEM consortium grants. These include: 1) adult retinal pigment epithelial stem cells as a replacement therapy for age-related macular degeneration (Sally Temple, PhD; Jeffrey Stern MD, PhD; Neural Stem Cell Institute, Rensselaer, NY) and 2) human oligodendrocyte progenitor cells as a means of restoring lost myelin in treating chronic progressive multiple sclerosis (Burk Jubelt, MD; Upstate Medical University, Syracuse, NY; Steve Goldman, MD, PhD; University of Rochester Medical Center).

The USCGF is a key component of URM's Stem Cell and Regenerative Medicine Institute and is a resource available to scientists in institutions throughout New York State.

(Supported by NYSTEM contract #C026713)

**(F3) NYSTEM HIGH-THROUGHPUT SCREENING AND CHEMISTRY SHARED FACILITY AT COLUMBIA UNIVERSITY**

Charles Karan<sup>1,8</sup>, Jan Michael Austria<sup>1,8</sup>, Andrea Califano<sup>1,2,8</sup>, Olivier Couronne<sup>1</sup>, Shi-Xian Deng<sup>3</sup>, Donald W. Landry<sup>3</sup>, Hai Li<sup>1,8</sup>, Sergey Pampou<sup>1,8</sup>, Ronald Realubit<sup>1,8</sup>, Alison Rinderspacher<sup>3</sup>, Arie Zask<sup>5,8</sup>, Brent R. Stockwell<sup>5-8</sup>, Christopher E. Henderson<sup>4,8</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 to 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 facility continues to expand its arsenal of small molecules including new libraries of biologically active small molecules and a new custom-designed 61,000-compound diversity set. Since 2011 the OCCC has provided 10 research labs with organic synthesis support. Of these projects 8 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>.

[Supported by NYSTEM contract # C026715; and by a grant from the Helmsley Trust]

**(F4) 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 the 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.

Available libraries include human and mouse whole genome (with custom updated RefSeq targets) siRNA, and miRNA mimic and inhibitors for reverse genetic screens. Cell-based assays are optimized and developed with the support of Core staff. Our instrumentation includes a multimode filter-based plate reader, quad-monochromater, infrared plate reader for In-Cell Westerns, and a high content imaging microscope.

A summary of stem cell related projects will be presented as well as best practices for assay development and optimization. Data highlighting and comparing the capabilities of assay instrumentation will also be presented. Our streamlined data workflow will emphasize the importance of data visualization and analysis.

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.

[Supported by NYSTEM contract #C026719]

#### **(F5) PLURIPOTENT STEM CELL PRODUCTION AND CHARACTERIZATION OF STEM CELLS DERIVATIVES IN THE HELMSLEY STEM CELL CORE FACILITY AT COLUMBIA**

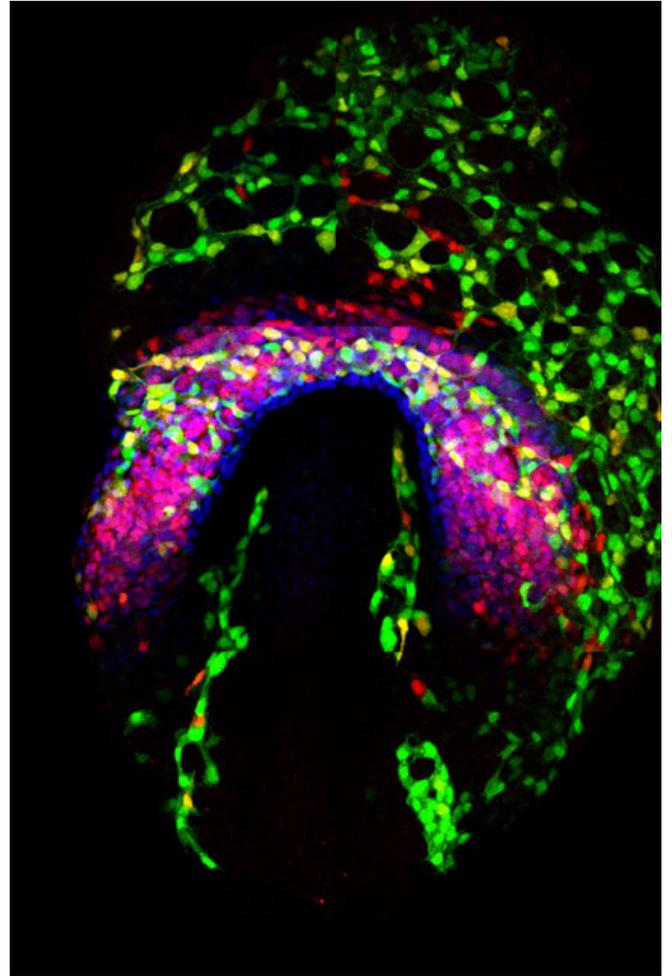
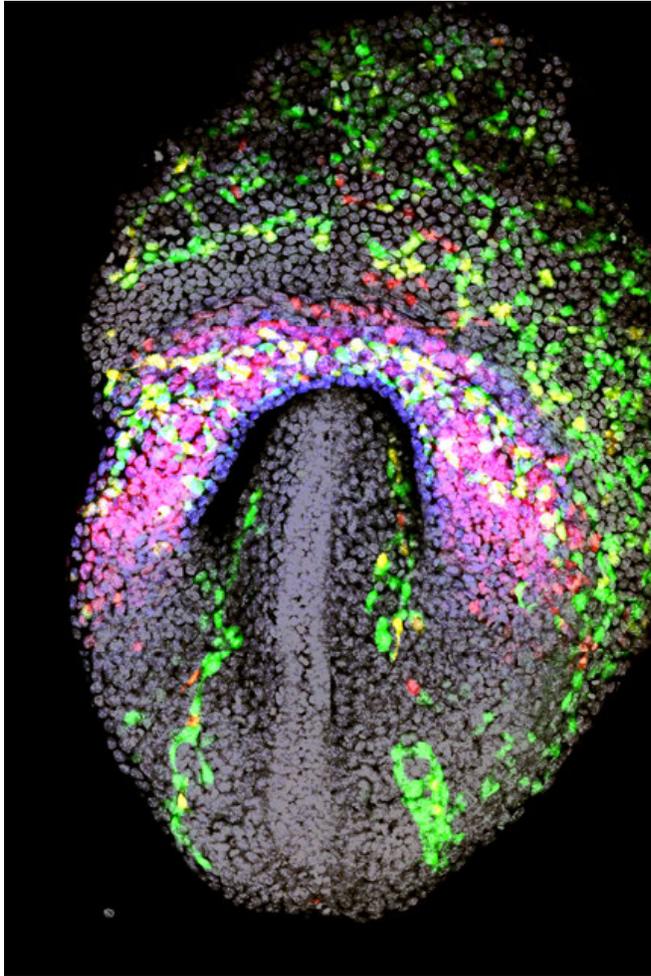
Damian Williams<sup>1,2,3</sup>, Dario Sirabella<sup>1,2,4</sup>, Dosh Whye<sup>1,2</sup>, Kevin Sampson<sup>1,2,5</sup>, Ray Funahashi<sup>1,2</sup>, Robert Kass<sup>1,2,5,6</sup>, Amy MacDermott<sup>1,2,6,7</sup>, Christopher E. Henderson<sup>1,3,6,8</sup>, Hans-Willem Snoeck<sup>1,2,9</sup>, Gordana Vunjak-Novakovic<sup>1,2,4</sup> and Barbara Corneo<sup>1,2</sup>

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A generous grant by the Helmsley Foundation has allowed the establishment of the Helmsley Stem Cell Core Facility at Columbia University, a multi-service production and characterization facility at Columbia University Medical Center. The facility has two primary aims: first, to give our users access to quality-controlled pluripotent stem cells and stem cell-derivatives prepared using standardized approaches and, second, to provide access to electrophysiological and Ca<sup>2+</sup> imaging methods for functional characterization of these cells. The Cell Production Section of the Core Facility offers to its users maintenance and supply of mouse and human ESC/iPSC; derivation and characterization of hiPSC; and differentiation of mouse and human pluripotent stem cells into functional, mature, highly purified progenies (including neuronal, cardiac, retinal pigment epithelium and endoderm-derivatives). The Electrophysiology and Ca<sup>2+</sup> Imaging Section of the Core Facility is available for assessing physiological properties of these cells using a wide range of electrophysiological and Ca<sup>2+</sup> imaging techniques. These include high-throughput automated patch systems and conventional whole-cell patch recording, and low-resolution Ca<sup>2+</sup> imaging. Some of the equipment, including the conventional electrophysiology rigs, qRT-PCR machine, plate readers and picking hood are also available for rental. The core also serves as a forum for workshops, training sessions and informal interactions between scientists from multiple laboratories working on different stem cell models. Sharing of reagents and protocols will make research involving pluripotent stem cells more standardized, rigorous and affordable.

The Helmsley Stem Cell Core Facility is located in room 1109, Black Building, 650 W 168<sup>th</sup> St, New York. A website that outlines all services on offer is currently in preparation [www.columbiastemcell.org/stemcellfacility](http://www.columbiastemcell.org/stemcellfacility). You can contact us at (212) 422-4406 or [bc2599@cumc.columbia.edu](mailto:bc2599@cumc.columbia.edu) with your research questions.

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**E8.5 mouse embryo.**

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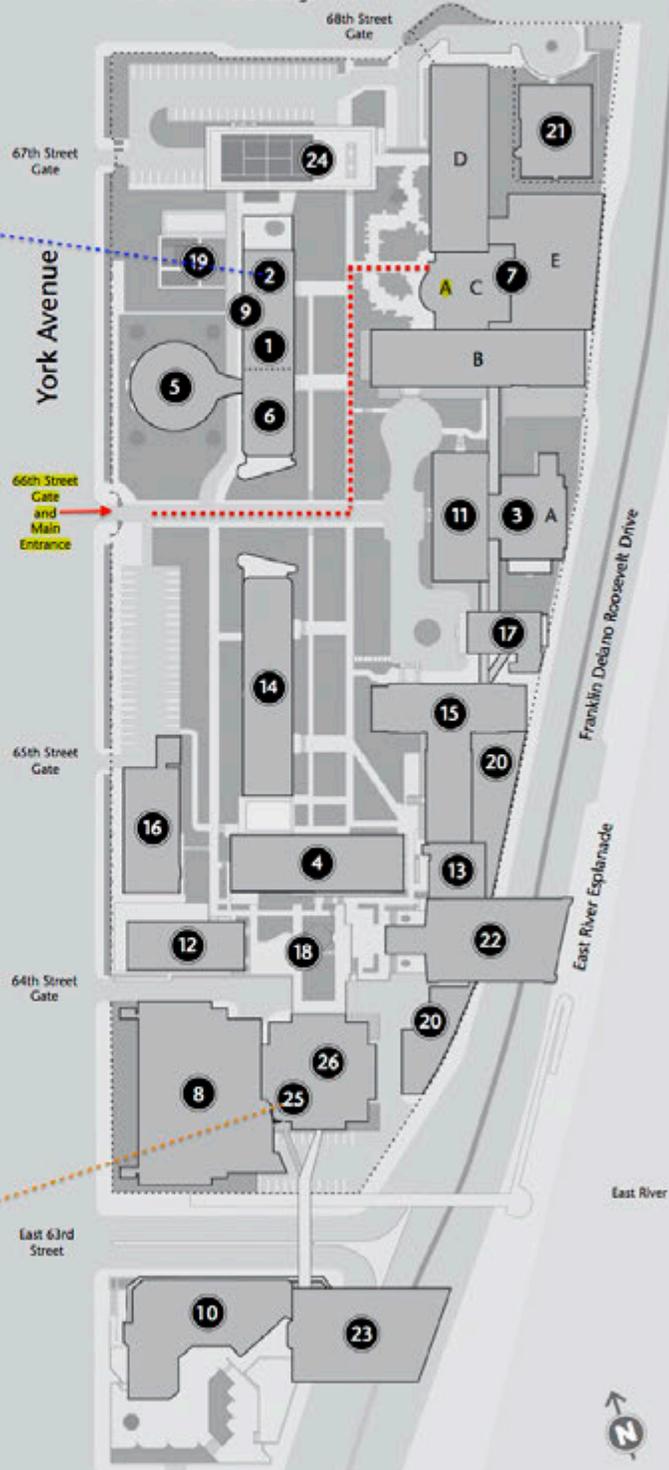






NewYork-Presbyterian Hospital and  
Weill Cornell Medical College

1. Abby Aldrich Rockefeller Hall
2. **Abby Dining Room**
3. Bass Center
  - A. Welch Hall (Markus Library)
4. Bronk Laboratory
5. Caspary Auditorium
6. Caspary Hall
7. Collaborative Research Center
  - A. **Carson Family Auditorium**
  - B. Flexner Hall
  - C. Greenberg Building
  - D. Smith Hall
  - E. Smith Hall Annex
8. Comparative Bioscience Center
9. Faculty and Students Club
10. Faculty House
11. Founder's Hall
12. Fricke Hall
13. Gasser Hall
14. Graduate Students Residence
15. Hospital (Heilbrunn Outpatient Research Center)
16. IT Pavilion
17. Nurses Residence
18. Peggy Rockefeller Plaza
19. Philosophers Garden
20. Power House
21. President's House
22. Rockefeller Research Building
23. Scholars Residence
24. Tennis Court
25. **Weiss Café**
26. Weiss Research Building



## NYSTEM 2014

Thursday lunch sponsored by LONZA for attendees of the lunchtime presentation

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Thursday reception cosponsored by Stemcell Technologies



### Additional Sponsors



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### On Your Own Thursday Lunch options:

- “A” level of CRC building, just upstairs from the Carson Family Auditorium: a ‘Starbucks-like’ Café for to go order, offering soups/salads of the day, sandwich station, desserts, beverages, coffee etc.
- A cafeteria in WEISS located at the south end of the campus (corresponding to 64<sup>th</sup> St.) with a choice of menu, salad bar, sandwich bar, grill and hot food.
- A formal restaurant with buffet, with reservation only (seated is limited): the ABBY DINING ROOM (the full menu there is \$16.95/person, desserts optional).

## **PROGRAM AT A GLANCE**

### **THURSDAY, MAY 29, 2014**

9:30 AM	REGISTRATION AND POSTER SESSION I SETUP
10:30 – 10:40	OPENING REMARKS
10:40 – 12:20	PLENARY I – GENOME EDITING & TECHNOLOGY
12:20 – 1:20	LUNCH BREAK
1:20 – 1:30	REMARKS AND INTRODUCTION OF KEYNOTE
1:30 – 2:30	KEYNOTE ADDRESS
2:30 – 2:45	TRIBUTE AND PRESENTATION TO LAWRENCE S. STURMAN
2:45 – 3:15	BREAK AND POSTERS
3:15 – 4:55	PLENARY II – STEM CELLS IN CANCER
5:00 – 6:30	POSTER SESSION I & RECEPTION

### **FRIDAY, MAY 30, 2014**

8:30 AM	REGISTRATION, BREAKFAST AND POSTER SETUP
9:00 – 11:00	SPECIAL SESSION – OVERCOMING BARRIERS TO TRANSLATING BENCH RESEARCH TO THE CLINIC
11:00 – 12:00	POSTER SESSION II
12:00 – 1:00	LUNCH
1:00 – 1:15	POSTER WINNERS ANNOUNCED
1:15 – 3:15	PLENARY III – DISEASE MODELING
3:15	ADJOURN