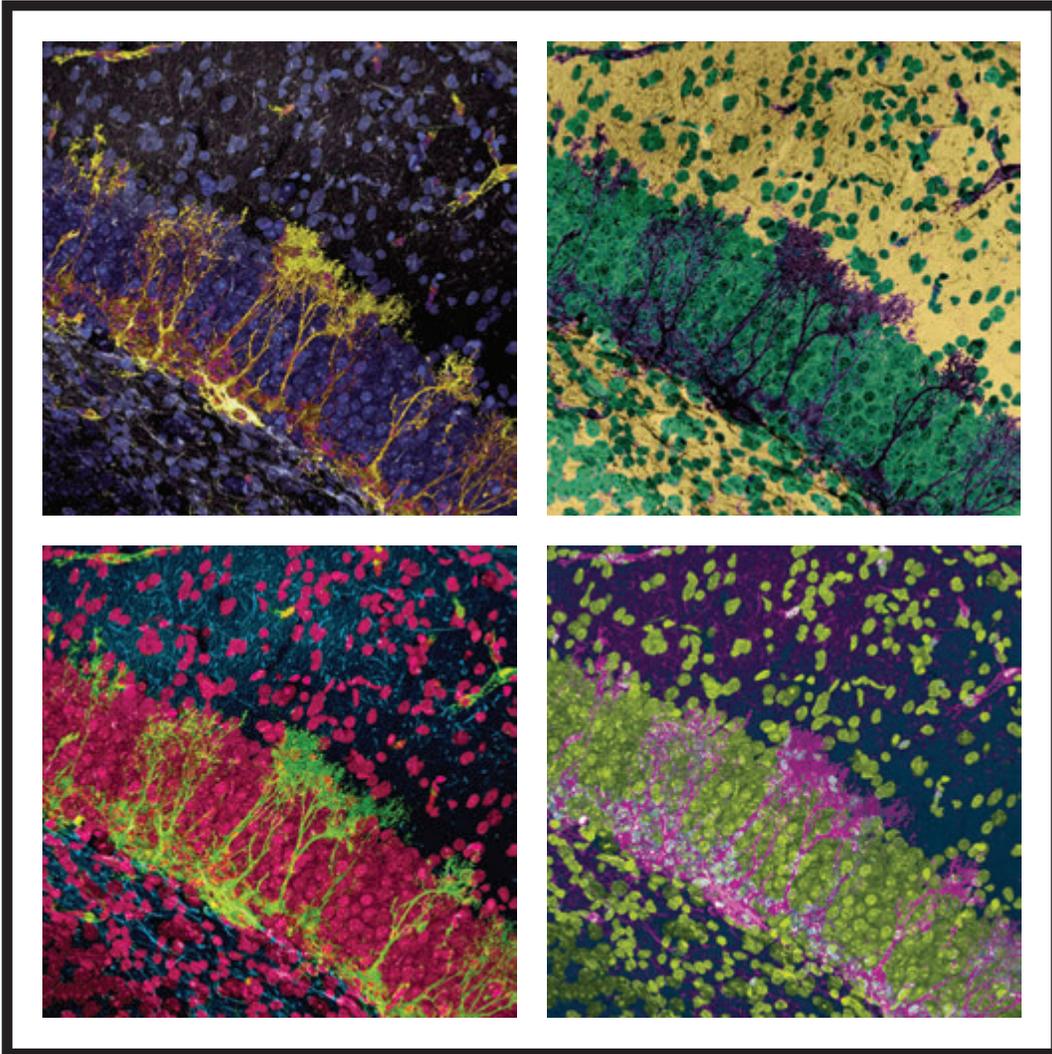


NYSTEM 2012



WINDOWS OF OPPORTUNITY

May 23 & 24, 2012

Windows of Opportunity
CUNY Graduate Center

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GENERAL INFORMATION

Sessions

Except where otherwise indicated, sessions will convene in the Harold M. Proshansky Auditorium.

Meals

Thursday breakfast and lunch will be served in the Concourse Lobby. Meal service will require your registration badge.

Posters

The poster sessions are in the breakout rooms across the Concourse Lobby from the auditorium. Posters should be put up by 1:00 pm on May 23rd, prior to the start of the Opening Remarks and Keynote Address, and should remain up through the second day. Please refer to the Poster Abstracts section of the program book for poster assignments. If your poster is assigned an odd number, please be prepared to present it at Poster Session I, 4:20 pm - 6:00 pm on May 23. If your poster is assigned an even number, please be prepared to present it at Poster Session II, 11:10 am - 1:10 pm on May 24. Posters must be removed by 5:00 PM Wednesday.

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

PROGRAM SCHEDULE

WEDNESDAY, MAY 23, 2012

- 12:00 PM Registration and Poster Set-up
- 1:00 – 1:10 **OPENING REMARKS**
- 1:10 – 2:00 **KEYNOTE:** Update on NIH CRM Efforts
Mahendra Rao, *NIH Center for Regenerative Medicine*
- 2:00 – 2:30 **BREAK AND POSTERS**

PLENARY I: Stem Cell Biology

- 2:30 – 4:20 Chair: **Asa Abeliovich**, *Columbia University*
- 2:30 – 3:00 **John Schimenti**, *Cornell University*
Sensitivity of Germline Stem Cells to DNA Replication Errors
- 3:00 – 3:20 **Lisa Dailey**, *NYU School of Medicine*
Tracking Transcriptional Regulatory Modules in Pluripotent Cells
- 3:20 – 3:40 **Philipp Voigt**, *NYU School of Medicine*
Asymmetrically Modified Nucleosomes May Function To Establish Bivalent Domains in Embryonic Stem Cells
- 3:40 – 4:00 **Jianlong Wang**, *Mount Sinai School of Medicine*
Reprogramming Logic from the Nanog/Oct4 Interactome
- 4:00 – 4:20 **Igor Matushansky**, *Columbia University Medical Center*
Terminal Differentiation and Loss of Tumorigenicity of Human Cancers via Pluripotency Based Reprogramming
- 4:20 – 6:00 **POSTER SESSION I and RECEPTION**

THURSDAY, MAY 24, 2012

- 8:00 AM Registration, Breakfast and Poster Viewing

PLENARY II: Neural Stem Cells and the Stem Cell Niche

- 9:00 – 11:10 Chair: **Steven Goldman**, *University of Rochester Medical Center*
- 9:00 – 9:20 **Ting Chen**, *The Rockefeller University*
An RNAi Screen Unveils a New Player in Stem Cell Self-Renewal and Long-Term Regeneration
- 9:20 – 9:40 **Carlos Clavel**, *Mount Sinai School of Medicine*
Programming the Hair Follicle Stem Cell Niche in Uncommitted Skin Fibroblasts

- 9:40 – 10:00 **Michal Stachowiak**, *University at Buffalo*
A Novel Nuclear FGF Receptor-1 Partnership with Retinoid and Nur Receptors in Neuronal Programming of Embryonic Stem Cells
- 10:00 – 10:20 **Lindsay Tannenholz**, *The New York State Psychiatric Institute*
Nr2b-Dependent Plasticity is Critical for the Impact of Adult Born Granule Cells on Pattern Separation
- 10:20 – 10:40 **Deanna Thompson**, *Rensselaer Polytechnic Institute*
Neural Progenitor Cell Response to Hemodynamically-Stimulated Endothelial Derived Extracellular Matrix and Soluble Factors
- 10:40 – 11:10 **Fiona Doetsch**, *Columbia University*
Stem Cells and Their Niche in the Adult Mammalian Brain
- 11:10 – 12:00 **POSTER SESSION II**
- 12:00 – 1:10 **LUNCH and POSTER SESSION II**

PLENARY III: Cardiovascular and Hematopoietic Systems

- 1:10 – 2:50 Chair: **Ira Cohen**, *Stony Brook University*
- 1:10 – 1:30 **Saghi Ghaffari**, *Mount Sinai School of Medicine*
Regulation of hESC Pluripotency and Lineage Specification by FOXO Transcription Factors
- 1:30 – 1:50 **Shahin Rafii**, *Weill Cornell Medical College*
Development of a Vascular Niche Platform for the Clinical-Scale Expansion of Repopulating Human Cord Blood Stem and Progenitor Cells
- 1:50 – 2:10 **Pratima Chaurasia**, *Mount Sinai School of Medicine*
Ex Vivo Expansion of Hematopoietic Stem Cells by Chromatin Modifying Agents
- 2:10 – 2:30 **Ann Foley**, *Weill Cornell Medical College*
Sinoatrial Node-Specific Differentiation of Cardiomyocytes by TGF β -Activated Kinase1 (TAK1/MAP3K7)
- 2:30 – 2:50 **Donald Freytes**, *Columbia University*
Effects of Macrophage Polarization on Mesenchymal Stem Cells in the Context of Myocardial Regeneration
- 2:50 – 3:20 **BREAK and POSTERS**

PLENARY IV: Disease Modeling and Therapeutic Approaches

- 3:20 – 4:50 Chair: **Alexander Nikitin**, *Cornell University*
- 3:20 – 3:40 **Lan Wang**, *Sloan-Kettering Institute*
Effects of AML1-ETO on leukemia stem cell maintenance are mediated by its target gene *Id1*

- 3:40 – 4:00 **Mark Noble**, *University of Rochester Medical Center*
Targeting Cancer Stem Cells in Glioblastoma and Breast Cancer
- 4:00 – 4:20 **Dieter Egli**, *The New York Stem Cell Foundation Laboratory*
Human Oocytes Reprogram Somatic Cells to a Pluripotent State
- 4:20 – 4:50 **CAPSTONE:** Applications of Human Pluripotent Stem Cells in the Nervous
System – From Pain to Therapeutic Gain
Lorenz Studer, *Sloan-Kettering Institute*
- 4:50 – 5:00 **CLOSING REMARKS**
- 5:00 **ADJOURN**

Keynote Address by Mahendra Rao, M.D., Ph.D.

NIH CRM – An Update

The NIH CRM is a common fund initiative that was established a year ago to accelerate efforts in translating the rapid advances being made in this field to the clinic. We have used the resources available to the NIH to help set up tissue sourcing, establish repositories and develop engineering methodologies and develop control and reference lines. We have begun working with StemBook to develop protocols and an ebook of stem cell chapters written by experts in the field. I will present an update to our efforts to obtain freedom to operate for iPSC technology and our efforts with licensing reagents. I will use examples of efforts from our own laboratory to describe how we expect these efforts to help accelerate the development of screening tools and clinical grade cell lines.



Mahendra S. Rao, M.D., Ph.D., is internationally renowned for his research involving human embryonic stem cells (hESCs) and other somatic stem cells. He has worked in the stem cell field for more than 20 years, with stints in academia, government and regulatory affairs and industry. He received his M.D. from Bombay University in India and his Ph.D. in developmental neurobiology from the California Institute of Technology, Pasadena. Following postdoctoral training at Case Western Reserve University, Cleveland, he established his research laboratory in neural development at the University of Utah, Salt Lake City. He next joined the National Institute on Aging as chief of the Neurosciences Section, where he studied neural progenitor cells and continued to explore his longstanding interest in their clinical potential. He then spent six years as the vice president of Regenerative Medicine at Life Technologies, Carlsbad, Calif. Most recently, he returned in August 2011 to the National Institutes of Health (NIH), as Director of the new NIH Center for Regenerative Medicine (NIH CRM). He co-founded Q Therapeutics, a neural stem cell company based in Salt Lake City. He also served internationally on advisory boards for companies involved in stem cell processing and therapy, on committees including the U.S. Food and Drug Administration's Cellular Tissue and Gene Therapies Advisory Committee chair, and as the California Institute of Regenerative Medicine and International Society for Stem Cell Research liaison to the International Society for Cellular Therapy.

SENSITIVITY OF GERMLINE STEM CELLS TO DNA REPLICATION ERRORS

John Schimenti

Cornell University, Ithaca, NY

High fidelity transmission of genetic material through the generations, with minimal deleterious mutations, is of paramount importance to species. The germline - from primordial germ cells (PGCs) through the eggs and sperm - are the carriers of the genetic material. More so than somatic cells, it is important that germline stem cells have effective mechanisms for minimizing mutations that would be deleterious to offspring. Since most mutations occur from errors during DNA replication, this process is critical to the health and reproductive success of organisms. In the germ lineage, DNA replication is of particular importance is during the rapid proliferation of Primordial Germ Cells (PGCs) as they colonize the primitive gonads during embryonic development, destined to ultimately form cells that can become oocytes or sperm. To understand how the germline stem cells protect the genome against transmissible mutations, we have isolated and examined mutations in DNA replication genes affecting the germline. The phenotypes of mutant mice bearing mutations in two genes, *Mcm9* and *Fancm*, reveal that DNA replication-linked quality control mechanisms are especially important for germline stem cells, and that defects to DNA repair mechanisms trigger cell death more readily than somatic cells, presumably as an evolutionary protection against transmission of offspring bearing birth defects.

TRACKING TRANSCRIPTIONAL REGULATORY MODULES IN PLURIPOTENT CELLS

Matthew Murtha¹, Francesco Strino², N. Sumru Bayin¹, Yatong Wang¹, Xiangmei Xi¹, Claudio Basilico¹, Yuval Kluger², Lisa Dailey¹

¹*Department of Microbiology, NYU School of Medicine, New York, NY;* ²*Department of Pathology and Department of Computational Biology and Bioinformatics, Yale University, New Haven, CT*

Pluripotent murine embryonic stem cells (ESCs), derived from the inner cell mass of pre-implantation embryos, are characterized by a unique chromatin landscape that is permissive for the expression of self-renewal genes, and suppressive or 'poised' for lineage-specific gene expression. Key ESC transcription factors (TFs) such as Oct4, Sox2, and Nanog, are thought to facilitate the establishment of this chromatin landscape, and ESC gene expression patterns, by targeting chromatin-modifying complexes to specific genomic loci. More recently, cultured pluripotent cells have also been established from post-implantation mouse embryos. These epiblast stem cells (EpiSCs) represent a distinct, more mature pluripotent state just proximal to gastrulation, and are more similar to human ESCs than are mouse ESCs. Interestingly, ESCs and EpiSCs utilize the same core TFs but display distinct gene expression patterns, suggesting the existence of additional ES- or EpiSC-specific transcriptional subcircuits acting in parallel to— or together with—the core TFs. To uncover these putative stage-specific components, we have undertaken two approaches. First, we have generated a genome-wide atlas of open chromatin in ESCs-, EpiSCs, MEFs, and NSCs, and have identified a number of Epi (or ES)- specific open chromatin regions that may correspond to stage-specific enhancers, and permit identification of novel TFs. Ongoing analyses are integrating these open chromatin maps with available gene expression and ESC ChIP data to track the character and fate of elements in ESCs that may be destined for activation or silencing upon differentiation. Secondly, we have developed a high-throughput functional method for the high resolution identification of transcriptional regulatory modules in ESCs. Changes in the activity of these modules upon *in vitro* differentiation of the ESCs towards the EpiSC state are being used to identify stage-specific transcriptional regulatory elements and their cognate TFs. These combined analyses will provide insights into distinguishing determinants of these two pluripotent states.

[Supported by NYSTEM contract C024322]

ASYMMETRICALLY MODIFIED NUCLEOSOMES MAY FUNCTION TO ESTABLISH BIVALENT DOMAINS IN EMBRYONIC STEM CELLS

Philipp Voigt¹, Gary LeRoy², William J. Drury III¹, Barry M. Zee², Jinsook Son¹, David B. Beck¹, Benjamin A. Garcia², Danny Reinberg¹.

¹Howard Hughes Medical Institute (HHMI), New York University School of Medicine, Biochemistry Department, New York, NY; ²Princeton University, Molecular Biology, Princeton, NJ

Histone posttranslational modifications (PTMs) are key elements in the regulation of gene expression and chromatin structure. Mononucleosomes, the basic building blocks of chromatin, contain two copies of each core histone H2A, H2B, H3, and H4. It is currently 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 trimethylation of lysine 4 on histone H3 (H3K4me3) and the repressive mark H3K27me3. Recent evidence indicates that these PTMs, however, cannot co-occur on the same histone tail, rendering both their nucleosomal conformation and the mechanism for their establishment elusive. Here we aim to assess whether, in general, nucleosomes are modified in a symmetric or asymmetric fashion for a given modification and whether the symmetry state of marks might impinge on the generation of bivalent domains. By employing an approach based on modification-specific antibodies and mass spectrometry, we show that chromatin consists of both symmetric and asymmetric populations of mononucleosomes *in vivo*. The H3K27me2/3 marks placed by PRC2 are mostly present on both copies of H3 in nucleosomes carrying the mark in ES cells. For H4K20me1, we likewise observed a combination of symmetric and asymmetric nucleosomes for ES cells, HeLa cells, and MEFs. To explore implications of nucleosomal asymmetry, we analyzed co-occurrence of histone marks and obtained direct physical evidence for bivalent nucleosomes carrying H3K4me3 or H3K36me3 along with H3K27me3, albeit on opposite H3 tails. PRC2-mediated methylation of H3K27 was inhibited when nucleosomes contain symmetrically, but not asymmetrically, placed H3K4me3 or H3K36me3. These findings uncover a potential mechanism for the incorporation of bivalent features into nucleosomes and show how asymmetry might set the stage to diversify functional nucleosome states.

[Supported by NYSTEM contract C026880]

REPROGRAMMING LOGIC FROM THE NANOG/OCT4 INTERACTOME

Junjun Ding¹, Miguel Fidalgo¹, Francesco Faiola¹, Huilei Xu², Arven Saunders¹, Avi Ma'ayan², Jianlong Wang¹

¹*Department of Developmental and Regenerative Biology, Black Family Stem Cell Institute, Mount Sinai School of Medicine, New York, NY;* ²*Department of Pharmacology and Systems Therapeutics, Mount Sinai School of Medicine, New York, NY*

Oct4 and Nanog are well-known transcription factors that play fundamental roles in stem cell self-renewal, pluripotency and somatic cell reprogramming. However, limited information is available on Oct4 and Nanog associated protein complexes and their intrinsic protein-protein interactions that dictate the critical regulatory activities of Oct4 and Nanog. We have employed an improved affinity purification approach combined with mass spectrometry to purify Oct4 and Nanog associated protein complexes in mouse embryonic stem cells (ESCs), and discovered many novel Oct4 and Nanog partners important for self-renewal and pluripotency of ESCs. Notably, we found that Oct4 and Nanog are associated with multiple chromatin modifying complexes with documented as well as newly proven functional significance in stem cell maintenance and somatic cell reprogramming.

We will present our new studies demonstrating the physical and functional link of novel genetic and epigenetic regulators to the pluripotency network. Specifically, we will provide the functional validation of critical genetic-epigenetic interconnections that could serve as either a roadblock or a facilitator for efficient somatic cell reprogramming. Our study establishes a solid biochemical basis and provides molecular insights into the genetic and epigenetic mechanisms governing stem cell pluripotency and somatic cell reprogramming.

[Supported by NYSTEM contract C026420]

TERMINAL DIFFERENTIATION AND LOSS OF TUMORIGENICITY OF HUMAN CANCERS VIA PLURIPOTENCY BASED REPROGRAMMING

Xi Zhang, Filemon Dela Cruz, Melissa Terry, Fabrizio Remotti, Igor Matushansky

Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, New York, NY

Pluripotent cells can be derived from various types of somatic cells by nuclear reprogramming using defined transcription factors. It is however unclear whether human cancer cells can be similarly reprogrammed and subsequently terminally differentiated with abrogation of tumorigenicity. Here, using sarcomas we show that human derived complex karyotype solid tumors: (1) can be reprogrammed into a pluripotent state as defined by all *in vitro* criteria used to define pluripotent stem cells generated from somatic cells; (2) can be terminally differentiated into mature connective tissue and red blood cells; and (3) terminal differentiation is accompanied with loss of both proliferation and tumorigenicity. We go on to perform the first global DNA promoter methylation and gene expression analyses comparing human cancers to their reprogrammed counterparts and report that reprogramming/differentiation results in significant epigenetic remodeling of oncogenes and tumor suppressors; while not significantly altering the differentiation status of the reprogrammed cancer cells, in essence de-differentiating them to a state slightly before the mesenchymal stem cell differentiation stage. Our data demonstrates that direct nuclear reprogramming can restore terminal differentiation potential to human derived cancer cells, with simultaneous loss of tumorigenicity, without the need to revert to an embryonic state. We anticipate that our models would serve as a starting point to more fully assess how nuclear reprogramming overcomes the multitude of genetic and epigenetic aberrancies inherent in human cancers to restore normal terminal differentiation pathways. Finally, these findings suggest that nuclear reprogramming may be a broadly applicable therapeutic strategy for the treatment of cancer.

AN RNAi SCREEN UNVEILS A NEW PLAYER IN STEM CELL SELF-RENEWAL AND LONG-TERM REGENERATION

Ting Chen, Evan Heller, Slobodan Beronja, Naoki Oshimori, Nicole Stokes, Elaine Fuchs*

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

Adult stem cells (SCs) sustain tissue maintenance and regeneration throughout the lifetime of an animal. They often reside in specific signaling niches that orchestrate the stem cell's balancing act of transitions from quiescence to cell cycle re-entry in order to fulfil a demand for tissue regeneration. How SCs maintain this remarkable capacity to self-renew for long-term tissue regeneration is still poorly understood. Here, we use RNA interference (RNAi)-based loss-of-function screening as a powerful approach to uncover transcriptional regulators governing SC self-renewal and long-term regenerative potential. Hair follicle SCs provide an ideal paradigm, since they've been purified and characterized from their native niche *in vivo*, and in contrast to their rapidly dividing progeny, can be maintained and passaged long-term *in vitro*. Focusing on nuclear proteins/transcription factors enriched in SCs versus progenies, we screened ~2,000 shRNAs for their impact on long-term but not short-term self-renewal *in vitro*. To address the physiological relevance of our findings, we selected one candidate, *Tbx1*, surfacing in the screen. Expressed in many tissues, transcription factor *Tbx1* has not been studied in the context of SC biology. By conditionally ablating *Tbx1 in vivo*, we show that tissue regeneration during homeostasis occurs normally but is dramatically delayed. Devising an *in vivo* assay for SC self-renewal, we then show that when challenged with repetitive bouts of regeneration, the *Tbx1*-deficient SC niche becomes progressively depleted. Addressing mechanism, we discover that *Tbx1* acts as an intrinsic rheostat to control BMP signalling at the heart of the transition between SC quiescence to self-renewal in hair follicles. Our results validate the RNAi screen and underscore its power in unearthing new players governing SC self-renewal and tissue-regenerative potential.

[Supported by NYSTEM Scholar Award C026722 (T.C.)]

PROGRAMMING THE HAIR FOLLICLE STEM CELL NICHE IN UNCOMMITTED SKIN FIBROBLASTS

Carlos Clavel^{1,2}, Brittany Russo^{1,2}, Amelie Rezza^{1,2}, & Michael Rendl¹⁻³

¹*Black Family Stem Cell Institute*, ²*Department of Developmental and Regenerative Biology*,
³*Department of Dermatology, Mount Sinai School of Medicine, NY*

Stem cells are essential for proper formation of all organs during embryogenesis and for maintenance and renewal in the adult. In many tissues this is orchestrated by signals from supporting cells within the stem cell microenvironment, also called niche. How supporting niche cells acquire their specialized niche status during development is largely unknown. Here, we strive to determine how dermal papilla (DP) niche cells are programmed to activate hair follicle stem cells during formation and regeneration. We have previously identified highly enriched transcription factors (TFs) in a DP gene signature, suggesting a specialized function in regulating the cell fate of DP cells. We find that many DP signature features are lost in culture during the *in vivo/in vitro* transition, including the vast majority of DP signature TFs. By modulating 3D-clustering, we can partially rescue the DP molecular identity, including the majority of signature TFs. We now isolate fibroblasts from double-transgenic fluorescent DP fate reporter mice to overexpress and reprogram DP niche fate with selected TFs. With gene expression analysis we have uncovered significant upregulation of DP signature genes in fibroblasts overexpressing single or combinations of TFs. In addition, upregulation of DP signature genes is enhanced by inhibitors of histone modifiers. Our preliminary results suggest that by uncovering the right combination of DP TFs we will achieve programming DP niche fate and increasing our understanding of how the hair follicle stem cell niche is regulated for stem cell activation and maintenance.

[Supported by NYSTEM contract C026411]

A NOVEL NUCLEAR FGF RECEPTOR-1 PARTNERSHIP WITH RETINOIC AND NUR RECEPTORS IN NEURONAL PROGRAMMING OF EMBRYONIC STEM CELLS

Yu-Wei Lee, Christopher Terranova, Barbara Birkaya, Sridhar Narla, Abhirath Parikh, John M. Aletta, Emmanuel S. Tzanakakis, Ewa K. Stachowiak, Michal K. Stachowiak
Western New York Stem Cells Culture and Analysis Center, SUNY, Buffalo, NY

FGF receptor 1 (FGFR1), a membrane-targeted protein, is also involved in independent direct nuclear signalling. We show that nuclear accumulation of FGFR1 is a common response to retinoic acid (RA) in pluripotent embryonic stem cells (ESC) and neural progenitors and is both necessary and sufficient for neuronal-like differentiation and accompanying neuritic outgrowth. Dominant negative nuclear FGFR1, which lacks the tyrosine kinase domain, prevents RA-induced differentiation while full-length nuclear FGFR1 elicits differentiation in the absence of RA. Immunoprecipitation and GST assays demonstrate that FGFR1 interacts with RXR, RAR and their Nur77 and Nurr1 partners. Conditions that promote these interactions decrease the mobility of nuclear FGFR1 and RXR in live cells. RXR and FGFR1 co-associate with 5'-Fluorouridine-labeled transcription sites and with RA Responsive Elements (RARE). RA activation of neuronal (*tyrosine hydroxylase*) and neurogenic (*fgf-2* and *fgfr1*) genes is accompanied by increased FGFR1, Nur and histone H3.3 binding to their regulatory sequences. Reporter-gene assays show synergistic activations of RARE, NBRE and NurRE by FGFR1, RAR/RXR and Nurs. As shown for mESC differentiation, FGFR1 mediates gene activation by RA and augments transcription in the absence of RA. Cooperation of FGFR1 with RXR/RAR and Nurs at targeted genomic sequences offers a new mechanism for developmental gene regulation during neuronal differentiation.

[Supported by NYSTEM contracts C026415 and C026714 (to MKS) and C024355 (to EST)]

NR2B-DEPENDENT PLASTICITY IS CRITICAL FOR THE IMPACT OF ADULT BORN GRANULE CELLS ON PATTERN SEPARATION

Mazen A. Kheirbek^{1,2}, Lindsay Tannenholz^{2,3}, René Hen¹⁻³

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Adult generated granule cells (GCs) in the dentate gyrus (DG) exhibit a period of heightened plasticity four to six weeks post-mitosis. The functional contribution of this critical window to hippocampal neurogenesis or behavior remains unknown. Here, by targeting NR2B-containing NMDA receptors on adult born GCs, we show that this window of heightened plasticity is not essential for the survival or maturation of adult-born GCs, yet is critical to their impact on pattern separation.

[Supported by NYSTEM contracts C024330 and C026430]

NEURAL PROGENITOR CELL RESPONSE TO HEMODYNAMICALLY-STIMULATED ENDOTHELIAL DERIVED EXTRACELLULAR MATRIX AND SOLUBLE FACTORS

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This study demonstrates that adult neural progenitor cells exhibit a differential response to endothelial cell-derived extracellular matrix produced under hemodynamic flow compared to statically-produced extracellular matrix. *In vivo*, neural stem cells reside in close proximity to blood vessels, suggesting that endothelial-produced factors may influence neural stem cell fate. *In vitro*, Shen *et al.* (2004) demonstrated that soluble factors released by endothelial cells stimulate self-renewal and inhibit differentiation of embryonic neural stem cells. It is well known that the endothelial phenotype is dependent on the local hemodynamic environment and can alter both extracellular matrix and soluble factors. However, neural stem cell response to endothelial derived products produced under hemodynamic stimulation has not been explored. In this work, we examined neural progenitor response to endothelial-produced extracellular matrix. Briefly, confluent mouse brain microvascular endothelial cells (mbEnd.3) were either (1) exposed to shear stress (10 dynes/cm²- dynamic) or (2) traditionally cultured (static). Both endothelial cell-produced extracellular matrix and soluble factors can be isolated for analysis or for use in culture with neural stem cells. Dynamic culture results in significant changes to the matrix composition: a 2.7 fold increase in laminin, a 1.9 fold increase in collagen IV and a 2.2 fold increase in fibronectin, as well as differences in microstructure. Adult primary murine neural stem cells were isolated from the sub-ventricular zone, expanded to generate neurospheres, dissociated and seeded onto the ECM substrates. Neural progenitor cells attached, spread, and infiltrated the dynamically-produced ECM, while the statically-produced ECM generated only small neurospheres at the ECM surface. Significant changes in cell morphology are observed between on the cell-produced biomaterials and while both materials support significant increases in cell number, no changes in cell differentiation were observed during the 7 days. Current work is focused on examining changes in the endothelial-produced soluble factors generated dynamically and the neural progenitor cell response. This work will generate a better understanding of the neural stem cell response to endothelial-derived cues.

STEM CELLS AND THEIR NICHE IN THE ADULT MAMMALIAN BRAIN

Fiona Doetsch

*Columbia University, Departments of Pathology and Cell Biology, Neurology, and Neuroscience
Columbia Stem Cell Initiative, New York, NY*

Stem cells reside in specialized niches that support their self-renewal and differentiation. A balance between intrinsic and extrinsic signals mediates stem cell quiescence, activation and proliferation. In the adult mammalian brain, neural stem cells in the subventricular zone (SVZ) continuously generate neurons destined for the olfactory bulb. The stem cells exhibit molecular and ultrastructural features of astrocytes, glial cells largely considered support cells in the brain. A major limitation in the neural stem cell field has been the ability to prospectively purify stem cell astrocytes from other astrocytes in the brain. We have developed a strategy to simultaneously purify each SVZ cell type in the lineage by means of fluorescence activated cell sorting, including for the first time quiescent stem cells and activated stem cells. Quiescent and activated stem cells have strikingly different functional properties in vivo and in vitro. Transcriptional profiling further highlights their unique physiological states. We have also begun to elucidate the role of two different compartments in the SVZ stem cell niche, the vasculature and the cerebrospinal fluid, and their differential effect on each stage of the stem cell lineage. Together, our findings are providing novel insights into the integration of intrinsic and extrinsic signals in the regulation of adult neural stem cell quiescence and activation.

[Supported by NYSTEM contract C026401 and C024287, NIH NINDS]

REGULATION OF hESC PLURIPOTENCY AND LINEAGE SPECIFICATION BY FOXO TRANSCRIPTION FACTORS

Xin Zhang, Valentina D'Escamard, Jose Luis Garrido, Saghi Ghaffari

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FoxO transcription factors are key regulators of pluripotency in embryonic and adult stem cells. FoxO1 is essential for the control of pluripotency in hESC through direct transcriptional regulation of OCT4 and SOX2 master regulators of pluripotency (Zhang *et al.*, Nature Cell Biology, 2011). Similarly FoxO3 is critical for the maintenance of adult blood and neural stem cells as well as leukemic stem cells. In addition, we found FoxO3 to be key in the regulation of mouse embryonic stem cell pluripotency (Zhang *et al.*, Nature Cell Biology, 2011). Expression of FoxO3 first decreases and then increases with differentiation and mesoderm specification in both hESC and mESC. In addition, FoxO3 is almost entirely cytoplasmic and presumably inactive in pluripotent hESC, and translocates to the nucleus during mesoderm induction, whereas FoxO1 moves to the cytosol in this process, further suggesting activation of FoxO3 and inhibition of FoxO1 during the mesoderm specification. Importantly, FoxO3's expression is upregulated during both hematopoietic and cardiac differentiation from hESC in agreement with critical functions FoxO3 exerts in both these tissues. Stable knock-down of FoxO3 in hESC lines using two distinct shRNA resulted in over 75% reduction in the expression of FoxO3 protein within 48 hours associated with strong induction of hematopoietic markers KDR (FLK1, VEGFR2), CD31 and CD34 and strong reduction in cardiac markers such as Troponin T, NKX2.5 and Myocardin. These results were obtained in two distinct hESC expressing shRNAs targeting FoxO3 and highly comparable in two distinct hESC (H1 and HES2) and only seen in shRNA targeting cell lines and not in control scrambled control shRNA-expressing cells altogether strongly suggesting that (a) FoxO3 is a critical regulator of ESC mesoderm specification and differentiation specifically into hematopoietic and cardiac lineages and (b) a coordinated FoxO1/FoxO3 regulation is implicated in the maintenance of ESC pluripotency versus differentiation.

DEVELOPMENT OF A VASCULAR NICHE PLATFORM FOR THE CLINICAL-SCALE EXPANSION OF REPOPULATING HUMAN CORD BLOOD STEM AND PROGENITOR CELLSJason M. Butler, Eric J. Gars, Joseph Scandura, Shahin Rafii*Department of Genetic Medicine Weill Cornell Medical College, New York, NY*

Within the mouse bone marrow, endothelial cells serve as specialized niche cells that balance self-renewal and differentiation of the hematopoietic stem cell (HSC) into lineage-specific progenitor cells (HPCs). However, the inability to maintain human endothelial cells (hECs) in culture conditions that permit proliferation of the hematopoietic cells has hindered the studies to define the role vascular cells in supporting homeostasis of human hematopoietic stem and progenitor cells (HSPCs). We have devised an approach to cultivate primary hECs in xenobiotic-free conditions in the absence of serum and endothelial growth factors. We demonstrate that hECs in the presence of minimal concentrations of thrombopoietin, stem cell factor and Flt3-ligand can perform as an instructive vascular platform to efficiently expand cocultured human cord blood cells (hCBs) in serum-free culture conditions. While incubation of CD34⁺ hCB cells with cytokines alone resulted in rapid attrition of hematopoietic cells beyond 12 days of culture, coculturing of the same number of CD34⁺ hCBs with hECs and minimal cytokines resulted in a 400-fold expansion of total CD45⁺ nucleated cells, 150-fold expansion of CD45⁺CD34⁺ hematopoietic progenitor cells, and a 22.5-fold expansion of Lineage⁻CD34^{hi}⁺CD45^{RA}⁻CD49^f⁺ HSPCs beyond 12 days that have enhanced multi-lineage engraftment when transplanted into primary and secondary immunocompromised recipient mice. Limiting dilution, competitive repopulation transplantation assays demonstrate that CD34⁺ hCBs cells that have been co-cultured with hECs results in a 7.5-fold increase in the number of human cord blood cells capable of long-term repopulation as compared to unmanipulated CD34⁺ hCBs. Therefore, hECs establish an ideal niche cell for long-term expansion and differentiation of repopulating HSC and HSPCs setting stage for the therapeutic use of human HSPCs for treatment of hematological disorders.

EX VIVO EXPANSION OF HEMATOPOIETIC STEM CELLS BY CHROMATIN MODIFYING AGENTS

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Epigenetic modifications play an important role in lineage determination and expansion of pluripotent hematopoietic stem cells (PHSC) and hematopoietic progenitor cells (HPC). We hypothesize that histone deacetylase inhibitors (HDACIs) and histone methyl transferase inhibitors might serve as pharmacological agents to generate a greater numbers of PHSC/HPC. We developed serum free culture conditions supplemented with cytokines (SCF, FLT-3, TPO and IL3) and assessed effect of several commercially available chromatin modifying agents such as valproic acid (VPA), scriptaid (SCR), CAY10398 (C398), CAY10433 (C433), CAY10603 (C603) and UNC0224 (UN024) for their ability to promote *ex vivo* generation of HSC/HPC from cord blood (CB)-CD34+ cells. Addition of VPA promoted the greatest expansion of the absolute number of CD34+, CD34+CD90+ and CD34+CD90+CD184+ cells as compared to cytokines alone (CA). VPA also led to a 36-fold greater expansion of CD34+ and aldehyde dehydrogenase (ALDH)+ cells as compared to CA. VPA treatment resulted in an 81-fold increase in BFU-E and CFU-mix colonies as compared to CA. Gene expression profiling (Illumina, Inc.) of CD34+reisolated following a week of exposure to VPA, SCR and CA revealed 587 differentially expressed genes (DEG) with a ≥ 2 -fold expression difference. A distinctive gene signature distinguishes SCR and VPA from CA-treated CD34+ cells as well as primary cells. DEG analysis using GeneGo(MetaCore) algorithm revealed a distinct network of many significantly regulated genes, receptors, ligands, kinases, phosphatases and pathways known to be involved in stem cell maintenance, self-renewal and expansion following treatment with SCR and VPA. *In vivo* studies demonstrated that VPA treatment of CD34+ cells resulted in the expansion of long term-NOD/SCID-marrow repopulating cells (SRC) which were capable of multi-lineage hematopoietic differentiation and generating circulating myeloid and lymphoid cells in the blood of recipients 13-weeks after transplantation. Our results suggest that VPA is a most promising HDACI which can be utilized to promote *ex vivo* expansion of CB-PHSC/HPC for eventual use as allogeneic stem cell grafts.

[Supported by NYSTEM contract C026431]

SINOATRIAL NODE-SPECIFIC DIFFERENTIATION OF CARDIOMYOCYTES BY TGF β -ACTIVATED KINASE1 (TAK1/MAP3K7)

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In vivo, cardiomyocytes comprise a heterogeneous population of contractile cells each with unique markers, morphologies and physiologies, however the mechanisms that direct myocardial cells to specific subtypes are poorly understood. In particular, the differentiation of embryonic stem (ES) cells to specific myocardial lineages has been elusive. We have established several ES cell lines overexpressing TGF β -Activated Kinase (TAK1/Map3K7) and found that cardiac differentiation in these cells faithfully recapitulates the transcriptional program that directs sinoatrial node (SAN) differentiation in the embryo. Briefly, they upregulate the expression of *Tbx5*, *Shox2*, *Tbx3* and the expression of SAN markers but downregulate the expression of *Nkx2.5* and cardiac contractile proteins characteristic of atrial and ventricular cardiac cells. All cardiac cells in these cultures adopt markers such as CaV1.3 and HCN-2 and -4 and have cellular morphologies and electrophysiological phenotypes that are characteristic of the SAN. Our novel protocol has broad implications for the study of SAN differentiation and physiology. This finding will also play a key role in developing bioengineered grafts for regenerative medicine. In the near future we hope to translate these studies to human ES and patient-specific iPS cells. We would also like to carry out epigenetic profiling of these cells to identify SAN-specific enhancers and have identified potential collaborators for this project. We also plan to make conditional knockouts of this pathway in ES cells and mouse embryos to determine if this represents an endogenous pathway for SAN differentiation.

EFFECTS OF MACROPHAGE POLARIZATION ON MESENCHYMAL STEM CELLS IN THE CONTEXT OF MYOCARDIAL REGENERATION

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²*Albert Einstein University, Department of Microbiology and Immunology, Bronx, NY*

Introduction: The outcome of cardiac cell therapy will depend on the inflammatory environment at the site of injury. Following myocardial infarction, tissue repair is mediated by the presence of polarized macrophages, with pro-inflammatory (M1) and anti-inflammatory (M2) properties that are recruited during the early and late stages of cardiac ischemia, respectively. Repair cells delivered into the injured myocardium are inevitably subjected to this inflammatory milieu and may have profound impacts on their survival and function. The present study investigated how M1 or M2 environments affect mesenchymal stem cells (MSCs), which are the most frequently used cells in human clinical trials of heart repair.

Methods: Human MSCs were cultured with M1 and M2 macrophages (both in direct and indirect culture) and the cytokines they secrete. For co-cultures, monocytes were differentiated and polarized into M1 and M2 macrophages by the addition of LPS/IFN- γ and IL-4/IL-13 respectively. The effect of macrophage polarization on the growth and function of MSCs was determined by measuring cell growth and differentiation potential.

Results/Discussion: The M1 and M2 cytokines decreased and increased respectively the numbers of MSCs when compared to controls, and changed the differentiation potential of MSCs. There was a consistent increase in the number of MSCs during indirect and direct co-cultures with M2 macrophages.

Conclusions: Taken together, our data suggest that M2 macrophages augment survival and growth of mesenchymal stem cells, in contrast to M1 macrophages, which inhibit the growth of MSCs in certain conditions. There seems to be a dynamic and reciprocal effect between macrophages and MSCs that may ultimately dictate the type of reparative response.

Future Directions: Current work utilizes this system to study the effects of polarized macrophages on human embryonic stem cell derived cardiomyocytes.

[Supported in part by NYSTEM contract C026721]

EFFECTS OF AML1-ETO ON LEUKEMIC STEM CELL MAINTENANCE ARE MEDIATED BY ITS TARGET GENE ID1

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Leukemic stem cells (LSCs) drive the development of acute myelogenous leukemia (AML), and share two main characteristics with normal hematopoietic stem cells (HSCs), the ability to self-renew and the ability to proliferate. Unlike normal HSCs they lack the ability to fully differentiate, although their limited differentiation may be responsible for some of the cellular heterogeneity found in leukemia. AML is commonly associated with aberrant transcriptional regulation, and with high levels of Id1, a dominant inhibitor of basic-helix-loop-helix transcription factor, which confers a worse prognosis. We have recently shown that Id1 is required for adult, normal HSC self-renewal using a knockout mouse model, and that Id1 is upregulated by AML1-ETO in human CD34+ hematopoietic stem/progenitor cells isolated from cord blood. We found that AML1-ETO and the transcriptional co-activator p300 co-localize at regions within the Id1 promoter regulatory regions, which contains the AML1 consensus binding sites. We have also shown that lysine acetylated AML1-ETO may activate Id1 gene expression via the recruitment of TBP associated factors (TAFs). Others have shown that Id1 expression is upregulated by oncogenic tyrosine kinases. In this study, we address the relevance of Id1 in the initiation and maintenance of LSCs by the AML1-ETO9a and MLL-AF9 fusion proteins, to determine whether Id1 could be a suitable target for attacking LSCs. To understand the mechanism of the effects of Id1 on LSC biology we performed microarray analysis using LSCs that contain or lack Id1. Our results suggest that blocking Id1 can inhibit the formation or propagation of LSCs; the information generated by this study will be useful for developing targeted therapies for human acute leukemia.

TARGETING CANCER STEM CELLS IN GLIOBLASTOMA AND BREAST CANCER

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Our research is focused on the hypothesis that, just as normal stem and progenitor cells share several fundamental regulatory pathways, cancer stem cells from different tissues also share critical properties but that these differ from normal cells in ways that allow selective elimination of cells essential for tumorigenesis. Identifying such properties would enable selective targeting of tumor cells in a way that could greatly enhance cancer treatment.

Our studies on glioblastomas (GBMs), the most malignant glial tumors, and normal glial progenitor cells identify Cool-1-mediated suppression of the c-Cbl ubiquitin ligase as one of the most effective control points discovered thus far for selectively inhibiting GBM cancer stem cell function. Cool-1-mediated inhibition of c-Cbl is critical in regulating GBM cell migration, chemosensitivity, promotion of epidermal growth factor receptor degradation by pro-oxidant stimuli, cancer stem cell antigen expression, and tumor spheroid generation *in vitro*. *In vivo*, Cool-1-mediated inhibition of c-Cbl is so critical that Cool-1 knockdown cells cannot form tumors unless they re-express Cool-1. In contrast, Cool-1/c-Cbl complexes are not found in normal brain cells and Cool-1 knockdown does not reduce normal progenitor division, indicating this interaction selectively targets GBM cells. Thus, inhibition of c-Cbl function integrates multiple critical properties of GBMs, is essential for cancer stem cell function and offers a selective target for these otherwise refractory tumors.

To investigate whether c-Cbl sequestration represents a general principle in cancer stem cell biology, we also examined c-Cbl function in basal-like (triple-negative) breast cancer cells. Here too, inhibition of c-Cbl function is critical for chemoresistance and tumor initiation.

We next identified pharmacological agents that are suitable for clinical development. One of these agents is even able to render basal-like breast cancer cells sensitive to tamoxifen, a finding that would greatly enhance treatment of these otherwise untreatable tumors.

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HUMAN OOCYTES REPROGRAM SOMATIC CELLS TO A PLURIPOTENT STATE

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The exchange of the oocyte's genome with the genome of a somatic cell, followed by the derivation of pluripotent stem cells, could enable the generation of specific cells affected in degenerative human diseases. Although it is now possible to induce pluripotent stem cell (iPS) formation by forced expression of transcription factors in somatic cells, differences between iPS- and blastocyst-derived stem cells have been reported for gene expression, DNA methylation, and differentiation potential. In addition, reprogramming to iPS cells may compromise genomic integrity, introducing de novo mutations and copy number variations. These differences could affect the use of iPS cells in research as well as for therapeutic purposes. In contrast, in animal models, reprogramming using oocytes consistently yields fully reprogrammed stem cells. Our aim was to determine whether human oocytes have the ability to reprogram somatic cells to a pluripotent state, and to compare the resulting cells to iPS cells of the same genotype. In initial experiments, we found that the development of human oocytes after genome exchange arrests at late cleavage stages in association with transcriptional abnormalities. In contrast, if the oocyte genome is not removed and the somatic cell genome is merely added, the resultant triploid cells develop to the blastocyst stage. Stem cell lines derived from these blastocysts differentiate into cell types of all three germ layers, and a pluripotent gene expression program is established on the genome derived from the somatic cell. This result demonstrates the feasibility of reprogramming human cells using oocytes and identifies removal of the oocyte genome as the primary cause of developmental failure after genome exchange.

[Supported by NYSTEM contract C026184]

**APPLICATIONS OF HUMAN PLURIPOTENT STEM CELLS IN THE NERVOUS SYSTEM –
FROM PAIN TO THERAPEUTIC GAIN**

Lorenz Studer

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SHARED FACILITIES

(SF1) CORNELL MAMMALIAN CELL REPROGRAMMING CORE

(SF2) EXPANSION OF PLURIPOTENT STEM CELL RESEARCH AT MSSM

(SF3) THE ROCKEFELLER UNIVERSITY HUMAN PLURIPOTENT STEM CELL CORE FACILITY

(SF4) STONY BROOK STEM CELL FACILITY CENTER

(SF5) UPSTATE STEM CELL cGMP FACILITY AT THE UNIVERSITY OF ROCHESTER

(SF6) NEURACELL BANK – A SOURCE OF MOUSE AND HUMAN CENTRAL NERVOUS SYSTEM STEM CELLS AND ASSOCIATED REAGENTS

(SF7) INTRODUCTION TO THE WESTERN NEW YORK STEM CELL CULTURE AND ANALYSIS CENTER (WNYSTEM)

(SF8) NYSTEM HIGH-THROUGHPUT SCREENING AND CHEMISTRY SHARED FACILITY AT COLUMBIA UNIVERSITY

(SF9) STEM CELL DERIVATION AND CHARACTERIZATION AT THE NEW YORK STEM CELL FOUNDATION

(SF10) RENSSELAER CENTER FOR STEM CELL RESEARCH (RCSCR)

(SF11) THE SKI STEM CELL RESEARCH FACILITY

(SF12) DEVELOPING AN INTEGRATED PLATFORM FOR siRNA AND shRNA-BASED GENOMESCALE SCREENING IN EUKARYOTIC STEM CELLS

POSTER ABSTRACTS

- (1)** ACCELERATED HIGH-YIELD GENERATION OF LIMB-INNERVATING MOTOR NEURONS FROM HUMAN STEM CELLS USING SMALL MOLECULES

- (2)** LINEAGE CHOICE REGULATION BY POLYMERIC SUBSTRATES

- (3)** MORE NEUROBLASTS IN THE HIPPOCAMPUS OF ANTIDEPRESSANT-TREATED MAJOR DEPRESSION

- (4)** REGULATION OF PLURIPOTENCY AND STEM CELL DIFFERENTIATION BY THE UBIQUITIN PROTEASOME SYSTEM

- (5)** DEVELOPMENT OF A VASCULAR NICHE PLATFORM FOR THE CLINICAL-SCALE EXPANSION OF REPOPULATING HUMAN CORD BLOOD STEM AND PROGENITOR CELLS

- (6)** THE BIOLOGY AND POLITICS OF STEM CELLS: INTRODUCING STUDENTS TO EXPERIMENTAL DESIGN HEURISTICS TO UNDERSTAND ETHICS

- (7)** DENTAL PULP STEM CELL DIFFERENTIATION REGULATED BY MECHANICS OF POLYBUTADIENE RUBBER FILMS

- (8)** COMPARATIVE GENOME-WIDE SCREENS IN MAINTAINED AND DIFFERENTIATING MURINE EMBRYONIC STEM CELLS

- (9)** THE ROLE OF BONE MARROW STROMAL CELLS IN MECHANICALLY INDUCED OSTEOGENESIS

- (10)** ROLE OF TUDOR AND MBT DOMAIN PROTEINS IN DROSOPHILA GERMLINE SPECIFICATION

- (11)** DERIVATION OF FUNCTIONAL CRANIAL PLACODE CELLS AND SENSORY NEURONS FROM HUMAN PLURIPOTENT STEM CELLS

- (12)** HEMATOPOIETIC DEPENDENT ACTIVATION OF ENDOTHELIAL CELLS IS ESSENTIAL FOR LIVER REGENERATION

(13) ENDOTHELIAL-DERIVED INDUCTIVE SIGNALS INITIATE AND SUSTAIN REGENERATIVE LUNG ALVEOLARIZATION

(14) A NEW MOUSE MODEL FOR HUMANIZATION OF THE LIVER WITH IPS CELL-DERIVED HUMAN HEPATOCYTES

(15) DISSECTING THE PROTEIN INTERACTION NETWORK OF ESC PLURIPOTENCY

(16) A ROLE FOR INTRACELLULAR SIGNALING IN IPS REPROGRAMMING

(17) MAXIMUM DIASTOLIC POTENTIAL OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES DEPENDS CRITICALLY ON I_{kr}

(18) ENHANCED EXPRESSION OF POLYSIALIC ACID IN ESC-DERIVED NEURON TRANSPLANTS IMPROVES THEIR HOST TISSUE INTEGRATION AND FUNCTIONAL EFFICACY

(19) ELECTRICAL CONDITIONING OF hESC DERIVED CARDIOMYOCYTES

(20) SCALABLE EXPANSION AND DIRECTED DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS IN STIRRED-SUSPENSION

(21) Zfp281 MEDIATES NANOG AUTOREPRESSION AND INHIBITS EFFICIENT REPROGRAMMING

(22) MOLECULAR TARGETS OF Bmi1 – A PcG MEMBER IN ADULT NEURAL STEM CELL REGULATION

(23) IDENTIFICATION OF ACUTE MYELOID LEUKEMIA STEM CELL PATHWAYS ASSOCIATED WITH THERAPEUTIC RESISTANCE

(24) IPS-DERIVED HUMAN BETA CELLS RECAPITULATE THE MODY 2 PHENOTYPE

(25) FUNCTIONAL STUDIES OF JARID2 AS LONG NON-CODING RNAs BINDING PROTEIN

(26) Fgf SIGNALING IS REQUIRED FOR MUTUAL EXCLUSION BUT NOT ONSET OF PRIMITIVE ENDODERM TRANSCRIPTION FACTOR PROGRAM WITHIN THE ICM OF THE MOUSE BLASTOCYST

(27) SMALL MOLECULE_MEDIATED DIRECTED DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS TOWARD VENTRICULAR-LIKE CARDIOMYOCYTES

(28) ZEBRAFISH REST REGULATES GENE EXPRESSION BUT NOT STEM CELL FATE DURING EARLY EMBRYOGENESIS

(29) *POLYCOMB* PROTEIN SCML2A RECRUITS PRC1 AND USP7 TO CHROMATIN AND IS DIFFERENTIALLY REGULATED DURING ERYTHROID DIFFERENTIATION

(30) INHIBITION OF THE ANDROGEN RECEPTOR SIGNALING BY A SMALL MOLECULE INHIBITOR OF NUCLEAR BETA-CATENIN AS A NEW TREATMENT APPROACH TO PROSTATE CANCER

(31) BACTERIAL PROTEIN AVRA TARGETS INTESTINAL STEM CELLS VIA BETA-CATENIN PATHWAY

(32) METASTATIC TERATOCARCINOMA INDUCTION UPON SIMULTANEOUS *PTEN* LOSS AND *KRAS* ACTIVATION IN POST-NATAL MURINE GERM CELLS

(33) DEVELOPING SIGNATURE GENE REGULATION PATTERNS FOR MONITORING MESENCHYMAL STEM CELL DIFFERENTIATION USING LIVE CELL ARRAYS

(34) THE MICRO-RNA-310/13 CLUSTER ANTAGONIZES β -CATENIN FUNCTION IN THE REGULATION OF GERMLINE STEM CELL DIFFERENTIATION IN *DROSOPHILA*

(35) IMPLANTABLE ARTERIAL VASCULAR GRAFTS BASED ON HAIR FOLLICLE DERIVED MESENCHYMAL STEM CELLS AND SMALL INTESTINAL SUBMUCOSA

(36) GENOME WIDE-ANALYSIS OF HUMAN OLIGODENDROCYTE LINEAGE TRANSCRIPTIONAL NETWORKS REVEALS CONSERVED TARGETS FOR MYELIN REPAIR

(37) HUMAN ESC-DERIVED HEMATOPOIETIC PROGENITORS EMERGE FROM HEMOGENIC ENDOTHELIUM AND UNDERGO LINEAGE PROGRESSION WITHIN THE VASCULAR NICHE

(38) IDENTIFYING MICRORNAS IN SKIN STEM CELLS

(39) ASTROCYTES BASED APPROACHES TO SPINAL CORD INJURY THERAPY

(40) CHARACTERISTICS OF THE TRANSIENT OUTWARD K⁺ CURRENT AND ITS CONTRIBUTION TO THE ACTION POTENTIAL IN hiPSC-DERIVED CARDIOMYOCYTES

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(42) SMALL MOLECULE-MEDIATED INDUCTION OF GLOBIN SWITCHING IN DIFFERENTIATING HUMAN EMBRYONIC STEM CELLS

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(44) ACQUISITION OF DEVELOPMENTAL COMPETENCE THROUGH INTEGRATION OF SIGNAL TRANSDUCTION PATHWAYS AND EPIGENETIC REGULATION

(45) DERIVATION OF XENOFREE HUMAN EMBRYONIC STEM CELL LINES FROM MINORITY POPULATIONS

(46) SINGLE TRANSCRIPTION FACTOR REPROGRAMMING OF HAIR FOLLICLE-DERIVED DERMAL PAPILLA CELLS TO INDUCED PLURIPOTENT STEM CELLS

(47) DEVELOPMENT AND CHARACTERIZATION OF BIOMIMETIC CARDIAC TISSUE ENGINEERED FROM HUMAN EMBRYONIC STEM CELLS

(48) CEREBROSPINAL FLUID REGULATION OF ADULT NEURAL STEM CELLS

(49) STEM CELL-BASED APPROACHES TO THE NEUROBIOLOGY OF OBESITY IN BARDETBIEDL SYNDROME

(50) INVESTIGATING THE EMERGENCE AND DYNAMICS OF THE PLURIPOTENT STATE

(51) CONTROL OF PRESYNAPTIC DIFFERENTIATION BY MUSCLE-DERIVED RETROGRADE SIGNALS

(52) Nfix REGULATES NEUROGENESIS IN POSTNATAL MOUSE BRAIN

(53) KRAB-MEDIATED MAINTENANCE OF GENOMIC IMPRINTING IN ES CELLS

SHARED FACILITIES

(SF1) CORNELL MAMMALIAN CELL REPROGRAMMING CORE

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The Cornell Mammalian Cell Reprogramming Core is interested in the use of ES and iPS cells as a source of stem cells for clinical therapy in a variety of animal models, and as a tool for understanding the maintenance of the pluripotent state and mechanisms of differentiation. For most researchers, however, there are significant barriers to entry in stem cell work which necessitate a core facility that can perform services for users from different disciplines while minimizing variance and maintaining a level of quality control necessary for success. Over the past two years the core has been developing a variety of services on a partial cost recovery basis to meet the needs of researchers in this area, including the derivation and validation of ES and iPS cell lines, and the production of viral vectors for stable transgene expression and reprogramming. More recently, the core took over the duties of the existing transgenics core and has been providing ES cell microinjection services, gene targeting, and in-vitro fertilization.

The core also has a dedicated bioinformatician offering a wide range of data analysis services to NYS stem cell consortium members and other Cornell University researchers. These analysis services handle heterogeneous biological data, including high-throughput next generation sequencing data, microarray gene expression data, and genotyping data. The focus is on the genomics and epigenomics data mining and integration, and custom bioinformatics analysis of other types of data. These services are provided as fee-for-service, grant application and publication support, and experimental design and consultation.

[Supported by NYSTEM contract C024174]

(SF2) EXPANSION OF PLURIPOTENT STEM CELL RESEARCH AT MSSM

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The main objective of the hESC/iPSC shared resource facility (SRF) is to make available the latest developments in the field of ESC/iPSC biology to scientists. We are currently exploring different methods to aid in the development of transgene-free iPSC lines using the Sendai virus and the mRNA/micro RNA methodology. The next step involves the differentiation of these cells into the diseased tissue-specific cells in order to recapitulate aspects of the disease. To facilitate the latter, we are generating various reporter systems using the latest Transcription Activator-Like Effector Nuclease (TALEN) technology. These reporters will allow us to establish conditions necessary to improve the differentiation of specific lineages and allow siRNA, micro RNA, small molecule/drug screens in novel cell populations. Our second objective is to quality control stem cell lines and reagents. These quality control services will include karyotyping, mycoplasma testing and supply of these cell lines to the community (MTA permitting). Scientists are also provided with tested reagents at vastly discounted pricing made possible due to the establishment of two stem cell supply centers, bulk purchasing and NYSTEM funding. Last but not the least, our third objective is to continue to conduct classes to teach iPSC generation and differentiation into the lineage of choice in order to increase in the number of labs doing stem cell science. Taken together, these services will have a three-fold benefit. Firstly, it will alleviate the quality control burden of individual scientists and allow them to concentrate on important scientific questions. Secondly, it will allow collaborative projects involving hES/iPS cell lines to be initiated with multiple laboratories by removing the prohibitive cost and providing the expertise required establishing and sustaining this technology and lastly it will provide the Core with a source of revenue to meet its expenditures.

[Supported by NYSTEM contract C024176]

(SF3) THE ROCKEFELLER UNIVERSITY HUMAN PLURIPOTENT STEM CELL CORE FACILITY

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Building on the derivation of several human embryonic stem cell (hESC) lines (RUES1-3; James *et al.*, 2005) and the development of an efficient, reversible method of transgenesis in hESCs (Lacoste *et al.*, 2009), the Rockefeller University Human Pluripotent Stem Cell (hPSC) Core Facility has continued investigations into pluripotency and directed differentiation, as well as adapting technology and techniques for hPSC work and providing reagents and training to hPSC researchers in the U.S. and abroad. Utilizing both transgenic and small molecule approaches, core facility-trained investigators and staff have revealed new insights into requirements for maintenance of pluripotency, as well as differentiation toward neural, mesodermal and extraembryonic lineages. A new vector has been developed for stable, conditional expression of both shRNAs and miRNAs. Further, core facility staff has worked with affiliated researchers to improve and validate conditions for live cell imaging and single cell resolution studies in both standard culture and microfluidics platforms. RU hESC lines, which were approved for the NIH registry in 2009 and 2010, as well as transposable elements for transgenesis, have been shared with numerous labs in the United States and overseas. Finally, updated procedures and training continue to be made available directly from core facility staff and via internet. Together, these efforts serve the Rockefeller University hPSC Core Facility mission of elucidating mechanisms of pluripotency maintenance and fate choice between embryonic lineages, developing new technologies, and providing the stem cell community with resources and training for the advancement of hPSC research.

[Supported by NYSTEM contract C024180]

(SF4) STONY BROOK STEM CELL FACILITY CENTER

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The Stony Brook Center was officially launched in August, 2011, and the first six months have focused on retro-fitting, renovation, and equipment purchase to provide state-of-the-art facilities related to development of three key Stem Cell Cores: 1) *Stem Cell Processing and Education*, 2) *Stem Cell Gene Transfer*, and 3) *Stem Cell Imaging and Analysis*. During these planning sessions, a highly innovative partnership was established that leveraged the vision of the newly-expanding 250-acre Stony Brook Research and Development Park with that of the Stony Brook Stem Cell Center. The Stem Cell Facility was established as a ~3,500 SF Center embedded within the newly created 45,000 SF Advanced Energy and Research Technology Center (AERTC). This strategy provided complementary synergisms to the nanoengineering and materials sciences research in AERTC. Both the New York State-funded AERTC and the state-supported NYSTEM centers share the same mission of promoting cutting-edge interdisciplinary research with the goal of economic development through industry partnership. By linking the core competencies within each Center, a large fraction of the imaging equipment purchased within the AERTC (such as the electron and confocal microscope, cryo-sample preparation, atomic force microscope, etc.) provided value-added enhancement of the Stem Cell Imaging and Analysis Core. Similarly, the interdisciplinary focus of the NYSTEM facility provided the logistical infrastructure for the biological component originally lacking in AERTC. The pooling of resources, development of shared facilities, and laboratory space has created novel synergies that will considerably enhance state-leveraged dollars with the goal of providing innovative infrastructures at the interface of tissue reengineering and stem cell biology. The Center will have a tremendous impact on creating an interdisciplinary research environment linking nanoengineering and regenerative medicine, thereby providing value-enhanced resources that are unique across New York State. The operationalization of all expanded services will proceed during the next funding period.

[Supported by NYSTEM contract C026716]

(SF5) UPSTATE STEM CELL cGMP FACILITY AT THE UNIVERSITY OF ROCHESTER

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The University of Rochester Medical Center (URMC) has received a grant from the Empire State Stem Cell Board for the construction of a new facility that will enable scientists to produce human stem cells suitable for testing in human subjects. The Upstate Stem Cell cGMP Facility (USCGF) is currently being constructed on the URMC campus and will provide approximately 3600 ft² of manufacturing and testing space for early phase clinical trial materials.

The facility is designed to be a multi-use, clean-room facility, with high flexibility and versatility, for the production of Phase I clinical trial materials. The facility contains three manufacturing laboratories each with its own HEPA filtered HVAC unit operating independently providing Class 10,000 (ISO Class7) air. MFG 1 will be equipped with bio-safety cabinets, a bio-safety cabinet/cell sorter system, dual chamber incubators, a WAVE bioreactor, downstream monoclonal antibody purification system, pass-throughs and stainless work tables. MFG 2 and MFG 3 will each be equipped with bio-safety cabinets, dual chamber incubators, a CliniMACs cell separation system, pass-throughs and stainless work tables. Intermediate entry and exit areas, designated as Class 100,000 (ISO Class 8), will be equipped with LN₂, -80°C and -20°C freezer space, refrigerator space, metro storage shelving, a work desk, and a sink. In addition the facility will contain an analytical QC testing laboratory as well as storage and office space.

The USCGF is a key component of the University of Rochester's Stem Cell and Regenerative Medicine Institute and will be a regional resource available to scientists in institutions throughout upstate New York. Construction is anticipated to be complete by summer 2012 with initial validation activities complete by fall 2012.

[Supported by NYS DOH C026713]

(SF6) NEURACELL BANK – A SOURCE OF MOUSE AND HUMAN CENTRAL NERVOUS SYSTEM STEM CELLS AND ASSOCIATED REAGENTS

Susan K. Goderie, Steve Lotz, Shiela Le, Christopher Fasano, Jeffrey H. Stern and Sally Temple
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There is a recognized need for stem cell research leading to therapies for neurological diseases of the central nervous system (CNS), such as Parkinson's Disease, ALS, Alzheimer's Disease, brain cancer, spinal cord injury, and macular degeneration. Approximately 25% of all stem cell research concerns the nervous system (ESSCB strategic plan). Research using nervous system (neural) stem cells (NSCs), neural progenitor cells (NPCs), and induced pluripotent cell (iPS) derived NSCs, provides a platform to advance therapeutics for these devastating neurological diseases and disorders.

We have created a centralized facility located in the Capital region, upstate NY, to maintain, store and supply CNS stem cells to other researchers. The 1500 sq ft laboratory is fully equipped to derive, culture, FACS characterize and enrich, and store these various neural-related stem cells. We can create cultures of numerous regions of mouse CNS from different stages of development. In addition we routinely prepare cultures from human cadaver tissue, creating retinal pigment epithelium (RPE) cultures, and human spinal cord cultures. Over the past three years we have created several human iPSC lines from ocular tissue, and now have several unique sets of genetically matched fibroblast and CNS-derived iPSC lines that enable epigenetic studies related to cell source. Several of these RPE-derived iPSC lines are from elderly donors, and to our knowledge are the only elderly CNS-derived iPSCs. Hence these could be particularly valuable to study diseases of the aging CNS.

Tissue preparations at different stages of isolation and culture can be shipped either as live cultures or frozen cells. Live RPE cell cultures have been successfully shipped from our facility to Germany, demonstrating robust survival. In addition, our facility generates media additives for CNS stem cell

cultures, such as NC27, similar to the commercially available B27, but produced using a modified recipe that generates a highly reproducible reagent.

The Neuracell resource is aimed at advancing neural stem cell research in New York State, enabling experiments to produce the challenging variety of nervous system cells, and providing a consistent source of NSCs that can be used to develop models for drug screening or replacement therapies for neurological disease. In addition, our facility can help introduce researchers not yet familiar with handling these cells to the neural stem cell field, by making the cells easily available and by sharing our expertise.

New York has a very strong position in NSC research, and the goal of the Neuracell Bank is to strengthen this position, to assure our future leadership and exciting results in the areas of CNS stem cell research and the emerging field of Neuro-regenerative Medicine.

[Supported by NYSTEM Contract NO. C024177]

(SF7) INTRODUCTION TO THE WESTERN NEW YORK STEM CELL CULTURE AND ANALYSIS CENTER (WNYSTEM)

Michael J. Buck, Jian Feng, Norma J. Nowak, Michal K. Stachowiak, E. (Manolis) S. Tzanakakis, Suzanne Batt, Andrew Bruno, Steven M. Gallo, Houbo Jiang, Sinae Kim, Sridhar Narla, Ewa Stachowiak, Sujith Valiyaparambil, Scott Wersinger, Jianbo Wu, Richard M. Gronostajski *Departments of Biochemistry, Chemical and Biological Engineering, Pathology & Anatomical Sciences, Physiology and the Developmental Genomics Group, New York State Center of Excellence in Bioinformatics and Life Sciences, Univ. at Buffalo (SUNY), Buffalo, NY*
<http://wnystem.buffalo.edu>

We have established the NYSTEM-funded Western New York Stem Cell Culture and Analysis Center at the University at Buffalo to promote and facilitate research in the use of mouse and human embryonic, adult, induced pluripotent, and cancer stem cells. Stem cells have tremendous potential to improve our understanding and treatment of human diseases, including diabetes, cancers, spinal cord injury, Parkinson's disease, cardiomyopathies, neurodegenerative diseases and the damage or degeneration of various organs due to aging or injury. The Center will promote stem cell research by providing specialized, well managed and easy to use facilities: 1) to obtain, culture, expand and store stem cells; 2) to generate new stem cells by genetic reprogramming of somatic cells; 3) to analyze stem cells in terms of their growth, differentiation, and tumorigenic properties and ability to repopulate and heal organs in mice; and 4) to determine the genes, regulatory regions and chromatin state responsible for the growth and differentiated characteristics of stem cells and their progeny. Specialized facilities are needed for these functions in order to speed and maximize research efforts by scientists currently using stem cells and to create an easy access point for new investigators to begin to use stem cells in their research. This Center will be used both by scientists in New York State and also by those in surrounding regions who want to begin using stem cells in their research. The Center consists of an Administrative Unit and four Core Facilities:

- 1) Stem Cell Culture, Banking and Training Facility (SCCF)
- 2) Induced Pluripotent Stem Cell Generation Facility (iPSF)
- 3) Stem Cell Engraftment Facility (SCEF)
- 4) Stem Cell Sequencing/Epigenomics Facility (SCSF).

WNYSTEM will ensure that all work is performed in keeping with current federal and state scientific and ethical standards.

(SF8) NYSTEM HIGH-THROUGHPUT SCREENING AND CHEMISTRY SHARED FACILITY AT COLUMBIA UNIVERSITY

Charles Karan^{1,8}, Andrea Califano^{1,2,8}, Olivier Couronne¹, Shi-Xian Deng³, Mandalina Ene^{5,8}, Donald W. Landry³, Hai Li^{1,8}, Sergey Pampou^{1,8}, Ronald Realubit^{1,8}, Alison Rinderspacher³, Rachid Skouta^{5,8}, Brent R. Stockwell^{5,8}, Christopher E. Henderson^{4,8}

¹Columbia Genome Center; ²Department of Biomedical Informatics; ³Department of Medicine; ⁴Departments of Regenerative Medicine, Pathology, Neurology and Neuroscience, Columbia University Medical Center; ⁵Chemical Probe Synthesis facility; ⁶Howard Hughes Medical Institute; ⁷Departments of Biological Sciences and Chemistry, Columbia University; ⁸Columbia Stem Cell Initiative, New York, NY

The NYSTEM High-Throughput Screening and Chemistry Shared Facility is designed to expedite new discoveries in stem cell science and catalyze new translational strategies. It will allow NYSTEM researchers access to all aspects of academic screening and related synthetic chemistry, including state-of-the-art laboratory automation and detection technologies, and an experienced staff will provide advice and oversight in all aspects of the process from assay design, data analysis, target identification and medicinal chemistry. The facility will offer access to small molecules, which could provide new investigational leads for drug discovery, and RNAi and cDNA libraries, which can be used for mechanistic analysis through functional genomics assays. The facility possesses multiple automated liquid handling systems, including a Cell::Explorer automation system designed to handle large scale high-throughput cellular assays, as well as state of the art detection systems with a Perkin Elmer Envision for plate-reader based assays and a GE IN Cell Analyzer 2000 for automated microscopy. During its first six months, the facility hired key personnel and purchased equipment essential to the fulfillment of its mission. It has also begun to design a chemical library for screening. We have established the capability of designing and synthesizing both chemical probes and therapeutic candidates, using computational and medicinal chemistry. The establishment of a screening center which can provide access to both small molecules and biological reagents will aid in the prosecution of new targets and provide new insights in the treatment of disease. For more information on services available from the screening center contact screening@columbia.edu. For information on the chemical probe synthesis facility, contact cps@biology.columbia.edu.

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

(SF9) STEM CELL DERIVATION AND CHARACTERIZATION AT THE NEW YORK STEM CELL FOUNDATION

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¹The New York Stem Cell Foundation, New York, NY; ²Department of Pediatrics and Naomi Berrie Diabetes Center, Columbia University, New York, NY; ³Stowers Medical Institute; ⁴Howard Hughes Medical Institute; ⁵Department of Stem Cell & Regenerative Biology, Harvard University, Cambridge, MA

NYSCF has established an independent, specialized facility in which researchers within the stem cell community are able to conduct projects that would be prevented by Federal funding restrictions that segregate human embryonic stem cell research from other forms of medical research. This environment is designed to facilitate collaborations across institutional boundaries. The NYSCF laboratory continues to expand to accommodate the increasing needs of external users in addition to NYSCF researchers' own projects in disease modeling. To date, many major collaborative projects have been initiated within the NYSCF laboratory, including projects for disease modeling for diabetes, cardiac disease, Schizophrenia, and several neurodegenerative diseases as well as efforts to engineer functional bone grafts. To support these efforts, we have established regulatory compliant protocols for generating human embryonic stem cell lines from normal and diseased embryos and continue to explore human embryonic stem cell derivation by a variety of techniques. For example, in addition to non-affected HESC lines, we have derived a number of HESC lines from embryos diagnosed for Spinal Muscular Atrophy and Huntington's Disease by Preimplantation Genetic Diagnosis. We are also deriving a bank of iPS cell lines, including a range of disease-specific lines. To aid these efforts, we have developed techniques that increase the efficiency of iPS generation, greatly facilitating the application of this technology for large-scale iPS cell banking programs and drug screening assays. Our aim is to serve the greater New York State research community by acting as a repository for high quality human pluripotent stem cell lines and make them available to researchers across the US. The facility has become a center for collaboration, exchange of information, and development of innovative techniques that plays an essential and integral role in the growing stem cell community.

[Supported by NYSTEM contracts C024179 & C026185]

(SF10) RENSSELAER CENTER FOR STEM CELL RESEARCH (RCSCR)

Glenn Monastersky¹ and Christopher Bjornsson²

¹PI and RCSCR Director, Director of Operations and Associate Center Director, Center for Biotechnology and Interdisciplinary Studies and Professor of Practice, Biomedical Engineering Center for Biotechnology and Interdisciplinary Studies; ²Associate RCSCR Director, Core Director, Microscopy and Cellular Imaging and Cell and Molecular Biology and Research Assistant Professor, Biology Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY

The Rensselaer Center for Stem Cell Research will begin operations in July 2012. The specific aims of this Shared Resource Center include: 1) the construction of a superbly equipped and efficiently operated shared-use facility to support the unique demands of high-quality stem cell research; 2) the integration of the facility into the existing research core infrastructure at Rensselaer; and 3) the reliable, high-quality operation, maintenance and ultimate sustainability of the facility. The double-laboratory facility will provide research support and training in stem cell culture, derivation, imaging and analysis for students and faculty from Rensselaer, the Capital Region of New York state and beyond. In addition to dedicated tissue culture, incubation and microscopy equipment, the Center will provide the following research platforms: Olympus VivaView O₂/CO₂ incubator (deconvolved fluorescence, brightfield, DIC) with MetaMorph[®] imaging software; Thermo Scientific Cellomics ArrayScan VTI High-Content Screening (HCS) platform (automated fluorescence, brightfield); a hypoxic incubation and microscopy chamber; Eppendorf 5070 liquid handling system; Olympus IX51 inverted microscope (fluorescence, brightfield, phase contrast); Olympus CKX51 inverted microscope (brightfield and phase contrast); Olympus SZX 16 Motorized Stereo Microscope (fluorescence, brightfield, darkfield, oblique, reflected); BD FACSAria flow cytometer; Amaxa Nucleofactor; PCR thermal cycler. Instrumentation and research assistance available to Center users in adjacent core laboratories includes a Zeiss LSM 510 META laser scanning confocal microscope, a Zeiss LSM 510 NLO multiphoton microscope, an Asylum Research MFP 3D AFM atomic force microscope, an Olympus IX81 Total Internal Reflection Fluorescence (TIRF) Microscope, a Scanco Medical Viva CT40 Scanner, an MMI Laser Microdissection - Optical Tweezers system, a GE Typhoon Trio+ confocal flatbed scanner digital imaging system, a Roche LightCycler[®] 480 RT-PCR system and a BD LSRII flow cytometer. Stem cell researchers also will have access to nearby histology, proteomics, NMR, analytical biochemistry and nanobiotechnology laboratories, a small animal surgery suite and an embryo cryopreservation facility.

[Supported by NYSTEM contract C026717]

(SF11) THE SKI STEM CELL RESEARCH FACILITY

Mark J. Tomishima, Viviane Tabar, Lorenz Studer

The Center for Stem Cell Biology and the Center for Cell Engineering, Developmental Biology Program, Sloan-Kettering Institute, New York, NY

The SKI Stem Cell Research Facility provides a number of services to the New York State stem cell community including: 1) *training*; 2) *genetic modification* of human pluripotent stem cells (hPSCs); 3) reprogramming, 4) *directed differentiation* of pluripotent stem cells, and 5) *high-content screening* capabilities. Here, we will provide an update to the NYS stem cell community about the ongoing work in our facility, providing a special emphasis on the recently implemented high content module.

[Supported by NYSTEM contract C024175]

(SF12) DEVELOPING AN INTEGRATED PLATFORM FOR siRNA AND shRNA-BASED GENOME-SCALE SCREENING IN EUKARYOTIC STEM CELLS

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The primary objective of our NYSTEM award is to establish a collaborative, multi-institutional, state-of-the-art, RNA-interference (RNAi)-based high-throughput screening platform that will be used to investigate the molecular/genetic regulation of embryonic stem cells (ESCs), and induced pluripotent cells (iPSCs).

To expand the capabilities of the NYU RNAi Core we have purchased human and mouse microRNA mimic and inhibitor libraries. To improve workflow and increase capacity, we have purchased liquid handling/plate reformatting automation, a technology upgrade for the Arrayscan automated microscope, and the Odyssey SA for high-throughput in-cell Westerns. We have also used NYSTEM funds to offer screening subsidies to potential screeners. These have been successful in attracting screeners from NYC institutions, including Columbia, Memorial Sloan-Kettering, and Rockefeller Universities, in addition to several from NYU Medical School.

The shRNA Core at Mt. Sinai School of Medicine (MSSM) has developed and streamlined their technologies for the generation and maintenance of ESCs and iPSCs. They are in the process of procuring shRNA libraries, and the equipment for making the lentiviral libraries for screening.

We plan to coordinate efforts between the NYU siRNA and MSSM shRNA screening facilities to identify shared areas of scientific focus to tackle the outstanding questions in stem cell biology, as well as to offer educational courses to the scientific community on the application of high-throughput screening technology towards stem cell biology.

The creation of this unique resource has provided an unprecedented access to state-of-the-art screening capabilities for stem cell researchers. The outcomes of several RNAi screens currently in the pipeline will provide novel insights into stem cell biology. Importantly, the integrated screening platform continues to foster interactions/collaborative ventures between researchers.

[Supported by NYSTEM contract C026719]

(1) ACCELERATED HIGH-YIELD GENERATION OF LIMB-INNERVATING MOTOR NEURONS FROM HUMAN STEM CELLS USING SMALL MOLECULES

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Human pluripotent stem cells are a promising source of diverse cells for developmental studies, cell transplantation, disease modeling and drug testing. However, their widespread use, even for intensely studied cell types like spinal motor neurons, is hindered by the long duration and low yields of existing protocols for *in vitro* differentiation and by the molecular heterogeneity of the populations generated. We report here a combination of small molecules that induce up to 50% motor neurons within 3 weeks from human pluripotent stem cells with defined subtype identities that are relevant to neurodegenerative diseases. Despite their accelerated differentiation, motor neuron subsets thus derived expressed combinations of HB9 and ISL1 and the columnar specific markers that mirror those observed *in vivo* in human fetal spinal cord. They also showed spontaneous and induced activity, and projected axons to muscles when grafted into developing chicken spinal cord. Strikingly, this novel protocol preferentially generates limb-innervating lateral motor column (LMC, FOXP1⁺) motor neurons, of both lateral and medial divisions (LMC_m and LMC_L), which are the most affected in amyotrophic lateral sclerosis (ALS). Access to high yield cultures of human limb-innervating motor neuron subtypes will facilitate in depth study of motor neuron subtype specific properties, disease modeling, and development of large-scale cell-based screening assays.

[Supported by NYSTEM contract C024415]

(2) LINEAGE CHOICE REGULATION BY POLYMERIC SUBSTRATES

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To test the combined effects of biochemical and biophysical cues on stem cell differentiation, we used the mouse cell line C9 which expresses rhBMP-2 under control of the doxycycline-repressible promoter, Tet-Off. Cultures grown with doxycycline served as control, as did the parent cell line (C310T1/2) grown with and without doxycycline. Each cell line was grown on Si wafers coated with spun cast monodisperse polymer films of either polybutadiene (PB) where the substrate mechanical response can be controlled by film thickness, or on partially sulfonated polystyrene (SPS) where surface charge is controlled by the degree of sulfonation. On PB, the moduli of cells expressing rhBMP-2 varied by day 1 by more than a factor of 2 on when the substrate modulus was quadrupled and decreased substantially by day 5. Minimal variation in the moduli was observed for either the C310T1/2 cells or the C9 cells in the presence of doxycycline. On SPS, the moduli of all cells was initially large, and but decreased by day 3. Cell differentiation was monitored by qRT-PCR (OSX, ALP, OCN, BSP, SOX9, COLIIA, COLX and ALP) and biomineralization by SEM/EDX. Analyses of 14 day cultures showed that: (1) cells expressing rhBMP-2 appeared osteogenic when cultured on SPS (BSP high, COLIIA high, ACAN low) and chondrogenic (ACAN high) when cultured on PB; (2) in the absence of doxycycline, the ECM deposited by the C9 cells was completely biomineralized with calcium phosphate on SPS, while amorphous carbonaceous deposits were observed for the cells cultured on PB; and (3) because occasional regions with small amounts of calcium phosphate deposition were also observed in C9 cultures grown with doxycycline and in C3H10T1/2 cultures grown with or without doxycycline, on SPS, some mineralization of the ECM may occur independent of differentiation. These data suggest a model where substrate chemistry may control lineage choice and differentiation.

(3) MORE NEUROBLASTS IN THE HIPPOCAMPUS OF ANTIDEPRESSANT-TREATED MAJOR DEPRESSION

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Divisions of ¹Molecular Imaging and Neuropathology and ²Integrative Neuroscience, New York State Psychiatric Institute, New York, NY; Departments of ³Psychiatry, ⁴Neuroscience, ⁵Pathology and Cell Biology, ⁶Pharmacology, Columbia University, New York, NY; ⁷Department of Neurological and Psychiatric Sciences, University of Florence, Florence, Italy; ⁸Macedonian Academy of Sciences & Arts, Republic of Macedonia

Adult neurogenesis is a mechanism of structural plasticity whereby neurons are generated from neural progenitor cells (NPCs) in neurogenic niches in mammalian brain. It is involved in antidepressant response and behavioral adaptation to stress in animals. We found more NPCs and mitotic cells in dentate gyrus (DG) of antidepressant-treated major depressive disorder (MDD). We sought to determine whether antidepressant treatment is also associated with more cells at later stages of neuronal maturation in the adult human DG postmortem.

Whole hippocampi from untreated MDD (n=10), MDD treated with either selective serotonin reuptake inhibitors (MDD*SSRI, n=7), or tricyclics (MDD*TCA, n=5) and untreated nonpsychiatric subjects (controls, n=9) were fixed, sectioned at 50µm and immunoreacted with anti-polysialylated neuronal cell adhesion molecule (PSA-NCAM) antibody. All cases were free of neuropathology, had brain and blood toxicology screening and were matched for age, sex and postmortem interval. We used stereology to estimate number of immunolabeled neuroblasts in the anterior (head), mid (body) and posterior (tail) portions of the DG at 2-mm intervals. Based on morphologic characteristics, neuroblasts were classified as immature, bipolar or pyramidal.

In the DG head: immature neuroblast number differ between untreated MDD, MDD*SSRI, MDD*TCA and controls (F=4.221, p=.014) with MDD*TCA having more than untreated MDD (p=.041) and controls (p=.009); pyramidal neuroblasts differ between groups (F=3.364, p=.033) with MDD*TCA having more than untreated MDD (p=.044) and MDD*SSRI (p=.037). In the DG tail, bipolar neuroblasts differ between groups (F=5.713, p=.007) with MDD*TCA having more than untreated MDD (p=.005), MDD*SSRI (p=.021) and controls (p=.009).

We present the first evidence that antidepressant treatment is associated with more immature neurons in the DG of adult humans with MDD, suggesting that antidepressant treatment is associated not only with more neuroprogenitors and mitotic cells, but also with increased maturation and survival of hippocampal stem cells in human.

[Supported by NYSTEM contract N08G-184]

(4) REGULATION OF PLURIPOTENCY AND STEM CELL DIFFERENTIATION BY THE UBIQUITIN PROTEASOME SYSTEM

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 *Contributed equally to the work

Pluripotent embryonic stem (ES) cells have the ability to both self-renew and differentiate. Accumulating evidence suggests that post-translational modifications are central in regulating stem

cell behavior. To study in detail the role of the ubiquitin-proteasome system (UPS) in ES cell pluripotency, we employed a combination of genomic and proteomic approaches. We mapped using mass-spectrometry, the dynamic changes in protein ubiquitination and additionally, we have performed siRNA based ubiquitin screens targeting the majority of members of the ubiquitin enzymes in both conditions of self-renewal and differentiation. These combined studies demonstrated that a significant number of members of the core pluripotency machinery are regulated by ubiquitination. Moreover, our functional screens identified a large number of putative novel ubiquitin enzyme regulators. Overall our functional studies suggest that the UPS plays a central role in maintenance of pluripotency and the establishment of differentiation. To further elucidate the importance of the UPS in regulating self-renewal, we focused on the transcription factor Nanog, a key factor that controls pluripotency. We found that the protein levels of Nanog are dynamically regulated in self-renewal by ubiquitination in comparison to early stages of differentiation, suggesting that regulation of Nanog levels may be crucial for fine-tuning pluripotency. Overall, this is the first characterization of the UPS as a key regulator of ES cell function opening the way for further characterization for mechanisms of pluripotency, self-renewal, and differentiation.

[Supported by NYSTEM contract C026880]

(5) DEVELOPMENT OF A VASCULAR NICHE PLATFORM FOR THE CLINICAL-SCALE EXPANSION OF REPOPULATING HUMAN CORD BLOOD STEM AND PROGENITOR CELLS

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Within the mouse bone marrow, endothelial cells serve as specialized niche cells that balance self-renewal and differentiation of the hematopoietic stem cell (HSC) into lineage-specific progenitor cells (HPCs). However, the inability to maintain human endothelial cells (hECs) in culture conditions that permit proliferation of the hematopoietic cells has hindered the studies to define the role vascular cells in supporting homeostasis of human hematopoietic stem and progenitor cells (HSPCs). We have devised an approach to cultivate primary hECs in xenobiotic-free conditions in the absence of serum and endothelial growth factors. We demonstrate that hECs in the presence of minimal concentrations of thrombopoietin, stem cell factor and Flt3-ligand can perform as an instructive vascular platform to efficiently expand cocultured human cord blood cells (hCBs) in serum-free culture conditions. While incubation of CD34⁺ hCB cells with cytokines alone resulted in rapid attrition of hematopoietic cells beyond 12 days of culture, coculturing of the same number of CD34⁺ hCBs with hECs and minimal cytokines resulted in a 400-fold expansion of total CD45⁺ nucleated cells, 150-fold expansion of CD45⁺CD34⁺ hematopoietic progenitor cells, and a 22.5-fold expansion of Lineage⁻ CD34^{hi}CD45^{RA}⁻CD49^f HSPCs beyond 12 days that have enhanced multi-lineage engraftment when transplanted into primary and secondary immunocompromised recipient mice. Limiting dilution, competitive repopulation transplantation assays demonstrate that CD34⁺ hCBs cells that have been co-cultured with hECs results in a 7.5-fold increase in the number of human cord blood cells capable of long-term repopulation as compared to unmanipulated CD34⁺ hCBs. Therefore, hECs establish an ideal niche cell for long-term expansion and differentiation of repopulating HSC and HSPCs setting stage for the therapeutic use of human HSPCs for treatment of hematological disorders.

(6) THE BIOLOGY AND POLITICS OF STEM CELLS: INTRODUCING STUDENTS TO EXPERIMENTAL DESIGN HEURISTICS TO UNDERSTAND ETHICS

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Recent advances in the life sciences present students with an opportunity to engage in real-life debates that require basic biological literacy, ethical reasoning, and communication skills. Through collaboration across the natural sciences, humanities, social sciences, and design we developed and implemented a semester-length non-majors interdisciplinary course titled “Stem Cells and Social Justice” using a case-based approach and a social justice framework. The curriculum is referred to as “Stem Cells Across the Curriculum” (SCAC), as it contains modules that have been used together, or separately, in courses that span the liberal arts. The content of the modules include social concepts such as distributive and procedural justice and biological concepts such as gene-environment interactions involved in cell plasticity. Each case study weaves the ethical dimension associated with procurement and testing of ESCs, ASCs, and iPSCs with the biological dimension such as differences in genetic content and environmental conditions (*in vivo* vs. *in vitro*). By teaching students how to use two common heuristics of experimental design, we help students develop a “habit of mind.” The first heuristic involves mapping out the scientific method for important historical experiments, while the second heuristic is employed each time a new stem cell technology is introduced such that students identify the source/sample, the experimental manipulation, the assay, method of detection, and the method by which the original cell will be traced in the experiment. By introducing non-majors to the experimental design heuristics that are commonly used in the life sciences, we hope to equip these students with the tools for lifelong learning so that they may follow advances in the field. By embedding these heuristics in the social context of the case studies we help them see how understanding biology is essential for making informed decisions regarding stem cell research on a social and personal level.

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(7) DENTAL PULP STEM CELL DIFFERENTIATION REGULATED BY MECHANICS OF POLYBUTADIENE RUBBER FILMS

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Undifferentiated mesenchymal stem cells isolated from dental pulp (dental pulp stem cells (DPSCs)) when grown in specific inducing media can differentiate and express markers of odontoblasts, osteoblasts, adipocytes or neuronal cells. The use of materials whose mechanical properties can regulate or direct stem cell proliferation and differentiation offers exciting applications. Here we introduce the use of polybutadiene, (PB), a biocompatible, rubber similar to the gutta-percha used in dentistry to obturate the root canal, which can be synthesized in the monodisperse form ($M_w=205K$, $M_w/M_n=1.49$, $T_g=-95C$), and used to produce substrates whose mechanical properties follow an inverse power law dependence on film thickness. Hence substrates with uniform or patterned heterogeneous mechanical response can be produced in a straightforward manner without any additional chemical modifications. We show that (a) DPSC can be grown on PB without additional adhesion or growth factors, (b) the cells can adjust their modulus, E_c to obey the same power law as that of the substrate, G , such that $E_c=0.50G$, for all film thicknesses and (c) a critical value, $G_o=0.5E_o$, exists such that for $G>G_o$, the cells differentiate without the inducing agent dexamethasone. Glancing incidence X-ray diffraction, electron microscopy, qRT-PCR, and scanning force microscopy confirmed that for $E>E_o$, osteogenic markers were upregulated and the cells produced crystalline hydroxyapatite, HA, deposits. Immunofluorescent staining showed that the differentiation marker osteocalcin was upregulated uniformly throughout all 3-5 layers of the

culture. Production of HA did not persist when the cells were transferred on PB with $G < G_0$, but resumed when transferred back onto PB with $G > G_0$, indicating that the influence of the surface is felt even in the absence of direct cell contact. Further work is in progress to identify the mechanisms involved in cell surface interactions, the potential for de-differentiation, and the influence of the surface in templating tissue organization.

[Supported in part by NYSTEM contract C026716]

(8) COMPARATIVE GENOME-WIDE SCREENS IN MAINTAINED AND DIFFERENTIATING MURINE EMBRYONIC STEM CELLS

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Pluripotency of embryonic stem cells is an essential feature in their potential for use in regenerative medicine. The maintenance of pluripotency in mouse embrionic stem cells (mESCs) is controlled by a transcriptional network regulated by three core transcription factors (TFs), Nanog, Oct4 and Sox2. The balance between pluripotency and differentiation is further influenced by extracellular signals, among which the Wnt/ β -catenin (β -cat) pathway plays a major role in cell fate decisions. Observations that Tcf-3 (a transcriptional effector of Wnt signaling) binds to target regulatory sequences of Oct4 and Nanog, and its loss-of-function results in robust pluripotency, points towards a connection between the Wnt pathway and the activity of the pluripotency maintenance transcriptional network (PTN). We are using a combinatorial approach of three distinct genome-wide screens in order to find functionally relevant genes in stem cell maintenance and differentiation. Furthermore, we are particularly interested in components of the Wnt signaling cascade, as these can typically promote stem cell maintenance through both transcriptional regulation and distinct cytoplasmic roles. In our ongoing RNAi-based functional screen aimed at testing components of the Wnt pathway in mESC pluripotency, we mainly identified components that regulate β -cat's stability and its adhesive functions but not the genes involved in modulating β -cat's nuclear signaling function. Interestingly, Wnt/ β -cat signaling has also been shown to promote mesoderm specific differentiation. These observations raise intriguing questions regarding the mechanism(s) by which β -cat can coordinate its function in both pluripotency and differentiation.

[Supported by NYSTEM contract C026880]

(9) THE ROLE OF BONE MARROW STROMAL CELLS IN MECHANICALLY INDUCED OSTEOGENESIS

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Osteoporosis occurs when the activity of bone-forming osteoblasts is insufficient to counteract that of bone-resorbing osteoclasts. Bone marrow stromal cells (BMSC) can differentiate into osteoblasts and *in vitro* studies show that physical stimulation plays a role. However, the contribution of BMSCs to loading-induced bone formation *in vivo* is unclear, and is the objective of this study. Wild type mice were irradiated and transplanted with GFP BMSCs. Ulnae were axially loaded with a load of 3N for 120 cycles for 3 days. Mice were then sacrificed to culture BMSCs and process bones for immunohistochemistry. GFP was detected in cultured BMSCs and in the bone marrow cavity through immunohistochemistry, indicating successful engraftment of donor cells. Furthermore, GFP was detected in bone lining cells in a loaded ulna. The presence of GFP-expressing bone lining cells suggests BMSCs contribute to bone formation. However, it is unclear how they sense mechanical loading. One candidate is the primary cilium, an antennae-like organelle protruding from the cell body. Work from our lab suggests primary cilia are important in BMSCs for detecting or responding to fluid flow *in vitro*. When primary cilia formation was inhibited, BMSCs exhibited a reduced osteogenic response. Future work includes determining the role of primary cilia in BMSCs in loading-induced bone formation *in vivo*. Our methods can be used to transplant BMSCs from

Kif3a fl/fl GFP+ mice. BMSCs from these donors are treated *ex vivo* with Cre recombinase to delete Kif3a, which is important for primary cilia formation, and then transplanted into WT mice. We are currently awaiting loading and analyses of chimeric animals that lack primary cilia only in BMSCs. This work could lead to exciting new insights into the mechanism by which BMSCs sense physical stimulation *in vivo* and how physical stimulation may influence differentiation.

[Supported by NYSTEM contract C024331]

(10) ROLE OF TUDOR AND MBT DOMAIN PROTEINS IN DROSOPHILA GERMLINE SPECIFICATION

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Germ Cells are often regarded as the “ultimate stem cells”, seeing as they are the only cell type capable of generating an entire organism. Although their specification can occur through various mechanisms, all germ cells are set aside from the soma early in development to retain pluripotency. In *Drosophila melanogaster* they are the first cells to form in the embryo before migrating to the forming gonads where they will differentiate into gametes. I am interested in understanding the mechanisms by which the germline is formed in the fruit fly, for this purpose I am using two approaches:

- (1) I am studying the role of tudor domain proteins in the assembly a specialized cytoplasm that is instructive for germ cell formation in the embryo: *Drosophila* germ cells arise from a set of maternal RNAs and proteins called the germ plasm. Tudor (Tud), the founder of the tudor domain family acts as a scaffold for protein and RNA components necessary for germ cell formation. I am investigating the molecular mechanisms by which Tud localizes maternal components that control germ cell specification. There are 23 tudor domain proteins in *Drosophila*, many of which are involved in the piRNA pathway, a germline specific RNAi mechanism that protects the genome integrity by silencing transposable elements. I am also conducting an RNAi based screen to identify tudor domain proteins that could function redundantly with Tud.
- (2) I am characterizing somatic “germline-like” tumors, due to mutations in the L3MBT transcriptional repressor; interestingly these tumors rely on expression of key germline genes for their formation and survival. I am testing if these tumors recapitulate key germline features and I am also investigating why mutations in the *l3mbt* gene lead to germline stem cell death.

[Supported by NYSTEM contract C026880]

(11) DERIVATION OF FUNCTIONAL CRANIAL PLACODE CELLS AND SENSORY NEURONS FROM HUMAN PLURIPOTENT STEM CELLS

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A major challenge in human embryonic stem cell research is to develop efficient differentiation protocols to derive specific cell types that can be translated directly into therapeutics. Here, we report for the first time on the *in vitro* derivation of cranial placodes from human embryonic stem cells (hESCs) and their further specification into diverse subplacodes, including trigeminal placodes, anterior pituitary placodes and lens placodes. Placode cells are the developmental precursors of sensory organs, including the eye, the nose and the inner ear. To date, relatively little is known about placode development because of the inaccessibility of fetal tissue and the lack of specific markers for the lineage. Most of our knowledge comes from species such as zebrafish, chick, and frog and, to a lesser extent, mouse. Our new protocol mimics

development, resulting first in the induction of placode progenitor cells expressing Six1, Eya1, and Dlx3 with a purity of 71%, which can be further differentiated into a pure population of sensory neurons expressing Isl1, Brn3a, Tuj1 and Peripherin. These cells are appropriate for modeling developmental diseases, transplantation and the testing of novel drugs involved in sensory function including pain.

(12) HEMATOPOIETIC DEPENDENT ACTIVATION OF ENDOTHELIAL CELLS IS ESSENTIAL FOR LIVER REGENERATION

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Background: Liver repair after injury is a complex process intertwined with regeneration and wound healing. The high regenerative ability of the liver is frequently overwhelmed by the scarring response, culminating in overt fibrosis and end stage cirrhosis. Development of effective therapeutic approaches for liver diseases is hampered by the undefined cellular and molecular effectors involved in the repair processes. Endothelial cells (ECs) establish an instructive vascular niche to stimulate liver regeneration through elaboration of paracrine (angiocrine) trophogens. However, the precise mechanism by which liver-specific subsets of ECs are activated upon injury to produce pro-regenerative angiocrine factors is unknown.

Results: Utilizing complementary models of hepatotoxin (carbon tetrachloride, CCl₄)-induced injury and cholestatic liver fibrosis, we have demonstrated the differential role of vascular and hematopoietic compartments in modulating regeneration and fibrogenesis after inflammatory insults. Acute inflammatory injury caused deposition of hematopoietic cells on liver sinusoidal endothelial cells (LSECs), which by deploying VEGF-A activates Id1 pathway in VEGFR2⁺ LSECs to generate endothelial-derived angiocrine factors such as hepatocyte growth factor (HGF) and Wnt2, thereby promoting hepatic regeneration. Further analysis revealed that recruited CD11b⁺ myeloid cells activate FGFR1 in LSECs and amplified LSEC-mediated liver regeneration after acute inflammatory insults. Notably, during chronic injury aberrant persistent activation of FGFR1 on LSECs by myeloid cells increases sinusoidal endothelial invasiveness and generation of pro-fibrotic factors, leading to excessive deposition of extracellular matrix proteins and promoting pathological fibrogenesis and end stage cirrhosis.

Conclusions: We have demonstrated the differential role of vascular and hematopoietic compartments in modulating regeneration of liver after inflammatory injury. Hematopoietic cell-mediated sinusoidal vascular activation in hepatic reparative processes elicits regeneration after acute insults, while provoking fibrogenesis during chronic injury.

Impact: Exploiting the potential of pro-regenerative angiocrine cues while diminishing pro-fibrotic vascular activation would provide for designing and optimizing clinical protocols for treatment of end stage liver diseases.

(13) ENDOTHELIAL-DERIVED INDUCTIVE SIGNALS INITIATE AND SUSTAIN REGENERATIVE LUNG ALVEOLARIZATION

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Background: The molecular and cellular pathways that regulate the regeneration of adult lungs are unknown. To define the regulatory mechanism(s) involved in alveolar regeneration, we employed a left unilateral pneumonectomy (PNX) model that induces the expansion of resident epithelial progenitor cells and functional reconstitution of lung mass and respiratory function.

Findings: Here, we show that after PNX, activated VEGFR2⁺FGFR1⁺ pulmonary capillary endothelial cells (PCECs) by production of paracrine (angiocrine) factors stimulate proliferation of epithelial progenitor cells, including bronchioalveolar stem cells (BASCs) and type II alveolar epithelial cells (AECIIs), supporting regenerative alveolarization. After PNX, endothelial-specific inducible genetic deletion of *Vegfr2* and *Fgfr1* in adult mice (*Vegfr2*^{iΔEC/iΔEC}*Fgfr1*^{iΔEC/+}) impaired production of angiocrine factors that stimulate regeneration of BASCs/AECs and inhibited restoration of respiratory functions. Among the alveologenic factors, MMP14 was specifically upregulated in wild-type, but not in *Vegfr2* and *Fgfr1* deficient PCECs. MMP14 stimulated the propagation of epithelial progenitor cells via unmasking the cryptic EGF-like growth factors through shedding heparin binding EGF-like ectodomain and cleaving laminin5 γ2 chain. Antibody-mediated neutralization of MMP14 abolished the reconstitution of epithelial cells and subsequent remodelling into functional alveolar sacs, while delivery of recombinant EGF or transplantation of inductive PCECs from wild type mice into the pneumonectomized *Vegfr2*^{iΔEC/iΔEC}*Fgfr1*^{iΔEC/+} mice restored alveologensis, lung compliance and inspiratory volume.

Conclusion: Activation of VEGFR2 and FGFR1 in PCECs supports PNX-induced alveologensis not only by vascular sprouting, but also via inductive production of MMP14 and activation of BASCs and AECIIs. Crosstalk between PCECs and lung epithelial progenitor cells orchestrates regenerative alveolarization.

Impact: The identified cellular and molecular players in regenerative alveolarization hold remarkable clinical promise to bolster therapeutic lung regeneration for debilitating diseases, including lung fibrosis and chronic obstructive pulmonary disease (COPD).

(14) A NEW MOUSE MODEL FOR HUMANIZATION OF THE LIVER WITH iPS CELL-DERIVED HUMAN HEPATOCYTES

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Background: Currently available mouse models for liver repopulation with human hepatocytes, e.g. uPA- transgenic and fumarylacetoacetate hydrolase (Fah)-deficient mice exhibit severe host liver injury and limited life-span. Based on our discovery that transgenic mice (PiZ) expressing mutant human α 1-antitrypsin-Z (ATZ) exhibit minimal hepatocellular abnormality, but are repopulated spontaneously by transplanted wildtype mouse hepatocytes, we hypothesized that similar repopulation could occur after transplanting human iPSC-derived hepatocytes into immunodeficient hybrid PiZ mice.

Methods and Findings: PiZ/SCID hybrids were generated by crossing PiZ mice with SCID mice. hiPSCs reprogrammed from adult human fibroblasts by expressing Yamanaka pluripotency genes were differentiated into hepatocyte-like cells. Immunostaining showed progressive loss of pluripotency markers and gain of hepatocyte markers (albumin, CK-18, UGT1A1 and asialoglycoprotein receptor (ASGPR)) during *in vitro* differentiation. Approximately 80% of the differentiated cells expressed albumin and exhibited LDL and indocyanin green uptake, and glycogen storage. The differentiated cells secreted albumin, AAT, C-reactive protein, transferrin and urea into the media at 23-26%, 8-16%, 0.41-0.87%, 84.04-142.86% and 46-51% of the respective rates observed with primary hepatocytes. Primary or iPSC-derived human hepatocytes (1×10^6) were transplanted into the SCID/PiZ mouse livers by intrasplenic injection, followed by the injection of 1×10^{11} particles of an adenovector expressing hepatocyte growth factor. Liver repopulation by human albumin and UGT1A1-positive donor cells was ~2-5%, 10% and 20% after 1, 2 and 3 months respectively. Alpha-fetoprotein expression was not detectable in the donor cell clusters. In contrast, there was no liver repopulation the in non-transgenic SCID mouse recipients.

Conclusion: SCID/PiZ mice are an excellent model for evaluating the ability of stem cell-derived human hepatocytes to engraft and proliferate. The "humanized" mice may also be a potential mouse model for developing novel therapies for human hepatocyte-specific infections and other pharmaceutical uses.

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(15) DISSECTING THE PROTEIN INTERACTION NETWORK OF ESC PLURIPOTENCY

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Oct4 and Nanog are well-known transcription factors that play fundamental roles in stem cell self-renewal, pluripotency and somatic cell reprogramming. However, limited information is available on Oct4 and Nanog associated protein complexes and their intrinsic protein-protein interactions that dictate the critical regulatory activities of Oct4 and Nanog. Here we employed an improved affinity purification approach combined with mass spectrometry to purify Oct4 and Nanog associated protein complexes in mouse embryonic stem cells (mESCs), and discovered many novel Oct4 and Nanog partners important for self-renewal and pluripotency of mESCs. Notably, we found that Oct4 and Nanog are associated with multiple chromatin modifying complexes with documented as well as

newly proved functional significance in stem cell maintenance and somatic cell reprogramming. This expanded Oct4 and Nanog interactome both include the Ten eleven translocation (Tet) family hydroxylases. We show that Tet1, in synergy with Nanog, enhances the efficiency of somatic cell reprogramming. Tet1 and Nanog co-occupy the promoters of genes associated with both maintenance of pluripotency and lineage commitment. Depletion of Nanog significantly reduces Tet1 binding to these target genes. We propose that Tet1 is directly recruited by Nanog to regulate expression of a subset of target genes, thereby contributing to the efficiency of induced pluripotency. Our study establishes a solid biochemical basis for genetic and epigenetic regulation of stem cell pluripotency and provides a framework for exploring alternative factor-based reprogramming strategies.

(16) A ROLE FOR INTRACELLULAR SIGNALING IN iPS REPROGRAMMING

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Background: Previously, we identified the PI3K/Akt/GSK3 β signaling cascade as an important downstream target of reprogramming factors (Oct4(O), Sox2(S), Klf4(K), Myc(M)). Furthermore, we identified molecules which may work in concert/cross-talk to the PI3K/Akt/GSK3 β pathway, and thus, also support iPS cell reprogramming. Our chemical library screen pointed towards the important role of chromatin state changes in iPS cell generation. Hit components of the iPSC colony formation screen included U73122 (PLC γ inhibitor) and PJ34 (Parp1 inhibitor). Both target molecules are known regulators of chromatin and may work in concert with the PI3K/Akt/GSK3 β pathway. As the importance of Poly(ADP-ribose) polymerase 1 (Parp1) in chromatin remodeling has been suggested, we studied the effect of pharmacological and genetic manipulations of Parp1 on reprogramming efficiency.

Important Findings: Parp1 overexpression increased iPS generation, while Parp1 inhibition (PJ34) and shRNA-mediated knockdown decreased iPS colony formation. Furthermore, we generated *-/-* Parp1 mice. Generation of iPS cells from *-/-* Parp1 MEF was very inefficient. As Parp1 improves the efficiency of iPS reprogramming, we characterized the iPS clones produced with Parp1 OSKM as compared to GFP OSKM. Parp1 OSKM iPS cells are pluripotent as shown by immunohistochemistry, gene expression, and pyrosequencing.

Future Directions: We are going to study the impact of the PI3K/Akt/GSK3 β pathway on Parp1 activity and vice versa. In addition, we are exploring the role of Parp1 during the early stages of reprogramming. We are going to examine the effect of Parp1 iPS cells in a murine Parkinsonism model (transplantation) and on tumor development in adult chimeric mice.

Impact: iPS cells hold great promise for regenerative medicine, thus, overcoming major limitations, such as low efficiency, are essential for future clinical applications. Therefore, our research is focused on improvement of iPS cell generation and modulating intracellular signaling seems to be a very promising tool to increase efficiency and safety of iPS cell production.

[Supported by NYSTEM contract C024403]

(17) MAXIMUM DIASTOLIC POTENTIAL OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES DEPENDS CRITICALLY ON I_{kr}

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Background: Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) hold promise for therapeutic applications. To serve these functions, the hiPSC-CM must recapitulate the electrophysiologic properties of native adult cardiomyocytes. This study examines the electrophysiologic characteristics of hiPSC-CM between 11 and 119 day of maturity.

Method: Embryoid bodies (EBs) were generated from hiPS cell line reprogrammed with Oct4, Nanog, Lin28 and Sox2. Sharp microelectrodes were used to record action potentials (AP) from spontaneously beating clusters (BC) micro-dissected from the EBs (n=87, 37±0.5°C). We also examined the response to 5 µM E-4031 (n=15) or BaCl₂ (n=15). Patch-clamp techniques were used to record I_{Kr}, I_f and I_{K1} from single cells enzymatically dissociated from 10 BCs (n=25, 36°C).

Results: Spontaneous cycle length (CL) and AP characteristics varied widely among the 87 preparations. E-4031 (5 µM; n=15) increased Bazett-corrected AP duration from 295.6±98.7 to 492.7±98.2 ms (p<0.01) and generated early afterdepolarizations (EAD) in 6/15 preparations. In 9/15 BCs E-4031 rapidly depolarized the clusters leading to inexcitability. BaCl₂, at concentrations that selectively block I_{K1} (50-100 µM) failed to depolarize the majority of clusters (8/12). Patch-clamp experiments revealed a negligible I_{K1} in 83% (10/12) of the cells studied, but presence of I_{Kr} in all (5/5). Consistent with the electrophysiological data, RT-PCR and immunohistochemistry studies showed relatively poor mRNA and protein expression of I_{K1}, but robust expression of I_{Kr}.

Conclusion: In contrast to recently reported studies, our data point to major deficiencies of hiPSC-CM, with remarkable diversity of electrophysiologic phenotypes as well as pharmacologic responsiveness among beating clusters and cells up to 119 days post-differentiation. The vast majority have a maximum diastolic potential that depends critically on I_{Kr} due to the absence of I_{K1}. Thus, efforts should be directed at producing more specialized and mature hiPSC-CM for future therapeutic applications.

[Supported by NYSTEM contract C026424]

(18) ENHANCED EXPRESSION OF POLYSIALIC ACID IN ESC-DERIVED NEURON TRANSPLANTS IMPROVES THEIR HOST TISSUE INTEGRATION AND FUNCTIONAL EFFICACY

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The effectiveness of cell therapy has been demonstrated in many disease models, including transplantation of ESC-derived dopamine neurons in Parkinson's disease. However, the limited dispersion and survival of grafted cells as well as their poor fiber outgrowth into host brain are still major limitations. The impact of such problems is likely to be more severe in the larger human nervous system. Polysialic acid (PSA) is a cell surface homopolymer that promotes plasticity of neural tissues, including cell migration and axon outgrowth. It is highly expressed in the embryo and in a few adult CNS regions that maintain plasticity. We used PSA enhanced expression to facilitate tissue repair in several applications, which illustrates its broad translational potential. Introduction of a polysialyltransferase gene into CNS tissue promotes axon regeneration and progenitor migration. Enhanced PSA expression in grafted Schwann cells used to build cellular bridges for lesioned axon results in an increase in their regenerative and functional efficacy. We have also tested the PSA augmentation of ESC-derived neurons both *in vitro* and in animal models. The results show that the PSA enhancement of transplanted cells increases their neurite outgrowth and host tissue innervation. Moreover, the improved fiber innervation correlates with enhanced functional restitution.

[Supported by NYSTEM contract 71840]

(19) ELECTRICAL CONDITIONING OF hESC DERIVED CARDIOMYOCYTES

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Development of methods for cardiomyocyte (CM) differentiation from human stem cells has enabled patient specific cardiac disease models and increased the promise of clinical application of cardiac tissue engineering for treating myocardial infarction. One major obstacle, however, is to mature the relatively nascent CMs and direct their functional assembly. Mimicking native development we hypothesized that induction of synchronous contractions by electrical stimulation will enhance the maturation and assembly of immature human CMs.

We report here the use of electromechanical regulation on newly generated hESC-CMs, by conditioning in an external electrical field. We explored three frequencies of stimulation: 0.5, 1 and 2 Hz, mimicking a range of physiologic human heart rates (30 bpm, 60 bpm, 120 bpm), each applied for one week. Beating embryoid bodies - clusters of differentiated CMs - were shown to entrain with the external field stimulus, beating synchronously at the rate of the applied stimulus even after the electrical stimulation was discontinued. Interestingly, the cells most affected were those stimulated at 2Hz, but, consistent with normal overdrive suppression, external field stimulation was not able to decrease the average beating rate below unstimulated controls.

Consistent with the known structure-function relationships for human CMs, immunostaining demonstrated increased organization and expression of Troponin T and Connexin 43, markers of phenotypic maturation, with increasing stimulation rates. Calcium dye studies revealed that both CMs as well as their support cells worked in tandem to affect calcium trafficking within the embryoid body. To further elucidate the mechanisms by which the cells respond to external field stimulation, microarray analysis revealed upregulation of specific voltage sensitive channels and cardiac hypertrophy markers. These studies indicate that hESC-CMs are capable of responding to electrical stimulation, which in turn improves the maturity of cells and tissue constructs.

[The work was funded by NYSTEM contract C026449 to GVN]

(20) SCALABLE EXPANSION AND DIRECTED DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS IN STIRRED-SUSPENSION

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Successful therapeutic application of human pluripotent stem cells (hPSCs) requires their large-scale generation as stable pluripotent cells and their differentiated functional progeny. We adopted the stirred-suspension bioreactor platform for propagating hPSCs in a chemically defined environment under self-renewing and directed differentiation modes. For microcarrier culture, the low adhesion of hPSCs on the beads is a significant bottleneck. Optimal conditions were identified and compared to our previous methodology, the efficiency of microcarrier seeding increased from 30% to over 50%. The distribution of cells on microcarriers was more even resulting in aggregate-free cultures. The concentration of cultured hPSCs under xeno-free conditions increased 30-40-fold over 8 days and hPSC pluripotency was ascertained by qPCR and flow cytometry. When hPSCs were subjected to differentiation they displayed markers characteristic of different lineages. Our studies support the use of stirred-suspension microcarrier bioreactors for the expansion of hPSCs under chemically defined conditions.

[Supported by NYSTEM contract C024355]

(21) Zfp281 MEDIATES NANOG AUTOREPRESSION AND INHIBITS EFFICIENT REPROGRAMMINGMiguel Fidalgo¹, Francesco Faiola¹, Christoph Schaniel¹, Jianlong Wang¹¹*Department of Developmental and Regenerative Biology, Black Family Stem Cell Institute, Mount Sinai School of Medicine, New York, NY*

Molecular control of the pluripotent state in embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) resides in a core circuitry of master transcription factors Oct4, Sox2 and Nanog that together with other genetic and epigenetic factors form a core regulatory pluripotency network. The homeodomain transcription factor Nanog plays an important role in ESC self-renewal and is essential for acquiring ground state pluripotency during reprogramming. Enforced expression of Nanog liberates ESCs from requisite LIF dependence and reprograms “primed” pluripotent mouse EpiSCs to “naïve” pluripotent ESCs. Understanding how *Nanog* is transcriptionally regulated is important to further dissect mechanisms of ESC pluripotency and somatic cell reprogramming. We have identified Zfp281, a Krüppel-like zinc finger transcription factor, as an interaction partner of Nanog. We performed chromatin immunoprecipitation experiments and microarray analysis, and demonstrated that Zfp281 is required for Nanog binding to its own promoter and keeping its expression in check, suggesting that Nanog-associated repressive complex(es) involving Zfp281 may fine-tune Nanog expression for pluripotency of ESCs. Using a genetic knockout mouse model and an RNAi mediated loss-of-function system we demonstrated that Zfp281 functions as a transcriptional repressor to restrict Nanog autoregulation (i.e., autorepression) in mouse ESCs. We also presented evidence that Nanog autorepression requires the coordinated action of Zfp281 and other co-repressor complexes associated with the Nanog interactome. Finally, we show that Zfp281-mediated Nanog autorepression may pose a roadblock to efficient iPSC generation during somatic cell reprogramming.

(22) MOLECULAR TARGETS OF Bmi1 – A PcG MEMBER IN ADULT NEURAL STEM CELL REGULATIONMythily Ganapathi¹, Nathan Boles², Sally Temple², Randall H. Morse¹¹*Laboratory of Molecular Genetics, Wadsworth Center, NY State Department of Health, Albany, NY;*²*Neural Stem Cell Institute, Rensselaer, NY*

Polycomb group (PcG) genes, which generally repress gene expression, have emerged as important regulators of stem cell maintenance and differentiation. Previous work from our lab showed a role for Bmi1, a PRC1 member, in self renewal and proliferation of embryonic and adult mouse neural stem cells (NSCs). When Bmi1 was inhibited in NSCs derived from the subventricular zone (SVZ) of mice, self-renewal was dramatically curtailed. Conversely, when Bmi1 was over expressed in these NSCs in culture, it drastically enhanced the capacity of the NSCs to proliferate and self renew. Although, Bmi1 has been implicated in multiple adult stem cell lineages as a regulator, its direct targets in these cells have not been identified. We are currently using molecular techniques to dissect the genes and pathways involved in Bmi1 regulation of NSC self-renewal and differentiation capacity. Our gene expression profiling studies using microarrays on NSCs over-expressing Bmi1 has revealed several novel candidates which could regulate adult NSC differentiation. We are following up those genes by performing gene knockdown studies in NSCs and during NSC differentiation. In addition, we are currently engaged in ChIP-seq experiments to identify the direct targets of Bmi1 and its associated histone modification H3K27me3, in our system. The data obtained from these experiments will shed light on the polycomb target genes which are crucial for neural stem cell maintenance.

(23) IDENTIFICATION OF ACUTE MYELOID LEUKEMIA STEM CELL PATHWAYS ASSOCIATED WITH THERAPEUTIC RESISTANCETzu-chieh Ho^{1,2}, Mark W. LaMere², Michael W. Becker²¹*Department of Pathology and Laboratory Medicine;* ²*The Wilmot Cancer Center, University of Rochester Medical Center, Rochester, NY*

Acute myeloid leukemia (AML) is a malignant hematopoietic disease characterized by abnormal proliferation and differentiation of neoplastic hematopoietic cells. Leukemia stem cells (LSCs) within AML are rare, chemotherapy resistant and thought to drive the disease relapse. Despite the promise of the AML-LSC model, LSC mechanisms relevant to drug resistance remain uncertain. AML LSCs were thought to be restricted to the CD34⁺CD38⁻ fraction however recent studies have challenged this hypothesis. We performed flow cytometry to characterize the immunophenotype of human leukemic bone marrow cells at diagnosis and relapse. Unlike in normal bone marrow (NBM) specimens, the expression of typical stem cell markers CD34 and CD38 varied significantly from patient to patient. We observed that the expression of CD34 and CD38 dramatically changed between diagnosis and relapse. In comparison to normal cells, previously published markers of LSCs (CD32, CD45RA, CD96 and TIM-3) were identified as being aberrantly expressed on a greater percentage of CD34⁺CD38⁻ as well as CD34⁺CD38⁺ leukemic blasts. While CD32, CD45RA and TIM-3 were maintained at relapse, CD96 expression was typically lost upon disease progression. Using xenotransplantation assay, we demonstrate that multiple fractions initiated tumor in NSG mice when AML samples were sorted based on CD34 and CD38 expression. AML LSCs expanded to phenotypically distinct populations between diagnosis and relapse. Little is known about the impact of disease state on the previously reported AML LSC specific signaling. To address this we developed a new technique to characterize gene expression profile in primary samples with limited cell numbers (e.g., 10,000 double sorted LSCs). Preliminary analysis of molecular pathways in functionally validated AML LSC populations yielded several candidate pathways such as actin cytoskeleton signaling and JAK-STAT signaling that were up-regulated in relapse. This data suggest that the immunophenotype of AML LSCs are more heterogeneous than previously thought, and may be affected by treatment and disease progression. The AML stem cell pathways identified by the comparison of paired diagnosis versus relapse patient specimens may facilitate to elucidate how LSCs resist chemotherapy, thus helping to develop new AML regimens in future studies.

(24) iPS-DERIVED HUMAN BETA CELLS RECAPITULATE THE MODY 2 PHENOTYPEHaiging Hua^{1,2}, Linshan Shang², Hector Martinez², Matthew Freeby¹, Thomas Ludwig¹, Liyong Deng¹, Ellen Greenberg¹, Robin Goland¹, Wendy Chung¹, Rudy Leibel¹, Dieter Egli^{1,2}¹*Division of Molecular Genetics, Department of Pediatrics and Naomi Berrie Diabetes Center, Columbia University, New York, NY;* ²*The New York Stem Cell Foundation, New York, NY*

MODY 2 is due to hypomorphic mutations of glucokinase (GCK), impairing the glucose-sensing function of this molecule in the beta cell. Clinically, this results in chronic mild hyperglycemia. We used stem cell technologies to generate MODY 2 patient-specific beta cells from skin fibroblasts; we also disrupted GCK function by homologous recombination. *In vitro*, we observed the anticipated impairment of glucose-induced insulin secretion; responses to arginine and potassium were preserved. When corresponding pancreatic progenitors were transplanted into mice for several months, more complete beta cell maturation was achieved. These cells displayed *in vitro* and *in vivo* phenotypes consistent with MODY 2. The ability to create such cells has implications for mechanistic studies of the human beta cell, and possibly for therapeutics.

(25) FUNCTIONAL STUDIES OF JARID2 AS LONG NON-CODING RNAS BINDING PROTEIN

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Long non-coding RNAs (lncRNAs) participate in many chromatin-mediated processes, including the epigenetic control of gene expression in multicellular organisms. Although the Polycomb-Repressive Complex 2 (PRC2) has been suggested to associate with numerous lncRNAs in mouse embryonic stem cells (mESC), the mechanisms by which lncRNAs regulate the functions of PRC2 remain elusive. Previously, we have shown that Jarid2, an ESC-specific subunit of PRC2 plays crucial roles for regulating gene expression.

Here we report that Jarid2 is capable of binding to lncRNAs. Using *in vitro* assays, we identified small domain responsible for the RNA binding activities of Jarid2. LncRNA facilitates interactions of Jarid2 with Ezh2, a catalytic subunit of PRC2 *in vitro*. Furthermore, RNA binding domain (RBD) of Jarid2 is required for lncRNA-mediated Jarid2 and Ezh2 interactions. Interestingly, excess amounts of lncRNA had no effects, suggesting regulatory roles of lncRNA for these protein-protein interactions.

We next established cell lines, which express Jarid2 mutants lacking RBD with the goal of determining the functional consequences of RNA binding. We found that whereas deletion of the RBD domain of Jarid2 did not alter its recruitment to genomic targets, but affected downstream events. To identify Jarid2-interacting RNAs we performed RNA immunoprecipitation followed by deep sequencing (RIP-seq) and used the RBD-less mutant proteins as specificity controls. We found that very specific interactions of Jarid2 with a handful of lncRNAs that may have important functions in ESC pluripotency and differentiation. Furthermore, Jarid2 depletion in mESCs resulted in reduced lncRNAs binding to PRC2 whereas Ezh2 is dispensable for lncRNAs binding to Jarid2, suggesting that these lncRNAs selectively interact with Jarid2 in mESC.

(26) Fgf SIGNALING IS REQUIRED FOR MUTUAL EXCLUSION BUT NOT ONSET OF PRIMITIVE ENDODERM TRANSCRIPTION FACTOR PROGRAM WITHIN THE ICM OF THE MOUSE BLASTOCYST

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The late/implanting blastocyst comprises three morphologically and molecularly distinct cell lineages: pluripotent epiblast (EPI), primitive endoderm (PrE) and trophectoderm (TE). Precisely how putative EPI and PrE cells arise within the inner cell mass (ICM) is not entirely clear, however, FGF/ERK signaling appears to be critical for this cell fate decision. To gain further insight, we investigated the phenotype resulting from the inactivation of *Fgf4* both *in vivo* and in embryo-derived stem cells. *Fgf4* mutant blastocysts lacked PrE entirely; a phenotype that could be rescued by addition of bFGF. Notably, an initial period of widespread marker expression was established, even in the absence of *Fgf4*. However, *Fgf4* mutant embryos exhibited a defect in the subsequent restriction phase, where fate commitment is likely to be acquired. These data suggest that FGF4 signaling though critical for cell fate choice is not required for initiation of transcription factor expression within the early embryo. Accordingly, *Fgf4*-deficient ES cells were isolated at higher efficiencies than wild-type cells and while refractory to EPI lineage differentiation they could be directed towards a PrE identity. Rescuing the phenotype of mutant embryos permitted the isolation of *Fgf4*-deficient XEN cells, which were indistinguishable from wild-type XEN cells. Taken together, our studies reveal key roles and sequential requirements for FGF/ERK signaling within the lineages of the ICM.

(27) SMALL MOLECULE-MEDIATED DIRECTED DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS TOWARD VENTRICULAR-LIKE CARDIOMYOCYTES

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The generation of human ventricular cardiomyocytes from human embryonic stem cells (hESCs) could fulfill the demand for therapeutic applications and *in vitro* pharmacological research. Nevertheless, the current cardiomyocyte differentiation protocols generate a low yield and/or a heterogeneous cell population consisting of atrial-, pacemaker- and ventricular-like cardiomyocytes. By combining small molecules and growth factors, we report a fully chemically defined, direct differentiation system that restricts the hESCs differentiation to the cardiac ventricular fate in a highly efficient, reproducible and scalable fashion. Molecular characterization revealed that the differentiation of the hESCs recapitulated the developmental stages of cardiovascular fate specification. Phenotypic analysis illustrated the generation of a highly enriched population of ventricular-like cardiomyocytes. These chemically induced ventricular-like cardiomyocytes (termed ciVCMs) exhibited the expected electrophysiological, calcium handling characteristics, the appropriate chronotropic responses to cardioactive compounds and homing to native myocardium. Taken together, these data suggest that the small molecule-mediated directed differentiation system mimics human embryonic cardiac developmental program and generates a nearly pure population of functional ventricular-like cardiomyocytes. The differentiation system provides reproducible, economical and efficient experimental platform that could advance basic developmental research, facilitate large-scale pharmacological screening as well as provide a valuable source of ventricular cardiomyocytes for cell replacement therapies.

(28) ZEBRAFISH REST REGULATES GENE EXPRESSION BUT NOT STEM CELL FATE DURING EARLY EMBRYOGENESIS

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REST (RE1 Silencing Transcription Factor also known as NRSF/Neural Restrictive Silencing Factor) has been proposed to be an important mediator of the epigenetic modifications that govern proliferation and differentiation of embryonic and neural stem cells. However, the requirement for Rest-mediated transcriptional regulation of these processes is poorly understood. Conflicting views of the role Rest activity plays in controlling stem cell fate have emerged from recent studies. To address these controversies, we examined the developmental requirement for Rest in zebrafish using zinc finger nuclease mediated gene targeting.

Despite derepression of target gene expression in *rest* mutant blastulae, we found no evidence that either embryonic or neural stem cells failed to differentiate or self-renew. Germ layer specification and neurogenesis progress normally in *rest* mutants. However, abnormalities are observed within the nervous system including defects in oligodendrocyte precursor cell development and a partial loss of facial branchiomotor neuron migration. We discovered that maternal *rest* is essential for repression of target genes during blastula stages, which clearly supports a role for Rest in embryonic stem cells.

Rest mutants progress normally through embryogenesis, but many die as larvae (>12 days). However, some mutants reach adulthood and are viable. We utilized a transgenic reporter system to dynamically monitor *Rest* activity. This analysis revealed *Rest* represses gene expression in mesodermal derivatives including muscle and notochord, as well as within the nervous system. Finally, we demonstrated that *Rest* is required for sustained long-term repression of target genes in non-neural tissues in adult zebrafish. Our results point to a broad role for *Rest* in fine-tuning neural gene expression, rather than as a widespread regulator of stem cell differentiation or renewal.

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(29) POLYCOMB PROTEIN SCML2A RECRUITS PRC1 AND USP7 TO CHROMATIN AND IS DIFFERENTIALLY REGULATED DURING ERYTHROID DIFFERENTIATION

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Polycomb group (PcG) proteins are key transcriptional repressors of genes involved in development and differentiation. The *Drosophila* PcG protein Sex Comb on Midleg (SCM) is essential for development through the repression of specific genes in cooperation with Polycomb Repressive Complexes PRC1, PRC2 and the Pho Repressive Complex PhoRC. There are two homologues of SCM in mammals: SCMH1 and SCML2. SCMH1 associates, and collaborates with PRC1, but very little is known about the functions of SCML2. Two different isoforms of SCML2 are expressed in human cells (SCML2A and SCML2B). Although they only differ in the C-terminal SPM domain, the subnuclear distribution and function of these two isoforms are different.

Fractionation experiments show that both isoforms are nuclear. SCML2B is mainly nucleoplasmic, where it plays a role in the regulation of the cell cycle. SCML2A is associated to chromatin, and this localization is dependent on the presence of an RNA binding domain. The purification of SCML2A from nuclear extract and chromatin fractions reveals that it interacts with PRC1 and USP7, a ubiquitin protease that has been shown to act on p53, PRC1 and histone H2B. CHIP-seq experiments show that SCML2A co-localizes with PRC1 and USP7 on chromatin. Deletion of the RNA binding domain blocks the binding of SCML2A to its target genes, leading to the loss of PRC1 and USP7 in these regions. In a more physiological setting, the expression of SCML2 is specifically downregulated when K562 cells are induced to differentiate towards a megakaryocytic phenotype, while SCML2 levels are slightly increased during erythroid differentiation. We are currently investigating whether the downregulation of SCML2 alters the genome-wide localization of PRC1. Our data show that SCML2 recruits PRC1 and USP7 to target genes through the binding to non-coding RNA, and that SCML2 may play a role in erythroid differentiation.

(30) INHIBITION OF THE ANDROGEN RECEPTOR SIGNALING BY A SMALL MOLECULE INHIBITOR OF NUCLEAR BETA-CATENIN AS A NEW TREATMENT APPROACH TO PROSTATE CANCER

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The androgen receptor (AR) is the major therapeutic target in aggressive prostate cancer. However, targeting AR alone can result in drug resistance and disease recurrence. Therefore, simultaneous targeting of multiple pathways involved in prostate cancer could be an effective new approach to treating prostate cancer. Here we provide proof of concept that a small molecule inhibitor of nuclear beta-catenin (called C3) can inhibit both AR and Wnt signaling pathways that are often mis-regulated in prostate cancer. The idea underlying the approach takes advantage of the fact that the AR interacts with, and is transcriptionally regulated by beta-catenin, the main effector of the Wnt pathway. Treatment with C3 ablates prostate cancer cell growth by disruption of both beta-catenin/TCF and beta-catenin/AR interactions, in light of the fact that TCF and AR have overlapping

binding sites on beta-catenin. C3 treatment results in decreased occupancy of beta-catenin on the AR promoter and diminished AR and AR target gene expression. C3 also reduces expression of beta-catenin target genes, such as c-myc, an oncogene frequently up-regulated in prostate cancer.

Growing evidence shows that Wnt/beta-catenin signaling is highly active in “cancer stem cell (CSCs)”, a small population of cancer cells defined by their ability to self-renew as well as differentiate to a daughter cell type. In prostate cancer, CSCs have been suggested to be resistant to conventional AR antagonist treatment resulting in disease recurrence. Thus it is important to develop therapeutic approaches to selectively target the stem cell population. Treatment of C3 to prostate cancer cells blocks CSCs self-renewal, and knockdown of beta-catenin showed that beta-catenin mediates this inhibitory effect of C3 on CSCs survival. Importantly, C3 inhibited tumor growth in an *in vivo* xenograft model of castration-resistant prostate cancer, indicating its promising therapeutic potential.

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(31) BACTERIAL PROTEIN AVRA TARGETS INTESTINAL STEM CELLS VIA BETA-CATENIN PATHWAY

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Salmonella infection can become chronic and increase the risk of cancer. Moreover, alive and mutated *Salmonella* is used as a vector to target cancer cells. However, studies are lacking on chronic infection with *Salmonella* in the host. *Salmonella* AvrA is a multifunctional protein that acts as a deubiquitinase to regulate beta-catenin ubiquitination and stabilize beta-catenin, a key player in development and tumorigenesis. We hypothesize that AvrA activates the beta-catenin pathway to promote colonic tumorigenesis. In a colitis-associated cancer model, mice were colonized with *Salmonella* AvrA- sufficient or deficient strains then stimulated with a carcinogen azoxymethane (AOM) and dextran sodium sulfate (DSS, induced colitis). We found that the tumor incidence in the AvrA+ infected group is 100% compared to 51.4% in the AOM/DSS only group and 56.3% in the AvrA- infected mice. The AOM/DSS mice had most of the tumors close to the distal colon. In contrast, the location of tumors in the infected colon was moved closer to proximal colon, where has more bacterial growth and fermentation. AvrA expression increased the expression of phosphorylated-beta-catenin (ser552), indicating involvement in inflammation-induced stem-progenitor cell expansion. AvrA directly activates beta-catenin signaling by enhancing nuclear beta-catenin in the infected colon. We confirmed the colonization of *Salmonella* and found *Salmonella* was only at the top of the villus, but also at the basal side, stem cell niches of the intestinal epithelium. Inflammatory cytokine IL-6 was increased by infection. Overall, we demonstrated bacterial AvrA activation of the beta-catenin pathway in colonic tumorigenesis. Our findings provide important insights into how a bacterial protein contributes to the development of cancer. This study allows for a better understanding of intestinal stem cells targeted by a bacterial protein. It will bring us one step closer to understanding the therapeutic strategies that may hamper dysregulation of stem cells in infection and cancer.

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(32) METASTATIC TERATOCARCINOMA INDUCTION UPON SIMULTANEOUS *Pten* LOSS AND *Kras* ACTIVATION IN POST-NATAL MURINE GERM CELLS

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Testicular germ cell tumors (TGCTs) are the most common cancers in young men, and have remarkably high cure rates following treatment with DNA-damaging chemotherapies. Since TGCTs express pluripotency markers such as OCT4 and often contain teratoma components, we hypothesized that their exceptional sensitivity to chemotherapy might be due to distinctive, stem cell-like properties of the germ cells from which TGCTs arise, including unique responses to DNA damage. In order to test this hypothesis, we generated a novel mouse model of malignant TGCTs in which the *Pten* tumor suppressor and *Kras* oncogene were simultaneously altered via *Cre/Lox* recombination specifically in differentiating spermatogonia and spermatocytes. Such manipulation resulted in rapid testicular teratocarcinoma formation in 85% of mice, most of which harbored bilateral tumors with extensive hemorrhage and necrosis. Tumors in these mice exhibited varying degrees of differentiation into tissues derived from all three germ layers, with an abundance of early neural-like tissue, and to date, metastases have been identified at distant sites including lymph nodes and pancreas. *Kras* activation alone did not lead to tumor formation in these mice, but significantly increased the frequency and reduced the latency of germ cell tumorigenesis driven by *Pten* loss; only 29% of *Pten* single mutants developed testicular tumors, and these tended to be unilateral with longer latency. Since the tumors in this *Pten/Kras* TGCT model are more malignant than those of current mouse models, which primarily feature highly differentiated teratomas, they may provide a better tool for understanding testicular tumorigenesis in humans, including the role of the DNA damage response during malignant transformation and as a determinant of chemotherapeutic sensitivity. This model additionally holds promise for providing insights into the origin of TGCTs, and suggests that these neoplasms can originate not only from primordial germ cells, as previously proposed, but also from post-natal spermatogonia.

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(33) DEVELOPING SIGNATURE GENE REGULATION PATTERNS FOR MONITORING MESENCHYMAL STEM CELL DIFFERENTIATION USING LIVE CELL ARRAYS

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Lentiviral microarrays (LVMAs) are promising for live-cell, high-throughput and real-time gene profiling mammalian cells. However, spot-to-spot transduction variation and cross-contamination may hinder the uses of LVMAs. To address these two issues, we firstly have engineered a dual promoter lentiviral vector—LVDP containing a specific transcriptional regulatory element encoding for green fluorescence protein for monitoring gene regulation and a constitutive promoter driving red fluorescence protein as the internal standard that allows us to quantify the gene dynamics independent of virus titer. Second, we have genetically engineered the lentiviral envelope VSV-G for covalent immobilization of lentivirus to fibrin minimizing cross-contamination between spots and maximizing signal/noise ratios. As a proof of concept, we utilized these methods to study the differentiation of mesenchymal stem cells. We developed a library of lineage specific promoters (such as adiponectin, SOX9 and Runx2 for adipogenesis, chondrogenesis and osteogenesis respectively) and transcriptional factor response motifs (vitaminD, SMADs, C/EBP, etc.). By applying automated fluorescence microscopy, we established the dynamics of lineage specific reporters in two different MSCs derived from hair follicles or bone marrow. Our results demonstrate differential reporter activation between the two cell types as seen for example in the case of adiponectin during adipogenesis suggesting that the regulatory pathways controlling differentiation

may vary between MSCs from different anatomic locations. To further identify key molecules affecting the differentiation, we screened 20 chemical inhibitors each controlling different regulatory pathway and monitored their effects on responses of our reporters. Inhibition of the PI3 kinase and glucocorticoids readily suppressed adipogenic reporters, while inhibition of JNK and ERK blocked the expression of osteo-specific reporters such as vitaminD, OCBOX and Runx2. Our system can be applied in screening small-molecule libraries to identify compounds that affect differentiation, as well as optimization of differentiation conditions targeting specific stem cell fates.

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(34) THE MICRO-RNA-310/13 CLUSTER ANTAGONIZES β -CATENIN FUNCTION IN THE REGULATION OF GERMLINE STEM CELL DIFFERENTIATION IN *DROSOPHILA*

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Background: microRNAs (miRNAs) are regulators of global gene expression, and function in a broad range of biological processes. Recent studies have suggested that miRNAs can function as tumor-suppressors or oncogenes by modulating the activity of evolutionarily conserved signaling pathways that are commonly dysregulated in human cancer, and are linked to cancer stem cells.

Key Findings: We report the identification of miR-310/13 cluster as a novel antagonist of *Wnt/wingless (wg)* pathway activity in a comprehensive functional screen for *Drosophila* microRNAs. We demonstrate that the miR-310/13 cluster can directly target the 3'-UTRs of β -catenin (β -cat)/Armadillo (*arm*) and dTCF. Overexpression of the miR-310/13 cluster *in vivo* generates phenotypes that mimic *wg/arm* loss-of-function. Moreover, misexpression of the miR-310/13 within clones of cells exhibiting ectopic *wg/arm* signaling, can rescue the *arm* gain-of-function phenotypes. miR-310/13 deficient flies exhibit abnormal stem cell differentiation in the *Drosophila* male germline, which can be rescued by reducing Arm protein levels or activity. Surprisingly, reducing E-cadherin levels in miR-deleted testes also results in a significant rescue of the abnormal differentiation phenotype. Our results implicate a function for miR-310/13 in dampening the activity of Wg/Arm in *Drosophila* gonad stem, or early progenitor cells, whereby inappropriate activation of Arm-mediated signaling as well as cell-cell adhesion may impact the normal progression of male germ cell differentiation. In the future, we plan to further investigate the regulation/function of the distinct pools of β -catenin in the nucleus and at cell-cell junctions in stem cell homeostasis, both in the *Drosophila* model and in murine ESCs.

Shared Resource Description: The miRNA screens were performed at the NYU-RNAi screening facility at the NYU School of Medicine.

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(35) IMPLANTABLE ARTERIAL VASCULAR GRAFTS BASED ON HAIR FOLLICLE DERIVED MESENCHYMAL STEM CELLS AND SMALL INTESTINAL SUBMUCOSA

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Due to rise in cardiovascular disease throughout the world, there is increasing demand for development of engineered tissues that can be used as replacement grafts. Our laboratory has shown that hair follicle is a source of multipotent mesenchymal stem cells (HF-MSC) that can differentiate into adipocytes, chondrocytes, osteoblasts and smooth muscle cells (SMC). Using a tissue-specific promoter and flow cytometry sorting, SMC were successfully isolated from HF-MSC. HF-SMC expressed early and late myogenic differentiation markers, exhibited vascular contractility and demonstrated high proliferation and clonogenic potential, suggesting that the HF might be an easily accessible cell source for vascular engineering. To this end, we generated tissue engineered vessels (TEVs) using HF-SMC and a strong, natural biomaterial namely small intestine submucosa (SIS). Pulsatile pressure induced circumferential alignment of HF-SMC and generated tissues that exhibited vascular reactivity through receptor and non-receptor mediated pathways as well as secretion of collagen and elastin, the two major extracellular matrix components of the vessel wall. These constructs exhibited similar elasticity as native arteries and burst pressure of 1000mmHg, suggesting that they could be implanted into the arterial circulation. To this end, first we developed an *ex vivo* arteriovenous animal model to evaluate the thrombogenicity and mechanical properties of vascular grafts and compared them to native vessels. We found that endothelialization inhibited platelet adhesion significantly (n=8), while SIS alone showed significant platelet aggregation. Using the optimal parameters to engineer the vascular wall and lumen, we proceeded to implant TEVs into the left carotid artery of an ovine animal model. Doppler ultrasound and angiography showed that TEVs exhibited long-term patency (n=8) for the duration of the experiment (12 weeks). Explanted grafts showed infiltration of host cells, robust ECM remodeling and vascular reactivity similar to that of ovine arteries, demonstrating our strategy was successful in engineering implantable arterial substitutes.

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(36) GENOME WIDE-ANALYSIS OF HUMAN OLIGODENDROCYTE LINEAGE TRANSCRIPTIONAL NETWORKS REVEALS CONSERVED TARGETS FOR MYELIN REPAIR

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The directed induction of human oligodendrocyte fate has been impeded by a lack of understanding of the molecular similarities and differences between rodent and human. In this study, we sought to characterize the expression profiles of human oligodendrocyte progenitor cells (OPCs) during their specification and differentiation to oligodendrocytes. We first developed and characterized a novel prospective method for isolation of three stages of progenitor differentiation from fetal human forebrain using two-color FACS with PDGF α R/CD140a and O4 antigens. *In vitro* characterization revealed that CD140a⁺O4⁺ cells possessed an intrinsic bias to differentiate as oligodendrocytes, while CD140a⁺O4⁻ cells developed as either oligodendrocytes or astrocytes depending on their environment. We used Illumina whole genome microarray to analyze gene expression and compared the human profiles to mouse cell isolates (Cahoy *et al.*, *J. Neurosci.* 28:264-278, 2008). We identified the cholinergic receptor muscarinic type 3 (CHRM3) among the most significantly expressed receptors and found that activation of CHRM3 blocks oligodendrocyte commitment by human OPCs. In addition, we used magnetic cell sorting to isolate CD140a⁺ progenitors and analyze their gene expression profiles during oligodendrocyte commitment *in vitro*. To define transcriptional changes associated with oligodendrocyte differentiation, these profiles were analyzed

along with matched PDGF-treated cells, a known mitogen that delays oligodendrocyte development. The human data were then compared to a similar time-course data of rat oligodendrocyte differentiation (Dugas *et al.*, *J. Neurosci.* 26:10967-10983, 2006). In addition to gene-wise comparison, we used weighted gene co-expression network analysis to determine systems-level gene expression across species. These analyses revealed that, while a substantial number of transcripts were conserved between human and rodent species, there were significant differences between the two species. By identifying the specific pathways conserved between species, these data provide a valuable resource for the design of therapeutically relevant strategies for the stimulation of endogenous repair in demyelinating disease.

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(37) HUMAN ESC-DERIVED HEMATOPOIETIC PROGENITORS EMERGE FROM HEMOGENIC ENDOTHELIUM AND UNDERGO LINEAGE PROGRESSION WITHIN THE VASCULAR NICHE

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Hematopoietic cells have been shown to arise from a specialized endothelial subpopulation with hemogenic potential. In animal models, the transition from endothelial to hematopoietic identity has been directly observed both *in vitro* and *in vivo* using live and explanted embryonic tissues. However, the phenotypic progression of early human hematopoietic progenitor cells (HPC) during their expansion and lineage commitment remains undefined. To address this issue, we genetically modified human embryonic stem cells (hESC) to specifically express distinct fluorescent reporters in early endothelial (VE-cadherin) or hematopoietic (CD41a) derivatives. Using this dual reporting hESC line, we directly observed the transition of single embryonic endothelial cells (EC) to HPCs in a serum-free vascular feeder-based platform. These conditions stimulated robust expansion of hematopoietic derivatives, and fostered differentiation of distinct erythroid, megakaryocytic and myeloid signatures among HPC derivatives, defined by combinatorial expression of specific surface markers. Live confocal imaging and clonal analyses identified the developmental hierarchy among unique phenotypic intermediates of hESC-derived HPCs and revealed a temporal bias in lineage specific differentiation that resembles the discrete waves of lineage potential noted during mammalian hemogenesis. Specifically, HPCs isolated at later time points showed reduced capacity to form erythroid/megakaryocytic cells and instead exhibited a tendency toward myeloid fate. The increased differentiation to myeloid derivatives was specifically enabled by co-culture with hESC-derived vascular feeders. Together, these data clearly demonstrate endothelial origins of hESC-derived HPCs, provide a developmental framework for HPC lineage potential, indicate a bias in fate choice among hESC-derived HPCs isolated at different time points during differentiation and elucidate a molecular contribution from the vascular niche in promoting lineage progression.

[Supported by NYSTEM contract C026438]

(38) IDENTIFYING MICRORNAS IN SKIN STEM CELLS

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The correct regulation of processes that govern stem cell (SC) maintenance and differentiation is crucial to normal organ development and tissue homeostasis. In recent years, studies in mouse and human skin epidermis (which has high regenerative/proliferative potential), have identified the

“bulge” compartment of the hair follicle as a niche where follicular SCs reside. The regulation of expression of genes in the epidermal “bulge”-SCs that modulate their ability to self-renew or differentiate is actively being investigated. microRNAs (miRs) as global regulators of gene expression make them ideally suited to be involved in modulating the activity/expression of a network of genes involved in stem cell maintenance and differentiation. We are investigating which miRs are expressed or are specifically absent in the “bulge” SCs and what their function might be in the regulation of SC behavior. Eight week CD-1 mice were sacrificed and cells were stained with CD34-APC (for “stemness”) and CD-49PE (for $\alpha 6$ integrin, a marker of proliferating epidermal keratinocytes). SC (CD34hi, CD49hi) and NS cells (CD34low, CD49hi) were identified by flow cytometry and total RNA was isolated. miR levels were examined using TaqMan Low Density arrays. miR levels were also examined by ‘RNASeq’ to ascertain directly how many copies of each miR are present followed by microarray analysis. miRs judged to be of interest based on target sequences to which they bind (miRBase) and present in high enough copy number to be detected by *in situ* hybridization were then examined further by *in situ*. We intend to undertake overexpression and knockdown studies in appropriate cell types in culture to further characterize the role of these miRs in stem cells.

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(39) ASTROCYTES BASED APPROACHES TO SPINAL CORD INJURY THERAPY

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Every year about 300,000 patients in the U.S. suffer traumatic injury of the brain or spinal cord requiring hospitalization, of which 250,000 survive. Due to the limited regenerative capability of adult central nervous system (CNS), these patients are facing the debilitating consequence of their injuries. Taking advantage of the neuroregeneration promoting properties of glial cells, we have developed a cell therapy for SCI using a specific type of astrocyte. Previously we have shown that transplantation of BMP-induced astrocytes (GDAs^{BMP}) derived from either rat or human embryonic glial-restricted precursor (GRPs) cells promote extensive neuronal survival, axonal regeneration, and functional recovery in a rat spinal cord lesion model. With the recent advances in human embryonic stem cell (hESC) technology, we hypothesize that hESCs can be used as a source for generating astrocyte populations with therapeutic potential. Using step-wise differentiation through an intermediate neural progenitor stage, we have derived distinct types of human astrocytes from hESCs. Our data indicate that these hESC-derived astrocytes recapitulate characteristics of rodent precursor-derived astrocytes with respect to cell morphology and expression of glial specific genes. We have also developed a high-throughput *in vitro* neuronal survival and axonal outgrowth assay using the Cytellect Celigo adherent cell cytometer. Our results show that astrocytes derived from hESC by BMP induction selectively support the survival and axonal growth of embryonic cortical and dorsal root ganglion neurons. These results set the stage for testing the therapeutic potential of hESC-derived astrocytes for spinal cord injury regeneration *in vivo*.

[Supported by NYSTEM contract C026877]

(40) CHARACTERISTICS OF THE TRANSIENT OUTWARD K⁺ CURRENT AND ITS CONTRIBUTION TO THE ACTION POTENTIAL IN hiPSC-DERIVED CARDIOMYOCYTES

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Background: Human induced pluripotent stem cell derived-cardiomyocytes (hiPSC-CMs) have the potential to serve as *in vitro* models of human disease, thus contributing to our understanding of the pathophysiology and approach to therapy of ion channelopathies associated with sudden cardiac death, such as Early Repolarization Syndrome (ERS). Central to the development of ERS is the

transient outward K⁺ current (I_{to}). In this study we identify and characterize I_{to} in single hiPSC-CMs and determine its functional role in beating clusters (BCs).

Method: BCs were made from a hiPS-cell line reprogrammed with Oct4, Nanog, Lin28 and Sox2 using a serum-free differentiation protocol supplemented with growth factors for selective cardiac differentiation. Sharp microelectrodes were used to record action potentials (AP) from spontaneously-contracting BCs and whole-cell-patch-clamp techniques were used to record I_{to} in single hiPSC-CMs.

Results: BCs beat at a rate of 54.9±30.1-bpm and displayed maximum diastolic potential (MDP) of -65.6±9.3mV (n=122). A small phase-1-repolarization (3±1mV), was observed in 3 BCs (MDP -91±1mV, take-off potential: -75±3mV, V_{max}=169±7 V/s, BCL 2650±750ms), which was suppressed by the I_{to} blocker 4-aminopyridine (4-AP, 1mM). Interestingly, in single dissociated hiPSC-CMs, patch clamp analysis revealed a robust I_{to} (13.7±6.6pA/pF;+40mV, n=14) in the majority of cells studied. I_{to} inactivated with a time constant of 17.5±1.6 ms, half-inactivation voltage was -41mV and reactivation was very slow (τ=1972±116ms at -80mV). Mathematical modeling of APs from hiPSC-CMs confirmed that I_{to}, although present, does not meaningfully contribute to the action potential because of a depolarized MDP due to a deficient or absent I_{K1} and slow recovery of I_{to} from inactivation.

Conclusion: Our results point to an important deficiency of hiPSC-CMs in recapitulating the phenotype of adult native myocytes. Our ability to create human models of disease using hiPS-CMs for ERS or other channelopathies will depend on our ability to better direct differentiation or address these deficiencies by other means.

[Supported by NYSTEM contract C026424]

(41) THE MOLECULAR MECHANISM OF JARID2 AND AEBP2 ON PRC2 REGULATION

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Polycomb group (PcG) proteins are evolutionarily conserved transcriptional regulators that function via putative epigenetic processes. These proteins exhibit a critical role in major developmental pathways, stem cell differentiation and cancer. Polycomb Repressive Complex 2 (PRC2) comprises Enhancer of Zeste homolog (EZH), Suppressor of Zeste 12 (SUZ12), Embryonic Ectoderm Development (EED) and RbAp46/48. PRC2 catalyzes methylation of histone H3 at lysine 27 (H3K27me3) through its EZH constituent. There are two mammalian EZH, EZH1 that is expressed both in dividing and resting cells and EZH2 only in dividing cells. Several ancillary factors, including AEBP2 and JARID2, are associated with PRC2. Jarid2 is important for PRC2 recruitment to target genes. Jarid2, like Ezh2, is present only in actively dividing cells whereas AEBP2 is expressed in all tissues. We found that Jarid2 is required for PRC2/Ezh2 binding to nucleosomes, yet PRC2/Ezh1 binds to nucleosomes in its absence. Jarid2, AEBP2 and the product of PRC2 catalysis, H3K27me3, stimulate PRC2 enzymatic activity by different mechanisms. Our biochemical and *in vivo* studies allow us to propose a model whereby PRC2/Ezh2 is important for establishing *de-novo* H3K27me3 in dividing cells whereas PRC2/Ezh1 is required for the maintenance of this modification in resting cells.

(42) SMALL MOLECULE-MEDIATED INDUCTION OF GLOBIN SWITCHING IN DIFFERENTIATING HUMAN EMBRYONIC STEM CELLS

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During mammalian development, nascent erythrocytes undergo a series of reprogramming steps in the genes encoding their α - and β -globin chains. In humans, globin switching proceeds from ϵ -globin in the yolk sac, to γ -globin in the fetal liver, to β -globin in the bone marrow. Defects in the production of adult globin lead to hemoglobinopathies such as sickle cell disease and β -thalassemia. The debilitating consequences of these diseases, combined with the current lack of adequate treatments, call for the identification of alternative therapeutic approaches. We propose that by using human embryonic stem cells engineered to express identifiable reporters for fetal and adult globin expression, we can build a platform to identify potential inducers of globin switching within the context of the native human cellular environment in a large, yet highly sensitive screen.

Here we describe important steps toward this end. Culture conditions have been established that allow for maintenance of human embryonic stem cells in an undifferentiated, pluripotent state for greater than 25 passages. A mesodermal differentiation regimen has been optimized for the erythroid lineage, with over 30% of cells exhibiting expression of the appropriate cellular markers for erythropoiesis. As well, we have shown that these cells are positive for endogenous γ -globin expression. We have also achieved insertion of exogenous DNA into these cells at a transient efficiency of over 50%.

Currently, we are pursuing methods of YAC transgenesis and immunostaining in these human embryonic stem cells, with the ultimate goal of creating a reportable system in which to test small molecules for potential inducers of globin switching. By this means we hope to overcome the historical disadvantage of other model systems, such as erythroleukemic cell lines and transgenic mice, as they do not fully mimic the endogenous globin switching paradigm seen during normal human red cell development. Induction of β -globin in human cells in culture is a major impediment for the creation of transfusable blood *ex vivo*. Having a pool of β -globin expressing cells would also provide a directly relevant system for γ -globin inducer discovery. It is for these reasons that the identification of small molecules which can push cultured human cells to definitive erythropoiesis is of such critical importance.

[Supported by NYSTEM contract C026435]

(43) RAPIDLY CYCLING NAIL STEM CELLS GOVERN CONTINUOUS NAIL FORMATION DEPENDENT ON WNT SIGNALING

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The nail plate grows continuously throughout life in mice and humans by cells within the nail matrix, which have been largely uncharacterized until now. Current theory supposes the presence of NSCs in the most distal end of the nail matrix based upon their slow cycling nature, a classic characteristic of adult stem cells. Whether the slow cycling cells truly represent self-renewing stem cells responsible for continuous nail formation is undermined. Using genetic lineage tracing, we identify self-renewing nail stem cells (NSCs) within the proximal region of the nail matrix. NSCs are rapidly proliferating *in vivo* and exhibit high proliferative behavior *in vitro*. Microarray analyses revealed that Wnt signaling is suppressed in NSCs compared to the other matrix cells. Removal of β -catenin, a mediator of Wnt signaling, in NSCs did not preclude their maintenance and proliferation, but blocked their differentiation. Unexpectedly, this led to the regression of not only the nail but also the

underlying bone tissue, demonstrating that the nail epithelium regulates the maintenance of the underlying bone. These findings offer the first comprehensive model explaining nail growth and suggest that NSCs can be a novel therapeutic target for disorders of nail and associated digit bone.

(44) ACQUISITION OF DEVELOPMENTAL COMPETENCE THROUGH INTEGRATION OF SIGNAL TRANSDUCTION PATHWAYS AND EPIGENETIC REGULATION

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Pluripotent ES cells have a complex gene regulatory network that culminates in the remarkable ability to differentiate into distinct lineages, under the instructive influence of developmental signals. This necessitates a robust transcriptional and epigenetic program, the control of which governs the fine-balance between self-renewal and responsiveness to developmental programs. In particular, the extracellular signal-regulated kinase (Erk) signaling pathway plays a requisite role in triggering lineage commitment in mouse ES cells, the abrogation of which results in a blockage of differentiation. We propose to conduct a systematic investigation of how this critical signaling pathway orchestrates cell fate decision, through specific modulation of the transcriptional and epigenetic machineries. We provide preliminary evidence showing that recruitment of the Polycomb machinery itself is coupled to activation of Erk signaling. Furthermore, we show that Erk signaling regulates the establishment of H3K27me3-H3K4me3 bivalent chromatin domains, and alters the RNA Polymerase II (RNAPII) machinery, on developmental gene promoters. In summary, our data provide evidence for a deterministic role of Erk signaling in the establishment of transcriptionally poised developmental gene promoters, and current efforts are directed toward attaining a comprehensive assessment of the effects of Erk signaling on the global ES cell chromatin structure.

(45) DERIVATION OF XENOFREE HUMAN EMBRYONIC STEM CELL LINES FROM MINORITY POPULATIONS

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Pluripotent stem cells offer unrivaled potential in human therapies for disease, developmental defects or debilitating injuries. Human embryonic stem cells (hESCs) remain the most reliable source of these cells and a gold standard for pluripotency. The current hESC lines however, represent very limited ethnic diversity, particularly among minorities, and are not a representative balance of the current USA's racial distribution of the human population. Our goal is to make available to researchers a set of hESC lines derived under identical conditions, characterized using the same state-of-the-art platform and at the same passage. This will also allow us to determine what hESC characteristics and molecular features differ amongst ethnic backgrounds and will serve as an invaluable and unique resource for ethnic specific diseases. By the completion of this work we will derive hESC lines under defined conditions from up to six different ethnic minorities, provide detailed molecular genomic characterization of these hESC lines along with statistical analysis and a database for accessing this information. Our use of animal-free culture conditions will allow the ability to grandfather-in any given hESC line that we derive into GMP conditions for clinical trials and therapeutic applications. We are mindful of all minorities and best efforts are being made to have these minorities represented in the final array of hESC lines we derive. We currently have access to greater than 290 embryos for derivations from excess *in vitro* fertilization (IVF) procedures and are following consenting guidelines by NIH to allow their expected approval for addition into the NIH Stem Cell Registry of approved hESC lines for use in research.

[Supported by NYSTEM contact C026186]

(46) SINGLE TRANSCRIPTION FACTOR REPROGRAMMING OF HAIR FOLLICLE-DERIVED DERMAL PAPILLA CELLS TO INDUCED PLURIPOTENT STEM CELLS

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Reprogramming patient-specific somatic cells into induced pluripotent stem (iPS) cells has great potential to develop feasible regenerative therapies. Several issues need to be resolved, however, such as ease, efficiency and safety of iPS generation. Many different cell types from more or less accessible tissues have been reprogrammed, most conveniently even from peripheral blood mononuclear cells. However, they typically require the enforced expression of three or four transcription factors, posing significant security risks as exogenous genetic material, even if expressed transiently. To reduce this risk, iPS cells were successfully generated with fewer factors from cells that endogenously express already some of the transcription factors, most notably neural stem cells were reprogrammed with only Oct4. However, the inaccessibility of neural stem cells from brain and the difficulty and risk of obtaining such cells from patients make them an impractical source. Here we combine accessibility, efficiency and safer iPS generation and show that accessible dermal papilla (DP) cells from hair follicles in the skin can be reprogrammed with the single transcription factor Oct4. Reprogramming was achieved already after three to five weeks and with efficiencies similar to other cell types reprogrammed with all four factors. DP-derived iPS cells are comparable to ES cells with respect to morphology, gene expression pattern and pluripotent differentiation capacity *in vitro* and *in vivo*. Importantly, reprogramming accessible DP cells by ultimately replacing the remaining factor with small molecules in the future may enable safe generation of transplantable cells.

[Supported by NYSTEM contract C026410]

(47) DEVELOPMENT AND CHARACTERIZATION OF BIOMIMETIC CARDIAC TISSUE ENGINEERED FROM HUMAN EMBRYONIC STEM CELLS

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Traditional 2D cultures and animal models often inadequately recapitulate the human phenotype. This study aimed to demonstrate engineering of functional heart tissue using human embryonic stem cell derived cardiomyocytes (hESC-CM) and assess their structural, molecular, mechanical, pharmacological and electrical properties. hESC-CM (>85% α -sarcomeric actin positive) were mixed with type I collagen and Matrigel (1:8:1 ratio) and cultured in custom elastomer molds with flexible end posts, yielding spontaneously beating (~70bpm) thin cylindrical human engineered cardiac tissues (hECT). hECT were maintained in culture and remained viable for at least 28 days. Electrical pacing yielded 1:1 capture, and lost capture beyond 3.3Hz, demonstrating underlying cardiac refractoriness. Action potential duration (~245ms) measured by optical mapping, shortened at high pacing rates confirming cycle length dependence. Developed (systolic - diastolic) twitch force increased with stretch, indicating a Frank-Starling mechanism, and resulting in an average stress (force/area) of 580 $\mu\text{N}/\text{mm}^2$ at 95% L_{max} using a physiologic muscle bath system. Trends in developed force reflected the known drug effects on humans with positive inotropic response to CaCl_2 and negative to verapamil; dose response curves followed exponential pharmacological models with physiologic half maximum concentrations of 1.6 mM and 0.52 μM , respectively. Histology revealed axially aligned cells, expressing α -actinin, troponin T and connexin 43, with ultrastructural confirmation of organized sarcomeres, Z-discs with intervening myofibrils and cardiac

plasma membrane components including cell junctions and caveolae. Expression levels of cardiac genes α MHC, SERCA2a, and ACTC1 were similar (0.5x-2x) to mRNA from adult human left ventricular myocardium, and showed increasing maturation trends with time in culture. We demonstrated the creation of hECT that exhibit characteristics mimicking natural human myocardium. These hECT provide a biomimetic *in vitro* environment and may become a valuable preclinical tool to enhance the translation of novel therapies for cardiac disease.

(48) CEREBROSPINAL FLUID REGULATION OF ADULT NEURAL STEM CELLS

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The adult subventricular zone (SVZ) is a neurogenic niche adjacent to the lateral ventricles (LV) that contains a population of stem cells responsible for the generation of olfactory interneurons. SVZ stem cells reside in a specialized niche, which supports adult neural stem cell self-renewal and differentiation throughout life. An underappreciated component of the SVZ niche is the cerebrospinal fluid (CSF), a product of the choroid plexus (CP) that constantly flows through the ventricles. Ependymal cells line the length of the LV. SVZ stem cells contact the CSF via a process that intercalates between ependymal cells. As such, the CSF may be an important source of factors that regulate SVZ stem cells. We are characterizing the functional and molecular contribution of the CP and the CSF to the adult SVZ stem cell niche. Strikingly, FACS purified SVZ stem cells and transit amplifying cells form neurospheres when cultured with CSF or CP conditioned medium in the absence any exogenous growth factors. Moreover, novel signaling pathways are involved, as neurospheres are still formed when canonical signaling are blocked using pharmacological inhibitors. We are currently screening to identify candidate factors using proteomic profiling of the CSF and CP secretome as well as microarray analysis of the LV CP transcriptome. Our results provide the first evidence that adult CSF and LV CP modulate adult SVZ stem cell fate by supporting and modulating proliferation, self-renewal and differentiation of SVZ stem cells and transit amplifying cells.

[Supported by NYSTEM contract C026401]

(49) STEM CELL-BASED APPROACHES TO THE NEUROBIOLOGY OF OBESITY IN BARDET-BIEDL SYNDROME

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Bardet-Biedl Syndrome (BBS) is an autosomal recessive genetic disease, which is characterized by polydactyly, obesity, retina degeneration, renal cysts and mental retardation. BBS is caused by mutations in a group of proteins comprising the basal body of the primary cilium. *Bbs* null mice display impaired leptin receptor signaling and reduced number of hypothalamic POMC neurons. Leptin and insulin signaling are two key hypothalamic pathways involved in regulating energy homeostasis. To investigate the neurobiology of obesity in BBS, we reprogrammed skin fibroblasts from BBS10 and BBS1 patients into induced pluripotent stem cells (iPSCs) and further differentiated them into neurons. We first demonstrated that the ciliogenesis was not affected in BBS1 mutant iPSC-derived neurons while BBS10 mutant neurons displayed longer cilia. Furthermore, the insulin-induced AKT activation was greatly reduced in both BBS1 and BBS10 mutant neurons compared to control neurons. Leptin signaling was investigated in BBS mutant fibroblasts expressing exogenous LEPR, and we found that both BBS mutations impaired leptin mediated STAT3 activation. These data suggest that mutation of BBS proteins can affect both leptin and insulin signaling, which may contribute to obesity in BBS patients.

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(50) INVESTIGATING THE EMERGENCE AND DYNAMICS OF THE PLURIPOTENT STATE

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Pluripotent stem cell-based therapies hold enormous promise for patients with degenerative and other diseases. However, a deeper understanding of the mechanisms driving pluripotent cells to self-renew or differentiate into specific lineages is needed in order to use these cells for clinical applications in a safe and effective manner. To that end, it was recently demonstrated that pluripotent stem cell populations of the same genetic background exhibit heterogeneous expression of pluripotency-associated genes. Remarkably, these heterogeneities have been shown to correlate with dynamic fluctuations in the expression of certain pluripotency-associated factors, suggesting the existence of inter-convertible substrates within pluripotent stem cell compartments. It is believed that such heterogeneity and fluctuating substrates may represent a hallmark of all pluripotent cell types, reflecting a propensity for the decision to self-renew or differentiate. Interestingly, these heterogeneities have not been observed yet in the early mammalian embryo, from where pluripotent cells are derived.

An essential tool for investigating the relationship between heterogeneous gene expression and cell fate decisions is live cell imaging. By using BAC recombineering methods, we have developed a series of novel, universal, high-resolution live imaging reporters of the pluripotent state. We show that these pluripotency-associated imaging reporters are nuclear localized in cultures of mouse embryonic stem cells and provide excellent single-cell resolution and tracking of cells over time. We will use these imaging tools in order to probe heterogeneities and characterize fluctuating substrates in cultures of pluripotent stem cells *in vitro*. Moreover, these reporters will allow us to visualize the pluripotent stem cell lineage in the early embryo and examine whether heterogeneities exist *in vivo*. These studies should help elucidate how stem cells might commit to a specific lineage or decide to remain in a pluripotent state in cultures *in vitro* as well as within the developing embryo.

(51) CONTROL OF PRESYNAPTIC DIFFERENTIATION BY MUSCLE-DERIVED RETROGRADE SIGNALS

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The neuromuscular junction (NMJ) is the chemical synapse between motor neurons and muscle fibers. After motor axons reach their targets, Agrin is secreted from motor axon terminals and triggers phosphorylation of muscle specific kinase (MuSK) on muscle fibers, which results in acetylcholine receptor (AChR) clustering. We previously reported that LDL receptor related protein 4 (Lrp4) is a receptor for Agrin and mediates MuSK activation by forming a complex with MuSK. Upon arrival at targets, motor axons receive retrograde signals from muscle fibers that are essential for differentiation and stabilization of motor nerve terminals. Identification of these retrograde signals has proved elusive, but their production by muscle depends upon MuSK and Lrp4. To determine whether Lrp4 could also act as a retrograde signal, primary motor neurons co-cultured with non-muscle cells expressing Lrp4 or microspheres coated with extracellular region of Lrp4. We found that synaptic vesicles accumulated at the site of contact and they were released and recycled upon stimulation with high potassium. These data indicate that Lrp4 functions as a direct retrograde signal and induces functional presynapses in motor neurons *in vitro*. Lrp4 binds to motor axons and its synaptogenic activity is specific of Lrp4. In addition, Lrp4 mutant mice carrying musk overexpression transgenes restored postsynaptic differentiation but failed to restore presynaptic differentiation, suggesting Lrp4 is a long sought molecule that is essential for motor nerve terminal differentiation. We are now attempting to identify Lrp4-binding molecules in motor neurons by expression cloning with cDNA library of mouse ES-derived motor neurons, which also express Lrp4 receptors.

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(52) Nfix REGULATES NEUROGENESIS IN POSTNATAL MOUSE BRAIN

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Postnatal neurogenesis is important in learning, memory and recovery from brain injury. A number of transcription factors, including Nuclear Factor I X (NFI-X) have been implicated in postnatal neurogenesis. Previously we showed that the cerebral cortex of postnatal *Nfix*^{-/-} mice are significantly larger than those of wild-type (WT) mice. The hippocampus in *Nfix*^{-/-} mice are distorted compared to WT during postnatal development. In addition, excess cells are observed in postnatal *Nfix*^{-/-} subventricular zone (SVZ), a main niche for neural stem cells (NSCs). Many of those cells express Pax6 and DCX, markers for neural stem and progenitor cells (NSPCs) and migrating neuroblast and immature neurons, respectively. This suggests that *Nfix* may be involved in the regulation of neurogenesis during postnatal brain development.

We have determined that NFI-X is expressed in some putative NSCs and most transient amplifying cells and neuroblasts in the dentate gyrus (DG) of the hippocampus. By neurosphere assay, we have compared the proliferation and differentiation abilities of putative NSCs from WT and *Nfix*^{-/-} brains *in vitro*. In addition, to study the direct effect of *Nfix* on NSCs during postnatal development *in vivo*, we have generated Nestin-CreERT2 *Nfix* conditional knockout (Cond KO) mice. By comparing subpopulations of cells at different stages of neurogenesis around the SVZ and DG of WT and Cond KO brains, we assessed the proliferation and differentiation abilities of *Nfix*^{+/+} and *Nfix*^{-/-} NSPCs *in vivo*. Understanding the mechanism by which *Nfix* regulates neurogenesis may allow the development of improved therapies for neuro-degenerative diseases, ischemic stroke and brain injury. In addition, as *Nfix* has been strongly implicated as a putative oncogene or tumor suppressor gene in a mouse model of glioblastoma, studying the role of *Nfix* in neurogenesis may yield insights into the generation and proliferation of glioblastoma cells.

[Supported by NYSTEM contract C026429]

(53) KRAB-MEDIATED MAINTENANCE OF GENOMIC IMPRINTING IN ES CELLS

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ZFP57 is a maternal-zygotic effect gene and it maintains DNA methylation imprint at a large subset of imprinted regions in mouse embryos including the *Dlk1-Dio3* imprinted region. In this study, we demonstrate that it plays a similar role in embryonic stem (ES) cells. First, we constructed *Zfp57*-null ES cells through homologous recombination. As expected, these ES cells lose DNA methylation imprint at multiple imprinted regions. Multiple ES cell lines were also directly derived from blastocysts and the ES clones lacking ZFP57 exhibit loss of genomic imprinting at similar imprinted regions. To test if DNA methylation imprint could be reacquired in *Zfp57*-null ES cells after it had been lost, exogenous wild-type ZFP57 was re-introduced into these ES cells via homologous recombination. Interestingly, DNA methylation imprint remains absent in *Zfp57*-null ES cells even when the exogenous ZFP57 is expressed in these cells. These results suggest that the imprinting memory is missing in *Zfp57*-null ES cells that are generated via homologous recombination. We also re-activate the endogenous ZFP57 in the ES clones lacking ZFP57 that are directly derived from blastocysts. The results are similar and DNA methylation imprint cannot be re-established once it is lost in ES cells.

ZFP57 is a KRAB zinc finger protein. It binds to the imprinting control regions. We also find that KAP1/TIF1beta/Trim28 facilitates the interaction between ZFP57 and DNA methyltransferases. These results indicate that ZFP57 may maintain genomic imprinting in ES cells via KAP1/TIF1beta/Trim28. KAP1/TIF1beta/Trim28 is an essential gene and null ES cells for KAP1/TIF1beta/Trim28 could not be generated for analyzing DNA methylation imprint. Thus, an ES

cell system was established to examine whether exogenous ZFP57 can substitute for the endogenous ZFP57 in the maintenance of genomic imprinting. While expression of exogenous wild-type ZFP57 can substitute for endogenous ZFP57 in maintaining genomic imprinting in ES cells, expression of the mutant ZFP57 lacking the KRAB box, i.e. its interaction domain with its cofactor KAP1/TIF1beta/Trim28, does not prevent the loss of genomic DNA methylation imprint after the depletion of the endogenous ZFP57 in ES cells. This implies that the interaction between ZFP57 and KAP1/TIF1beta/Trim28 is required for the maintenance of DNA methylation imprint in ES cells.

It has been reported that DNA methylation imprint at the *Dlk1-Dio3* imprinted region is often improperly reprogrammed in induced pluripotent stem (iPS) cells. Those iPS cell lines with defective imprinting at this imprinted domain also have abnormal developmental potential. We are currently testing whether ZFP57 will play a similar role in iPS cells.

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PROGRAM-AT-A-GLANCE

MAY 23 – Concourse Lobby and Breakout Rooms, Proshansky Auditorium

- 12:00 PM Registration and Poster Set-up
- 1:00 PM Opening Remarks
- 2:00 PM Break and Posters
- 2:30 PM Plenary I: Stem Cell Biology
- 4:20 PM Poster Session I and Reception

MAY 24 – Concourse Lobby, Proshansky Auditorium

- 8:00 AM Registration, Breakfast and Poster Viewing
- 9:00 AM Plenary II: Neural Stem Cells and the Stem Cell Niche
- 11:10 AM Poster Session II
- 12:00 PM Lunch and Poster Session II
- 1:10 PM Plenary III: Cardiovascular and Hematopoietic Systems
- 2:50 PM Break and Posters
- 3:20 PM Plenary IV: Disease Modeling and Therapeutic Approach
- 4:50 PM Closing Remarks

