

NYSTEM 2010

Building the New York Stem Cell Community

MAY 26 & 27, 2010

CUNY Graduate Center
365 Fifth Avenue
New York, New York



NYSTEM NEW YORK STATE
STEM CELL SCIENCE

Adult neural stem cells. *Image source:* Qin Shen and Yue Wang,
New York Neural Stem Cell Institute, Regenerative Research Foundation

Building the New York Stem Cell Community
CUNY Graduate Center

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Gordana Vunjak-Novakovic, Ph.D., *Columbia University*

Scott Noggle, Ph.D., *New York Stem Cell Foundation (Facilities Workshop)*

Sally Temple, Ph.D., *New York Neural Stem Cell Institute (Facilities Workshop)*

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Tracy Godfrey

PROGRAM-AT-A-GLANCE

MAY 26 – Elebash Recital Hall

- 8:00 AM Registration Opens and Continental Breakfast – Recital Hall Lobby
- 9:00 AM Shared Equipment and Facilities Workshop – Part 1
- 10:15 AM Break
- 10:30 AM Shared Equipment and Facilities Workshop – Part 2
- 12:00 PM Lunch – Concourse Lobby (one floor down)
- 1:20 PM Opening Remarks
- 1:30 PM Session I: Neural Stem Cells
- 3:00 PM Break
- 3:30 PM Session II: Stem Cells and Cancer
- 5:00 PM Reception/Poster Session – Concourse Lobby (one floor down)

MAY 27 – Elebash Recital Hall

- 8:00 AM Registration Opens and Continental Breakfast – Recital Hall Lobby
- 9:00 AM Session III: Stem Cells in Diseases
- 10:30 AM Break
- 11:00 AM Session IV: Stem Cell Biology
- 12:30 PM Lunch – Ninth Floor Conference Room
- 1:30 PM Session V: Tissue Engineering and Technology
- 3:00 PM Break
- 3:30 PM Session VI: Pluripotency and Reprogramming
- 5:00 PM Adjourn

 **STATE OF NEW YORK**
DEPARTMENT OF HEALTH

Wadsworth Center The Governor Nelson A. Rockefeller Empire State Plaza P.O. Box 509 Albany, New York 12201-0509

Richard F. Daines, M.D.
Commissioner

James W. Clyne, Jr.
Executive Deputy Commissioner

Welcome from the Executive Director of NYSTEM

Welcome to NYSTEM 2010, “Building the New York Stem Cell Community,” our second annual scientific meeting. A key goal of the Empire State Stem Cell Board has been to connect members of the New York stem cell research community to catalyze and accelerate discoveries for the benefit of New York and of society. This conference is a centerpiece of that effort, and a landmark for NYSTEM because for the first time NYSTEM-funded individual investigators will be presenting their work. It is remarkable that in the few short years since its inception, findings of NYSTEM funded projects already have been widely published, and include landmark papers in the highest impact journals. This is a tribute to the breadth and quality of New York’s stem cell research community and biomedical institutions.

High quality science and energetic participants mark successful meetings. They provide participants with new insights that accelerate progress or suggest new directions, and contacts that lead to new collaborations. Achieving the goal of translating discoveries in stem cell science to new therapies will require multiple approaches and the combined efforts of basic, translational and clinical scientists. For this reason an important strategic objective for NYSTEM has been to bring participants from diverse scientific backgrounds together. Our outstanding Program Committee has worked hard to assemble an agenda filled with timely, quality science that reflects the diversity of NYSTEM-funded projects across the state. At last count, the presenters represent eighteen institutions, and cover topics ranging from fundamental stem cell biology to the roles of stem cells in understanding and treating neurological, cardiovascular, hematological and hepatic diseases, and cancer.

We invite you to enjoy the meeting, contribute to the discussion and share your ideas for future meetings. Thank you for your participation.

Sincerely,



Lawrence S. Sturman, M.D., Ph.D.
Executive Director of NYSTEM

Building the New York Stem Cell Community

PROGRAM SCHEDULE

Wednesday, May 26

- 8:00 AM Registration Opens and Continental Breakfast – Recital Hall Lobby
- 9:00 AM **SHARED EQUIPMENT AND FACILITIES WORKSHOP PART 1**
Chair: Gerold Feuer, SUNY Upstate Medical University, Syracuse, NY
- 9:00–9:15 AM **Christopher Fasano**, New York Neural Stem Cell Institute, Rensselaer, NY
Neuracell: A Centralized Neural Stem Cell Bank that will Maintain, Store and Supply Stem Cells to Other Researchers
- 9:15–9:30 AM **Julien Lajugie**, Albert Einstein College of Medicine, Bronx, NY
GenPlay, A New Analyzer and Browser for High-throughput Data
- 9:30–9:45 AM **Gerold Feuer**, SUNY Upstate Medical University, Syracuse, NY
Expansion of the Humanized SCID (HU-SCID) Mouse Center and Stem Cell Processing Laboratory at SUNY Upstate Medical University
- 9:45–10:00 AM **J. Chloë Bulinski**, Columbia University, New York, NY
Differentiation-specific Stem Cell Marker Proteins Detected by Proteomics of Adipose-derived Human Stem Cells in Hyperosmotic Conditions
- 10:00–10:15 AM **Panel Discussion**
Moderator: Sally Temple, New York Neural Stem Cell Institute, Rensselaer, NY
- 10:15 AM Break – Recital Hall Lobby
- 10:30 AM **SHARED EQUIPMENT AND FACILITIES WORKSHOP PART 2**
Chair: Scott Noggle, New York Stem Cell Foundation, New York, NY
- 10:30–10:45 AM **Mark Tomishima**, Sloan-Kettering Institute, New York, NY
The SKI Stem Cell Research Facility
- 10:45–11:00 AM **Eric Bouhassira**, Albert Einstein College of Medicine, Bronx, NY
Einstein Comprehensive Human Pluripotent Stem Cell Center
- 11:00–11:15 AM **Sunita D’Souza**, Mount Sinai School of Medicine, New York, NY
Diverse Stem Cell Projects Supported by the NYSTEM Shared Facility Grant at MSSM
- 11:15–11:30 AM **John Schimenti**, Cornell University, Ithaca, NY
The Cornell Mammalian Cell Reprogramming Core
- 11:30–11:45 AM **Scott Noggle**, New York Stem Cell Foundation, New York, NY
The NYSCF Shared Facility for Derivation, Distribution and Translational Research with Human Pluripotent Stem Cells
- 11:45–12:00 PM **Panel Discussion**
Moderator: Sally Temple, New York Neural Stem Cell Institute, Rensselaer, NY
- 12:00 PM Lunch – Concourse Lobby (one floor down)

- 1:20 PM Opening Remarks
- 1:30 PM **NEURAL STEM CELLS**
Chair: Steven Goldman, University of Rochester, Rochester, NY
- 1:30–2:00 PM **Andreas Kottmann**, Columbia University, New York, NY
SHH Expression by Mesencephalic Dopaminergic Neurons is a Sentinel for Neuronal Dysfunction and Influences Cell Fate Decisions in SVZ Neurogenesis in the Adult Brain
- 2:00–2:15 PM **René Hen**, New York State Psychiatric Institute and Columbia University, New York, NY
Hippocampal Stem Cells: Impact on Mood and Cognition
- 2:15–2:30 PM **Grigori Enikolopov**, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
Division-coupled Astrocytic Differentiation of Neural Stem Cells Determines Age-related Decline in Hippocampal Neurogenesis
- 2:30–2:45 PM **Edmund Au**, New York University Medical Center, New York, NY
Directing ES Cells to Cortical Interneuron Fates by Transcriptional Specification
- 2:45–3:00 PM **Stuart Chambers**, Sloan-Kettering Institute, New York, NY
A Highly Efficient Neural and Neuronal Induction of Human ES and iPS Cells through Combined Small Molecule Inhibition
- 3:00–3:15 PM **Sally Temple**, New York Neural Stem Cell Institute, Rensselaer, NY
Homing of Neural Stem Cells to the Vascular Niche is Regulated by Differential Responses to SDF1/CXCR4 Signaling
- 3:15 PM Break – Recital Hall Lobby
- 3:45 PM **STEM CELLS AND CANCER**
Chair: Julio Aguirre-Ghiso, Mount Sinai School of Medicine, New York, NY
- 3:45–4:00 PM **Paloma Bragado**, Mount Sinai School of Medicine, New York, NY
Plasticity Loss and Regain of CD49F and ALDHA1 Expression Directs Tumor-initiating Capacity in Head and Neck Squamous Carcinoma Cell Populations
- 4:00–4:15 PM **Sapna Vijayakumar**, Mount Sinai School of Medicine, New York, NY
Human Sarcomas of Multiple Lineages Exhibit Constitutive Canonical Wnt Autocrine Activation
- 4:15–4:30 PM **Tudorita Tumber**, Cornell University, Ithaca, NY
RUNX1 Directly Promotes Proliferation of Hair Follicle Stem Cells and Epithelial Tumor Formation in Mouse Skin
- 4:30–4:45 PM **Stephen Goff**, Columbia University, New York, NY
Embryonic Stem Cells Use ZFP809 to Bind the Proline tRNA Primer Binding Site and Silence Retroviral DNAs
- 5:00 PM Reception and Poster Session – Concourse Lobby (one floor down)

Thursday, May 27

- 8:00 AM Registration Opens and Continental Breakfast – Recital Hall Lobby
- 9:00 AM **STEM CELLS IN DISEASES**
Chair: Ira Cohen, Stony Brook University, Stony Brook, NY
- 9:00–9:30 AM **Ira Cohen**, Stony Brook University, Stony Brook, NY
Improving Cardiac Conduction with a Skeletal Muscle Sodium Channel by Gene and Cell Therapy
- 9:30–9:45 AM **Jonathan Lu**, Columbia University, New York, NY
KCNQ1/KCNE1 K⁺ Channels Associated with Long QT Syndrome are Expressed in Early Stage Human Embryonic Stem Cell-Derived Cardiomyocytes
- 9:45–10:00 AM **Yong Chen**, Albert Einstein College of Medicine, Bronx, NY
Hepatocytes Differentiated from Induced Human Pluripotent Cells Ameliorated Hyperbilirubinemia after Transplantation into the Liver of the Gunn Rat Model of Crigler-Najjar Syndrome-1
- 10:00–10:15 AM **Margaret Baron**, Mount Sinai School of Medicine, New York, NY
Red Blood Cell Development in Differentiating Embryonic Stem (ES) Cells and Mouse Embryos
- 10:15–10:30 AM **Ulrich Steidl**, Albert Einstein College of Medicine, Bronx, NY
Identifying Epigenomic Determinants of Hematopoietic Stem Cell Commitment
- 10:30 AM Break – Recital Hall Lobby
- 11:00 AM **STEM CELL BIOLOGY**
Chair: Ruth Lehmann, New York University School of Medicine, New York, NY
- 11:00–11:15 AM **Dirk Bohmann**, University of Rochester, Rochester, NY
Redox Regulation of Drosophila and Mammalian Stem Cells Mediated by NRF2 Transcription Factors
- 11:15–11:30 AM **Carl Schildkraut**, Albert Einstein College of Medicine, Bronx, NY
The Regulation of DNA Replication Initiation in Mouse and Human Embryonic Stem Cells
- 11:30–11:45 AM **Emily Bernstein**, Mount Sinai School of Medicine, New York, NY
Deciphering Polycomb-mediated Epigenetic Regulation during Embryonic Stem Cell Differentiation
- 11:45–12:00 PM **Elizabeth Lacy**, Sloan-Kettering Institute, New York, NY
Role of Nuclear Pore Composition in Embryonic and Adult Stem Cell Differentiation
- 12:00–12:15 PM **Jerome Artus**, Sloan-Kettering Institute, New York, NY
Directed Differentiation of Extraembryonic Endodermal Stem Cells
- 12:15–12:30 PM **Erika Bach**, New York University School of Medicine, New York, NY
CHINMO is a Functional Effector of the JAK/STAT Pathway that Regulates Eye Development, Tumor Formation and Stem Cell Self-renewal in Drosophila

- 12:30 PM Lunch – Ninth Floor Suite
- 1:30 PM **TISSUE ENGINEERING AND TECHNOLOGY**
Chair: Gordana Vunjak-Novakovic, Columbia University, New York, NY
- 1:30–2:00 PM **Stelios Andreadis**, University at Buffalo, State University of New York, Buffalo, NY
Hair Follicle Mesenchymal Stem Cells as a Source of Smooth Muscle Cells for Engineering Mechanically Robust and Vasoreactive Vascular Media
- 2:00–2:15 PM **Lye Lock**, University at Buffalo, State University of New York, Buffalo, NY
Expansion of Human Stem Cells in a Microcarrier Bioreactor and their Directed Differentiation toward Pancreatic Islet Cells
- 2:15–2:30 PM **Robert Linhardt**, Rensselaer Polytechnic Institute, Troy, NY
Proteoglycomics of Human Embryonic Stem Cells
- 2:30–2:45 PM **Xinping Zhang**, University of Rochester, Rochester, NY
Activation of HH Pathway Enhances Osteogenic and Chondrogenic Differentiation of Periosteum-derived Mesenchymal Stem Cells and is Involved in Postnatal Bone Repair
- 2:45–3:00 PM **Louis Terracio**, New York University, College of Dentistry, New York, NY
Isolation, Expansion in Culture and Characterization of Stem Cells from Skeletal Muscle (Satellite Cells)
- 3:00 PM Break – Recital Hall Lobby
- 3:30 PM **REPROGRAMMING AND PLURIPOTENCY**
Chair: Ihor Lemischka, Mount Sinai School of Medicine
- 3:30–3:45 PM **Jian Feng**, University at Buffalo, State University of New York, Buffalo, NY
Generation of iPS Cells from Parkinson's Disease Patients with Parkin Mutations
- 3:45–4:00 PM **Saghi Ghaffari**, Mount Sinai School of Medicine, New York, NY
Identification of a Novel Regulator of Human Embryonic Stem Cell Pluripotency
- 4:00–4:15 PM **Claudia Doege**, Columbia University, New York, NY
Improvement of Reprogramming via Chemical and Genetic Approaches
- 4:15–4:30 PM **Gist Croft**, Columbia University, New York, NY
Comparison of Motoneuron Differentiation Efficiency Between hES and hiPS Cell Lines Derived from ALS Patients and Controls
- 4:30–4:45 PM **Eirini Papapetrou**, Sloan-Kettering Institute, New York, NY
Therapeutic β -globin Expression in Thalassemia Patient Induced Pluripotent Stem Cells from Genomic Safe Harbors
- 4:45–5:00 PM **Gabsang Lee**, Sloan-Kettering Institute, New York, NY
Modeling of Neural Crest Disease in Patient-specific Human iPSC and PGD-hESC Lines for Familial Dysautonomia and Charcot-Marie-Tooth 1A
- 5:00 PM Adjourn

NEURACELL: A CENTRALIZED NEURAL STEM CELL BANK THAT WILL MAINTAIN, STORE AND SUPPLY STEM CELLS TO OTHER RESEARCHERS

Christopher A. Fasano, Steven Lotz, Sheila Le, Susan K. Goderie, Sally Temple
New York Neural Stem Cell Institute, Rensselaer, NY

Disease and damage of the central nervous system (CNS), including traumatic brain and spinal cord injury, Parkinson's Disease and Alzheimer's Disease, are a great burden to patients, their families and to the NYS healthcare budget. Finding ways to repair the CNS is of paramount importance to restore functionality, patient independence, and reduce the cost of patient care.

Unlike other tissues such as bone marrow and skin, which have robust stem cell populations, the mature CNS has few stem cells and limited ability to self-repair. Consequently, stem cell research is particularly needed for nervous system applications. Neural stem cells (NSCs) are the building blocks from which the nervous system arises. These cells are a valuable research tool for advancing our understanding of CNS disease, and for creating cell replacement strategies. Currently approximately 25% of all stem cell research is focused on nervous system applications. NSCs can either be derived from embryonic stem cells or extracted from the developing or mature nervous system.

Researchers at the New York Neural Stem Cell Institute have a wealth of experience in isolating, culturing and characterizing NSCs. This is a valuable resource for other labs that are entering the field. We received a shared facility grant from NYSTEM to create a Neuracell bank to store, characterize and supply NSCs. Over the past six months, we have established the lab infrastructure, hired two technicians, and created a FACS (cell sorting) service. We have already generated multiple NSC lines that we have distributed to other NYNSCI researchers and local laboratories. We are in the process of building the website that will facilitate cell ordering and plan to roll that out in the next two months. We look forward to expanding this much needed service to other NYSTEM recipients and labs beyond NYS.

GENPLAY, A NEW ANALYZER AND BROWSER FOR HIGH-THROUGHPUT DATA

Julien Lajugie¹ and Eric Bouhassira^{1,2}

¹*Department of Medicine and Department of Cell Biology and* ²*Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY 10461*

Rapidly decreasing sequencing cost due to the emergence and improvement of massively-parallel sequencing technologies has resulted in dramatic increase in the quantity of data that need to be analyzed. Therefore, software tools to process, visualize, analyze and integrate data produced on multiple platforms and using multiple methods are needed.

We will report on GenPlay, an extremely fast, easy to use and stable tool for rapid analysis and data processing that is being developed at Albert Einstein College of Medicine. Genplay, which is written in Java and runs on all major operating systems, can display data obtained either with micro-array based or sequencing based platforms. GenPlay displays tracks adapted to summarize gene structure, gene expression; repeat families, CPG islands, etc. GenPlay can also display custom tracks to show the results of RNA-seq, Chip-Seq or TimEX-seq analysis, for example.

GenPlay also offers a library of operations to process raw data (normalization, binding, smoothing) and to generate statistics. The tools provided include Gaussian filter, peak finders, signal saturation.

The browser is currently being tested and used for in-house studies. The library of operations is growing based on the emerging needs. Contributions to the development of the software are welcome. The development team aims to release a first version of the software within this year.

EXPANSION OF THE HUMANIZED SCID (HU-SCID) MOUSE CENTER AND STEM CELL PROCESSING LABORATORY AT SUNY UPSTATE MEDICAL UNIVERSITY

Gerold Feuer

Department of Microbiology and Immunology, Director, Humanized SCID Mouse Center and Stem Cell Processing Laboratory, SUNY Upstate Medical University, Syracuse, NY 13210

Severe combined immunodeficient (SCID) mice support the engraftment, expansion and maturation of human hematopoietic stem cells *in vivo*. Following injection with human hematopoietic stem cells (HSCs), SCID mice allow for maturation and development of all components of the human immune system, including human T and B cells, monocyte-macrophages, dendritic cells and, in some instances, RBCs. The existing Humanized SCID Mouse Center at SUNY UMSOM was established in 1997 and has fostered interdisciplinary research focused on developing and utilizing the HU-SCID mice, a novel *in vivo* model which has the potential to become a broad platform for investigations of hESC/iPS cell maturation profiles, human viral infections, gene therapies, cancer stem cell biology and in translational development of anti-virals and chemotherapeutics. The unique capabilities of the Center has allowed for successfully attracting pre-clinical contract studies from pharmaceutical and biotech companies. NYSTEM Institutional funds for Shared Facilities are currently being used to renovate, expand and equip the newly designed Center for Humanized SCID Mouse Models at SUNY UMSOM. The expanded and renovated HU-SCID facility is located on the 4th floor of Weiskotten Hall, will encompass ~4,000 ft² and will be operated under BL-2 containment, allowing for the infection and manipulation of mice with human pathogens. The renovated facility is scheduled for completion in February 2011. In addition to significantly increased capacity for the performance of HU-SCID mouse research, technical support will be provided by the Center to assist individual investigators with *in vivo* manipulations and in the generation of HU-SCID mice. This facility will also house an *in vivo* imaging system (IVIS200) to allow *in vivo* tracking of transduced human hematopoietic, embryonic and iPS cells *in vivo*.

DIFFERENTIATION-SPECIFIC STEM CELL MARKER PROTEINS DETECTED BY PROTEOMICS OF ADIPOSE-DERIVED HUMAN STEM CELLS IN HYPEROSMOTIC CONDITIONS

Elizabeth S. Oswald¹, Lewis M. Brown², J. Chloë Bulinski², Clark T. Hung¹

¹*Department of Biomedical Engineering and* ²*Department of Biological Sciences, Columbia University, New York, NY*

At the Comparative Proteomics Center, we study proteins with differential occurrence in cells, tissues or affinity purified samples. NYSTEM funding with matching funds from Columbia University allowed us to acquire a NanoAcquity liquid chromatograph and a Synapt QTOF mass spectrometer. This equipment allows us to use a label-free technique for mass spectrometry-based shotgun proteomics. This versatile and sensitive methodology allows flexible experimental design, and is ideally suited to studies of stem cell biology. This resource is available on an equal basis to researchers at Columbia University and across New York State.

In this study, multipotential adipose-derived stem cells were subjected to hyperosmotic (400 mOsm/kg, versus control, 300 mOsm/kg) culture conditions during cell expansion as a means to coax them toward a chondrocyte lineage. Adipose tissue is an attractive source for allogeneic or autologous stem cells, due to its accessibility and abundance resulting from the prevalence of elective cosmetic procedures. Numerous proteins were clearly demonstrated to be differentially expressed in the two osmolarity groups. For example, aldose reductase increased 4-fold at the higher osmotic pressure. The fact that this protein was previously reported to increase in level as a result of hyperosmotic stress confirms the ability of this technique to identify known biomarkers of particular biological processes and provides an orthogonal validation. Another prominent differentially-expressed protein was fibronectin, which we detected in eight of nine replicate chromatograms for the control and in no chromatograms for the high osmolality. A number of markers of stem cell biology were detected; these provide initial insight into the biology of these cells, with implications for future monitoring of differentiation states. This technique allowed us to identify and quantify similar protein isoforms: Transgelin and transgelin-2 were differentially expressed, but varied inversely in response to hyperosmotic culture conditions.

THE SKI STEM CELL RESEARCH FACILITY

Mark J. Tomishima, Viviane Tabar, Hakim Djaballah, Lorenz Studer
Sloan-Kettering Institute, New York, NY

The SKI Stem Cell Research Facility was established in 2007 at the Sloan-Kettering Institute, the research branch of Memorial Sloan-Kettering Cancer Center in Manhattan. The goals of the facility are to: maintain a quality-controlled inventory of human pluripotent stem cells (hPSCs); teach interested investigators basic techniques for culturing hPSCs; derive differentiated progeny from hPSCs; and genetically modify hPSCs. NYSTEM support has allowed the facility to enhance and expand our capabilities in two important areas. We described a transgenic method using bacterial artificial chromosomes (BACs) in human embryonic stem cells. Such engineered cells fluoresce when they express a gene of interest. The facility continues to refine this technology, attempting to make it more time and cost effective. One important application of BAC transgenics is in the second key area enabled by NYSTEM support: high-throughput screening. We will discuss our progress in establishing a high-throughput module within the lab. Once complete, our facility will be capable of creating disease-specific cell lines that fluoresce when they become the clinically-relevant cell type. High-throughput screening of such cells could identify drugs, compounds or genes that destroy or preserve the relevant cells, ultimately helping to reveal disease mechanisms or identifying therapeutic compounds.

EINSTEIN COMPREHENSIVE HUMAN PLURIPOTENT STEM CELL CENTER

Nathalie Lailier, Zipora Etzion, Guillermo Simkin, Sanjeev Gupta, Vladik Sandler, Uli Steidl, Eric E. Bouhassira
Albert Einstein College of Medicine, Bronx, NY 10461

The Einstein Comprehensive Human Pluripotent Stem Cell Center consists of three major components: a Pluripotent Stem Cell Unit, a Stem Cell Genomic Unit, and a Xenotransplant Unit.

The Human Pluripotent Stem Cell Unit provides undifferentiated hESC plates and hESC reagents, produces iPS, and offers a variety of cell differentiation (EB formation, Hematopoietic differentiation) and phenotyping services.

The facility has designed and acquired a novel automated cell culture system to semi-automatically grow and subclone hESC and iPS cells. The facility has a staff of three.

The Human Stem Cell Genomic Unit provides assistance to scientists in the analysis of their Genomic data, produced by various platforms such as micro-arrays, or DNA massively-parallel sequencing. Massively-parallel sequencing technologies have greatly improved over the last years, and have become more affordable, which results in a dramatic increase in the quantity of data that need to be analyzed. The Stem Cell Genomic Unit possesses a powerful computer platform, composed of both UNIX and Windows based systems, as well as a team of programmers and data analysts. This combination allows fast and reliable data analysis, for a large variety of data sets and formats. The Unit works in close collaboration with the Genomic Core Facility at The Einstein College of Medicine, managed by Dr. Shahina Maqbool. The Genomic Core Facility performs all the Massively-parallel sequencing and the Stem Cell Genomic Unit processes and analyses the data. One achievement of the Stem Cell Genomic Unit is the development of an interface called GenePlay, which allows users to visualize their genomic data as well as to perform a large range of treatments on these data (normalization, filtering, Gaussian convolution smoothing, indexation, etc.)

The Flow Cytometry and Xenotransplant Unit is equipped with two FACSAria II and is staffed with a FACS operator and an animal technologist. This Unit provides dedicated flow cytometry services for primary human stem cells and transplantation of human stem cells in mice. Several colonies of immunodeficient mice are kept in stock.

Number of users: In 2009 the Center served 21 users.

DIVERSE STEM CELL PROJECTS SUPPORTED BY THE NYSTEM SHARED FACILITY GRANT AT MSSM

Liang Dong¹, Vera Alexeeva¹, Sunita DSouza¹, Ihor Lemischka¹

¹*Department of Gene and Cell Medicine, Black Family Stem Cell Institute, Mount Sinai School of Medicine, New York, NY 10029*

The NYSTEM-funded, human embryonic stem cell (hESC)/induced pluripotent stem cell (iPSC) Shared Resource Facility (SRF) was established with the goal to promote hESC/iPSC research. Towards this goal the hESC SRF regularly conducts classes to teach iPSCs generation and hESC/iPSC differentiation into the lineage of choice. Scientists are also provided with tested stem cell reagents at vastly discounted pricing made possible due to the establishment of two stem cell supply centers, bulk purchasing and NYSTEM funding. Since not all labs have the necessary funds to establish ESC technology, the hESC SRF assists these labs by providing them with the cell types necessary for their studies. For e.g. cardiomyocytes are regularly provided to Dr. Sobie's Lab to aid in signaling studies and hepatocytes are regularly provided to Dr. Woo's lab to aid in the development of an *in vitro* hepatitis C virus (HCV) model of infection. To further the development of iPSC technology, the hESC SRF is constructing a humanized version of the murine piggyBac vector system in collaboration with Dr. Lemischka's lab. This technology will aid in the creation of patient-specific iPSCs lines that are completely free of exogenous DNA. The hESC SRF is actively collaborating with the MSKCC Core Facility to understand neuronal development. In exchange the hESC SRF provides them with necessary know-how in endoderm/cardiac differentiation studies. These collaborations are not restricted to academia but also extend to industry. The hESC SRF is currently involved in the construction of various reporter cell lines for Drs. Ihor Lemischka, Hans Snoeck, Valerie Evans and Dr. Woo in collaboration with Invitrogen and Global Stem Companies. In conclusion, significant progress has been made with the help of NYSTEM funding. It has allowed the hESC SRF to increase its user base while keeping abreast with the latest technology developments in the field of stem cell research.

THE CORNELL MAMMALIAN CELL REPROGRAMMING CORE

Christian Abratte, Lishuang Shen, John Schimenti

Cornell University, Ithaca, NY

The Core has 2 arms. One is the Induced Pluripotent Stem Cell (iPS) Core Laboratory, dedicated to performing a variety of services related to iPS cell line derivation from laboratory rodents and veterinary animals. To this end, the Cornell iPS core offers retroviral vectors for reprogramming somatic cells to pluripotency, established iPS cell lines, and a variety of services related to the characterization of the pluripotent state. While derivation of mouse iPS lines is routine, we are actively pursuing non-integrating reprogramming methods and the creation of canine and equine iPS cell lines. Finally, we offer as a service the "iPS assay," whereby the core measures the efficiency of iPS derivation from mouse embryonic fibroblasts carrying various mutations.

The second arm is dedicated to modern high throughput genomic analyses. A "next generation" Illumina DNA sequencer was installed, and a dedicated bioinformatician was hired to help scientists interpret the vast amounts of data generated by analysis and characterization of stem cells and their derivatives. Initial efforts have concentrated on developing and optimizing several in-house academic bioinformatics pipelines for NextGen sequencing data analysis. SLIM was chosen for sequence alignment, and SAMTools for post-processing of alignments. SNP call filters were optimized for coverage, read and alignment quality. Data storage solutions involve a combination of MySQL database and file systems in portable data formats. We are also evaluating a commercial tool from Softgenetics and will present a comparison to the in-house developed pipelines. Our results for both arms of the Core will be presented.

THE NYSCF SHARED FACILITY FOR DERIVATION, DISTRIBUTION AND TRANSLATIONAL RESEARCH WITH HUMAN PLURIPOTENT STEM CELLS

¹Faizzan Ahmad, ¹David Kahler, ¹Lauren Bauer, ¹Caroline Marshall, ¹Dieter Egli, ²Haiqing Hua, ¹Hector Martinez, ^{3,4}Kevin Eggan, ¹[Scott Noggle](#)

¹*The New York Stem Cell Foundation, New York, NY*, ²*Columbia University Department of Pediatrics and Molecular Genetics*, ³*Harvard Stem Cell Institute, Cambridge, MA*, ⁴*Harvard University Department of Molecular Biology, Cambridge, MA*

NYSCF identified a need for an independent, specialized facility in which researchers within the stem cell community would be able to conduct research projects that would be prevented by Federal funding restrictions that segregate human embryonic stem cell research from other forms of medical research. This environment would also facilitate collaborations across institutional boundaries. To proactively address this need, the NYSCF stem cell research laboratory opened its doors on March 1, 2006. To date, many major projects, most involving cross-institutional collaborations, have been initiated within the NYSCF laboratory. These projects include disease modeling for diabetes, cardiac disease, Schizophrenia, and several neurodegenerative diseases as well as efforts to engineer functional bone grafts. Importantly, we continue to explore new methods for generating human embryonic stem cell lines from normal and diseased embryos by a variety of techniques, including somatic cell nuclear transfer and parthenogenesis. We are also deriving a bank of iPS cell lines, including a range of disease-specific lines, and exploring better methods for derivation, quality control and characterization. The NYSCF laboratory/derivation facility serves the greater New York State research community by acting as a repository and rapid distribution center for human pluripotent stem cell lines to researchers across the US. Finally, to address the need for additional specialized stem cell training in the scientific community, NYSCF holds regular training courses in the derivation, characterization and maintenance of pluripotent stem cells derived from embryonic sources and by novel methods including reprogramming adult cells with defined genetic factors. The NYSCF laboratory continues to expand to cater for the increasing needs of external users in addition to NYSCF researchers' own projects in disease modeling. 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.

SHH EXPRESSION BY MESENCEPHALIC DOPAMINERGIC NEURONS IS A SENTINEL FOR NEURONAL DYSFUNCTION AND INFLUENCES CELL FATE DECISIONS IN SVZ NEUROGENESIS IN THE ADULT BRAIN

Miguel Verbitsky*, Luis E. Gonzalez*, Elizabeth Gregorutti, [Andreas H. Kottmann](#)

Department of Psychiatry, Division of Integrative Neuroscience and Center for Motor Neuron Biology and Disease, Columbia University, New York City, NY

Cell signaling by the morphogen Sonic Hedgehog (Shh) is essential for the coordinated generation of neuronal identities along the ventral neuraxis during embryogenesis. Shh signaling also plays a role in neurogenesis in the adult brain. With no apparent Shh expression around the SVZ, the source(s) and functional details of neurogenesis relevant Shh remain elusive, however. In the subventricular zone (SVZ) B- and C- cells express the Shh receptor Ptc1. B-cells, but not C-cells, elaborate a primary cilium into the ventricular lumen possibly exposing B-cells to Shh present in the cerebrospinal fluid. C- and A-cells receive organized innervations from several neuro-modulatory projection systems including mesencephalic dopaminergic (DA) neurons. We therefore explored whether Shh expressed by SVZ innervating projection neurons could influence SVZ physiology.

We show that all DA neurons of the mesencephalon express Shh throughout adult life. Interestingly, Shh expression by DA neurons is dynamically up-regulated in correlation with the severity of transiently induced neuronal dysfunction in synaptically connected neurons. Consistent with the demonstrated axonal transport of neuron produced Shh, and Shh function in early spinal cord development, we observe that the conditional ablation of Shh from mature DA neurons results in the production of increased numbers of Pax6⁺ - and a concomitant reduction in the numbers of Olig2⁺ - lineage precursor cells in the SVZ. The changes in relative proportions of SVZ resident precursor populations in the SVZ translate into altered olfactory bulb cyto-architecture, increased numbers of dopaminergic, periglomerular neurons and ER81+ granule cells, and olfactory dysfunction.

Our data suggest that Shh expression at different levels by DA neurons alters the qualitative outcome of SVZ neurogenesis. Current experiments make use of conditional, cell fate mapping strategies to determine whether the induction of subtle neuronal dysfunction induces the increased production of neuronal identities of current physiological need via the modulation of Shh expression by DA neurons.

HIPPOCAMPAL STEM CELLS: IMPACT ON MOOD AND COGNITION

Amar Sahay, Kimberly Scobie, Alexis Hill, [René Hen](#)

New York State Psychiatric Institute and Columbia University, College of Physicians & Surgeons, New York, NY

Adult hippocampal neurogenesis is a unique form of plasticity that generates new neurons in the dentate gyrus throughout life. Adult-born neurons have been implicated in both cognitive functions and in mediating the behavioral effects of antidepressants. However, it is not known whether stimulation of adult hippocampal neurogenesis is sufficient to improve cognition and mood. We used an inducible genetic gain-of-function strategy to cell autonomously augment adult neurogenesis. We show that mice in which the pro-apoptotic gene, *Bax*, is deleted specifically in adult progenitors have increased survival of adult-born dentate granule neurons, exhibit enhanced neurogenesis-dependent synaptic plasticity and discriminate between similar contexts more efficiently than controls. In contrast, increasing the number of adult-born neurons did not produce an antidepressant-like behavioral response. Our findings suggest therefore that strategies designed to specifically stimulate adult hippocampal neurogenesis are likely to have pro-cognitive effects associated with improved pattern separation, but may not be sufficient to enhance mood.

DIVISION-COUPLED ASTROCYTIC DIFFERENTIATION OF NEURAL STEM CELLS DETERMINES AGE-RELATED DECLINE IN HIPPOCAMPAL NEUROGENESIS

Juan M. Encinas¹, Tatyana Michurina¹, Natalia Peunova¹, June-Hee Park¹, Julie Tordo¹, Daniel A. Peterson², Gord Fishell³, Alex Koulakov¹, Grigori Enikolopov¹

¹*Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724*, ²*Center for Stem Cell and Regenerative Medicine and Department of Neuroscience, Rosalind Franklin University of Medicine and Science, North Chicago, IL 60064* and ³*Smilow Neuroscience Program, New York University School of Medicine, New York, NY 10016*

Aging is accompanied by a continuous decrease in the regenerative capacity of many organs. For the brain, aging is associated with a continuous decline in the number of new neurons in the hippocampus and age is the main factor contributing to the decrease in neurogenesis in the normal brain. Age-related decline in neurogenesis is observed across mammalian species, including primates; this decrease may limit plasticity and repair and contribute to age-related cognitive dysfunction. The mechanisms underlying the diminished production of new granule neurons are not understood and may be driven by a host of different processes, including a decrease of size of the neural stem cells pool, diminished proliferative capacity of stem cells or their progeny, reduced survival of stem cells or their progeny, or reduced neuronal lineage commitment.

Our results indicate that a continuously diminishing number of neural stem cells is the major cause of age-related decline in hippocampal neurogenesis. Furthermore, they indicate that the disappearance of neural stem cells is due to their conversion into mature hippocampal astrocytes. This astrocytic differentiation is coupled to a rapid succession of asymmetric divisions of the activated stem cells that had been quiescent during the postnatal period. Thus, unlike the periodic activation of quiescent stem cells in a range of tissues, hippocampal stem cells, once activated, leave the pool of stem cells. We describe the life cycle of an adult neural stem cell and propose a “disposable stem cell” model which reconciles the age-related decrease in neurogenesis, the age-related increase in astrocytes, the disappearance of hippocampal neural stem cells, and continuous remodeling of the neurogenic niche; this model highlights a complex strategy for supporting neurogenesis in the aging hippocampus in the face of an ever-diminishing pool of stem cells.

DIRECTING ES CELLS TO CORTICAL INTERNEURON FATES BY TRANSCRIPTIONAL SPECIFICATION

Edmund Au¹, Theofanis Karayannis¹, Lee Rubin², Gordon J. Fishell¹

¹*Smilow Neuroscience Program, NYU Medical Center, New York NY 10016* and ²*Harvard Stem Cell Institute, Department of Stem Cell and Regenerative Medicine, Cambridge MA 02138*

Cortical interneurons comprise 20% of the neurons in the cortex, and dysfunction within this cell class has been associated with disorders such as schizophrenia, bipolar disorder, autism and epilepsy. We aim to generate inhibitory cortical interneurons from mouse ES cells while addressing two of the central issues currently facing stem cell biologists: 1) increasing efficiency and uniformity of the differentiation process to a desired end cell type, and 2) rigorously test the properties of ES-derived cortical interneurons and compare them to their *in vivo* counterparts.

To those ends, we are utilizing two complementary approaches in parallel to increase ES cell differentiation efficiency to a cortical interneuron fate. The first approach is an unbiased small molecule screen to identify and optimize extrinsic factor combinations that favor interneuron differentiation. The second approach is to alter the intrinsic competence of neuralized ES cells, allowing them to respond more appropriately to extrinsic cues. Because of our lab's expertise in developmental genetics and *in utero* transplantation, we are well suited to characterize putative ES-derived interneurons and validate their phenotype and function in a stringent and rigorous manner.

To date, we have found that transcriptional specification of interneurons using factors relevant to their normal developmental program, expressed in the correct temporal sequence, greatly increases differentiation efficiency *in vitro*. When transplanted *in utero* and analyzed postnatally, we observe a concomitant increase in the number of interneurons that migrate dorsally into the cortex and a strong bias in the subtype of interneuron produced. In summary, mouse ES cells appear to follow similar programs for cortical interneuron production, as previously described *in vivo*. In the future, we plan on using ES cells *in vitro* model system for studying cell lineage specification in the ventral telencephalon as well as explore its applicability in mouse models of epilepsy.

A HIGHLY EFFICIENT NEURAL AND NEURONAL INDUCTION OF HUMAN ES AND IPS CELLS THROUGH COMBINED SMALL MOLECULE INHIBITION

Stuart M. Chambers¹, Yuchen Qi², Lorenz Studer^{1,3}

¹*Developmental Biology Program, Sloan-Kettering Institute,* ²*Weill Cornell Graduate School of Medical Sciences and* ³*Department of Neurosurgery, Sloan-Kettering Institute, New York, NY*

Current neural induction protocols in human ES cells (hESCs) rely on embryoid body formation, stromal feeder co-culture, or selective survival conditions; each strategy displaying significant drawbacks including poorly defined culture conditions, protracted differentiation or low yields of neural tissue. We have found the synergistic action of two inhibitors of SMAD signaling, Noggin and SB431542, induces a rapid and uniform neural conversion of hESCs without the need for embryoid body formation or stromal cell co-culture. This early neural tissue shares characteristics of hESCs, yet expresses rosette neural stem cell (rNSC) markers, and readily forms rosettes upon passage and represents a discrete intermediate progenitor cell population downstream of hESC capable of giving rise to early neuronal and neural crest lineages. When the efficiency of neural differentiation is measured, a homogenous neuroepithelium is formed and greater than 83% of HES5::GFP BAC transgenic reporter hESC cells indicate differentiation towards a neural stem and progenitor cell fate by day 11. Noggin/SB431542 based differentiation of numerous human induced pluripotent stem cell (h-iPSC) lines into neuroectoderm, rNSCs, neural crest, dopamine neurons, and motoneurons further confirms robustness and general applicability of this differentiation method.

We are improving the Noggin/SB431542 neural induction in two ways. First, Noggin has been replaced with the more cost-effective small molecules, dorsomorphin and LDN-193189, as alternate methods for blocking BMP signaling. The small molecule neural induction yields comparable numbers of neural cells with minimal or no Noggin required. Second, we have screened inhibitors of additional signaling pathways in > 400 conditions to accelerate neuronal differentiation to yield the maximum percentage of TUJ1+ neurons. These small molecule methods for differentiating hESC and hiPS are highly efficient, rapid, easily scalable, inexpensive, and eliminate the need for stromal feeders, embryoid body formation, or copious amounts of recombinant protein.

HOMING OF NEURAL STEM CELLS TO THE VASCULAR NICHE IS REGULATED BY DIFFERENTIAL RESPONSES TO SDF1/CXCR4 SIGNALING

Erzsebet Kokovay^{1,2}, Susan Goderie¹, Yue Wang¹, Steve Lotz¹, Gang Lin³, Yu Sun⁴, Badrinath Roysam³, Qin Shen^{1,2}, Sally Temple^{1,2}

¹*New York Neural Stem Cell Institute, Rensselaer, NY,* ²*Center for Neuropharmacology and Neuroscience, Albany Medical College, Albany, NY,* ³*Rensselaer Polytechnic Institute, Rensselaer, NY and* ⁴*Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA 02115*

Neural Progenitor cells (NPCs) are nestled within niches which regulate stem cell quiescence, self-renewal, proliferation and fate. Within the adult brain subventricular zone (SVZ) progenitor cells are associated with both the ependymal and the vasculature niches. Proliferating Epidermal Growth Factor receptor (EGFR) positive Type B stem cells and their progeny the transit amplifying Type C cells are associated with the SVZ vasculature indicating that the vascular niche is important in activation. However, the dynamism of NPCs between these niches is ill-defined. Here we show that transplanted NPCs associate closely with endothelial cells and that proliferative SVZ progenitor cells home to the vascular niche in a stromal-derived factor 1 (SDF1) and CXC chemokine receptor 4 (CXCR4) dependent manner. Adult SVZ progenitors express the chemokine CXC-motif receptor 4 (CXCR4) and vascular cells and ependyma secrete SDF1. SDF1 stimulates homing of activated Type B cells, Type C cells and neuroblasts but not of unactivated EGFR negative Type B cells. The blockade of CXCR4 results in a significant decrease in NPC integration into the SVZ and homing of NPCs toward endothelial secreted factors. SDF1 induced upregulation of alpha 6 integrin and EGFR on activated progenitor cells enhancing their activation state and ability to bind to laminin on the vascular plexus. These findings have important implications for understanding the normal dynamism of NSCs in SVZ sub-niches. They also further our knowledge about how transplanted NSCs become integrated into host tissues. Finally they have relevance to cancer states, as stem-like cancer cells spread through the brain via migration along blood vessels. Future studies will involve 2 photon confocal time-lapse imaging of the SVZ using transgenic mice that express green fluorescent protein in a subset of NPCs to capture movement of progenitor cells within the ependymal and vasculature niches and perturbations following various treatments.

PLASTICITY LOSS AND REGAIN OF CD49F AND ALDHA1 EXPRESSION DIRECTS TUMOR-INITIATING CAPACITY IN HEAD AND NECK SQUAMOUS CARCINOMA CELL POPULATIONS

Yeriel Estrada¹, Aparna Ranganathan¹, Paloma Bragado¹, Julio A. Aguirre-Ghiso^{1,2}

¹Division of Hematology and Oncology, Department of Medicine and Department of Otolaryngology and

²Tisch Cancer Institute at Mount Sinai, Mount Sinai School of Medicine, New York, NY

We tested whether tumor-initiating cells (TICs) occur stochastically and whether they depend on a metastable TIC program in head and neck squamous carcinoma cells (HNSCC). We show that in HEp3 HNSCC tumors TICs are defined as transiently G1-S arrested ALDH1^{high}/CD49^{high} or CFSE label retaining/CD49^{high} cells. Only enriched TICs showed enhanced proliferative efficiency in the first week of *in vivo* growth, and they required high CD49f expression. Reduced proliferative capacity of non-TICs was associated with reduced ERK and high p38 signaling (low ERK/p38 activity ratio). However, after serial transplantation in chicken embryos it appeared that non-TICs could regain tumorigenic potential. Further, both sorted TICs and non-TICs could be immediately expanded *in vitro*. Regardless of the generations *in vitro* TICs retained CD49^{high} expression and were tumorigenic when re-injected *in vivo*. Non-TICs expanded *in vitro* showed variable levels of ALDH1 and low CD49f expression. However, despite this variability non-TICs always regained tumorigenicity with similar kinetics as TICs after injection in chicken embryos or nude mice. This was linked to restoration of an ALDH1^{high}/CD49^{high} profile and a high ERK/p38 activity ratio. We have also found that T-HEp3 cell populations that reprogram into a dormant phenotype (D-HEp3) are mostly CD49^{low}/ALDH1^{low} and this is associated with high intracellular ROS levels. Loss of TIC markers in T-HEp3 cells could be induced by paraquat treatment (ROS donor), which resulted in a loss of tumor initiating potential of T-HEp3 tumors. We conclude that TIC markers define tumor cells with a more efficient short-term engrafting capacity but these properties can resurface in cells *a priori* defined by the operator as non-TICs. Further, stress signals such as ROS may induce a reversible loss of TIC potential. These findings suggest that the absence of stem cells markers in tumor cells in patients might not necessarily mean absence of TICs.

HUMAN SARCOMAS OF MULTIPLE LINEAGES EXHIBIT CONSTITUTIVE CANONICAL WNT AUTOCRINE ACTIVATION

Sapna Vijayakumar, Guizhong Liu, Ioana A. Rus, Shen Yao, Yan Chen, Gal Akiri, Luca Grumolato, Stuart A. Aaronson

Department of Oncological Sciences, Mount Sinai School of Medicine, New York, NY 10029

Wnt/ β -catenin signaling is important for normal tissue homeostasis and is also implicated in the genesis of several different types of carcinomas. Earlier, we had shown that high levels of Wnt signaling inhibit multi-lineage differentiation of human mesenchymal stem cells (hMSCs) *in vitro* and *in vivo*. Sarcomas are mesenchymal tumors that account for a majority of pediatric cancers. Based on genetic and histological features, sarcomas are classified into several distinct types including fibrosarcoma, liposarcoma, leiomyosarcoma, malignant fibrous histiocytoma and osteosarcoma. Recent studies indicate that at least certain sarcomas originate from hMSCs, which we have shown exhibit low levels of Wnt signaling. We demonstrate here that Wnt signaling is frequently upregulated in human sarcomas of several subtypes compared to levels in hMSCs. Furthermore, both genetic and epigenetic events lead to overexpression of Wnt ligands or receptors and/or downregulation of Wnt antagonists culminating in the upregulation of Wnt signaling through an autocrine mechanism. Downregulation of Wnt signaling via exogenous expression of dnTCF4, an inhibitor of Wnt/ β -catenin signaling induces growth inhibition of Wnt activated human sarcoma cell lines analyzed both *in vitro* and *in vivo* by mechanisms currently under investigation. Our findings establish the Wnt canonical pathway as a new potential therapeutic target for a diverse array of human sarcomas.

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RUNX1 DIRECTLY PROMOTES PROLIFERATION OF HAIR FOLLICLE STEM CELLS AND EPITHELIAL TUMOR FORMATION IN MOUSE SKIN

Tudorita Tumber¹, Charlene SL. Hoi¹, Song Eun Lee¹, Shu-Yang Lu¹, David J. McDermitt¹, Karen M. Osorio¹, Caroline M. Piskun¹, Rachel M. Peters², Ralf Paus³

¹*Department of Molecular Biology and Genetics,* ²*Department of Biomedical Sciences, Section of Anatomic Pathology, Cornell University, Ithaca, NY and* ³*Dept. of Dermatology, University of Luebeck, D-23538 Luebeck, Germany*

Runx1/AML1 is a transcription factor implicated in tissue stem cell regulation, which belongs to the small Runx family of cancer genes. In the hair follicle (HF) Runx1 epithelial deletion in morphogenesis impairs normal adult hair homeostasis (cycle) and blocks adult hair follicle stem cells (HFSCs) in quiescence. Here, we show that these effects are overcome later in adulthood. By deleting Runx1 after the end of morphogenesis, we demonstrate its direct role in promoting anagen onset and HFSCs proliferation. Runx1 deletion resulted in cyclin dependent kinase inhibitor Cdkn1a (p21) up-regulation. Interfering with Runx1 function in cultured HFSCs impaired their proliferation and normal G0/G1 and G1/S cell cycle progression. The proliferation defect could be rescued by Runx1 re-addition or by p21 deletion. Chemically induced skin tumorigenesis in mice turned on broad Runx1 expression in regions of the skin epithelium, in papillomas, and squamous cell carcinomas. In addition, it revealed reduced rates of tumor formation in the absence of Runx1 accompanied by decreased epithelial levels of Phospho (P)-Stat3. Runx1 protein expression was similar in normal human and mouse hair cycle. We propose that Runx1 may act as a skin oncogene by directly promoting proliferation of the epithelial cells.

EMBRYONIC STEM CELLS USE ZFP809 TO BIND THE PROLINE RNA PRIMER BINDING SITE AND SILENCE RETROVIRAL DNAs

Daniel Wolf and Stephen P. Goff

Howard Hughes Medical Institute, Department of Biochemistry and Molecular Biophysics, College of Physicians and Surgeons, Columbia University, New York, NY 10032

Embryonic stem (ES) cells and other primitive stem cells of mice have been known for more than 30 years to potently block retrovirus replication (Teich *et al.*, *Cell* 12 (4), 973-982 1977). Infection of ES cells by the murine leukemia viruses (MLVs) results in the normal establishment of integrated proviral DNA, but this DNA is then transcriptionally silenced, preventing further viral spread. The repression is largely mediated by *trans*-acting factors that recognize a conserved sequence element termed the primer binding site (PBS), an 18-bp sequence complementary to the 3' end of a cellular tRNA. A specific tRNA is annealed to the PBS sequence of the viral genomic RNA, and is used to prime DNA synthesis. This same sequence in the context of the integrated proviral DNA is targeted for silencing in ES cells. We recently showed that a large protein complex binding to the PBS in ES cells contains TRIM28, a well-characterized transcriptional co-repressor.

We now have identified the novel zinc finger protein ZFP809 as the recognition molecule that bridges the integrated proviral DNA and TRIM28. We show that expression of ZFP809 is sufficient to render even differentiated cells highly resistant to MLV infection. These results identify ZFP809 as a DNA binding factor that specifically recognizes a large subset of mammalian retroviruses and retroelements and targets them for transcriptional silencing. We propose that ZFP809 evolved as a stem cell-specific retroviral restriction factor and therefore constitutes a novel component of the intrinsic immune system of stem cells.

IMPROVING CARDIAC CONDUCTION WITH A SKELETAL MUSCLE SODIUM CHANNEL BY GENE AND CELL THERAPY

Jia Lu¹, Hong-Zhan Wang¹, Zhiheng Jia¹, Joan Zuckerman¹, Zhongju Lu¹, Yuanjian Guo¹, Peter R Brink¹, Richard B Robinson², Michael R Rosen², Emilia Entcheva¹, Ira S Cohen¹
¹SUNY Stony Brook, Stony Brook, NY and ²Columbia University, New York, NY

A common mechanism leading to cardiac tachyarrhythmias is reentry, which is associated with a reduced conduction velocity. Current therapy usually terminates reentrant arrhythmias by making slow conduction fail. Our group has proposed an alternative antiarrhythmic approach: increasing Na⁺ conductance to restore normal propagation of the action potential by using the skeletal muscle Na⁺ channel, Nav1.4, which has more favorable biophysical properties than the cardiac isoform Nav1.5. Nav1.4 should particularly enhance conduction in depolarized tissue. Our group previously identified that mesenchymal stem cells (MSCs) can be used as a gene delivery platform. In this study, instead of direct delivery of the gene into cardiac tissue by virus, we used transfected HEK293 cells and canine MSCs (cMSCs) to introduce the skeletal muscle Na⁺ channels into the cardiac syncytium via gap junctions. Here, we report that:

1. Nav1.4 has a more depolarized inactivation versus voltage curve and faster recovery from inactivation than Nav1.5 when expressed in our delivery cell lines.
2. When coupled with Nav1.4- expressing cells, cultured adult canine ventricular myocytes showed an increase in the dV/dt_{max} of the action potential in both normal (5.4mM K⁺) and depolarized (10.4mM K⁺) conditions. Expression of Nav1.5 had no such effect.
3. In an *in vitro* cardiac syncytium, co-culture of myocytes with Nav1.4-expressing but not Nav1.5-expressing cells significantly increased the conduction velocity under both normal and depolarized conditions.
4. In an *in vitro* reentry model induced by high frequency stimulation, the Nav1.4 co-culture has a faster angular velocity of the induced reentry than the control and Nav1.5 cultures, which should improve the possibility of normalizing conduction and preventing/terminating reentry.

In summary, cells carrying non-cardiac Na⁺ channel isoforms with a more depolarized inactivation versus voltage curve can improve cardiac excitability and conduction in our *in vitro* cardiac syncytium in both normal and depolarized conditions. This work was supported by NYSTEM grant CO24344.

KCNQ1/KCNE1 K⁺ CHANNELS ASSOCIATED WITH LONG QT SYNDROME ARE EXPRESSED IN EARLY STAGE HUMAN EMBRYONIC STEM CELL-DERIVED CARDIOMYOCYTES

Cecile Terrenoire¹, George Kai Wang¹, Eric Adler³, Kevin J. Sampson¹, Jonathan T. Lu², Robert S. Kass¹

¹Department of Pharmacology, ²Department of Medicine, College of Physicians and Surgeons, Columbia University Medical Center, New York, NY and ³Cardiovascular Institute, Mount Sinai School of Medicine, New York, NY

Human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) are not only a potential source of functional cardiac tissue that can be utilized for regenerative therapy, but also offer great potential in laboratory studies of congenital cardiac arrhythmias, including Long QT syndromes (LQTS). Several major cardiac ion currents have been reported to be expressed in human embryonic stem cell-derived cardiomyocytes (hESC-CMs). However, the presence of I_{Ks} (carried by KCNQ1 and KCNE1), a potassium current critical for cardiac repolarization, has not been reported. Mutations in KCNQ1 are associated with the most common form of congenital LQTS: LQT1. To understand whether cardiomyocytes derived from pluripotent stem cells, such as patient-specific induced pluripotent stem cells (iPSC), can serve as an adequate *in vitro* model of LQTS, we investigated the cellular electrophysiological properties of hESC-CMs during the first 34 days of cytokine directed differentiation, focusing primarily on I_{Ks}. All beating hESC-CMs studied had action potentials with cardiac phenotypes and expressed L-type calcium channels (n=26) and pacemaker channels (n=27) while 68% of cells (n=11 out of 16) expressed I_{Kr}, defined as E4031-sensitive outward current measured during prolonged depolarization. I_{Kr} is the potassium current carried by KCNH2 and KCNE2; mutations in KCNH2 are associated with LQT2, the second most common form of LQTS. I_{Ks} was identified as a slowly activating outward current during prolonged depolarization, insensitive to (5 μM) E4031 but blocked by (30 μM) Chromanol 293B. I_{Ks} was recorded in 29% of cells (n=5 out of 17). Quantitative PCR confirmed the presence of I_{Ks} channels • - (KCNQ1) and • - (KCNE1) subunits in these hESC-CMs. This is the first report of I_{Ks} channel expression in hESC-CMs providing strong evidence in support for their utility in mechanistic and pharmacological investigations of LQT1 and other arrhythmia syndromes linked to mutations in the genes coding for I_{Ks} channel subunits and/or accessory proteins.

HEPATOCTES DIFFERENTIATED FROM INDUCED HUMAN PLURIPOTENT CELLS AMELIORATED HYPERBILIRUBINEMIA AFTER TRANSPLANTATION INTO THE LIVER OF THE GUNN RAT MODEL OF CRIGLER-NAJJAR SYNDROME-1

Yong Chen^{1,4}, Chanjung Chang², Ketherine Atienza⁵, Xia Wang^{1,4}, Guha Chandan^{3,4}, Namita Roy-Chowdhury^{1,4}, Eric E. Bouhassira², Jayanta Roy-Chowdhury^{1,4}

¹Departments of Medicine and Genetics, ²Hematology, ³Radiation Oncology; ⁴Marion Bessin Liver Research Center, Albert Einstein College of Medicine, Bronx, NY and ⁵Department of Pediatrics, Montefiore Medical Center, Bronx, NY

Background: Hepatocyte transplantation is safe and effective in patients with liver-based metabolic diseases, but its application has been limited by a severe shortage of donor livers. Human somatic cells, reprogrammed to pluripotent stem cells (iPS), provide a potentially unlimited source of individual-specific hepatocytes. Toward this goal, we have generated iPS cells by gene transfer of OCT4, SOX2, KLF4 and cMyc into adult human fibroblasts, and have reported a 4-step procedure to differentiate human embryonic stem cells (hES-H1) to hepatocytes. Here we report a similar approach to differentiate our iPS cell line to hepatocytes. Metabolic function of the derived hepatocytes was tested after transplantation into UGT1A1-deficient jaundiced Gunn rats (model of Crigler-Najjar syndrome-1).

Methods: The iPS cells were cultured in the mTeSR to generate embryoid bodies (3 days). The cells were then cultured sequentially in Activin A (100ng/ml) plus bFGF (100ng/ml) (4 days), HGF 100ng/ml plus 1% DMSO for 4 days and dexamethasone (3 days). The cells were then transduced with a lentivector, expressing GFP from an albumin promoter (~30% efficiency) and the GFP⁺ cells were sorted. Gene expression at each step was analyzed by qPCR, immunofluorescence and Western blot. The derived hepatocytes were engrafted into Gunn rat livers by intrasplenic injection. The recipient rats received preparative irradiation to the median liver lobe (50Gy) and an adenovector, expressing HGF for mitotic stimulation, and tacrolimus (2mg/kg) to prevent xenograft rejection.

Results: After differentiation of the iPS, 11% cells expressed albumin (GFP⁺). The yield was comparable with that obtained from hES-H1. Albumin expression increased progressively during differentiation, although some alpha-fetoprotein-positive cells persisted after cell sorting. Livers of the recipient Gunn rats contained human UGT1A1-positive cell clusters and serum bilirubin levels declined by 30-70%.

Conclusion: The iPS-derived human hepatocytes ameliorated hyperbilirubinemia in Gunn rats should be explored as a potential source of transplantable hepatocytes.

RED BLOOD CELL DEVELOPMENT IN DIFFERENTIATING EMBRYONIC STEM (ES) CELLS AND MOUSE EMBRYOS

Stuart T. Fraser^{1,2}, Joan Isern¹, Zhiyong He¹, Margaret H. Baron^{1,2}

¹Medicine/Hematology-Oncology and ²Black Family Stem Cell Institute, Mount Sinai School of Medicine, New York, NY

Primitive erythroid cells (EryP) are the first differentiated cell type to form in the mammalian embryo and in ES cell-derived "embryoid bodies (EBs)." They are an outstanding model for erythroid differentiation: the cells develop synchronously and in a stepwise, developmentally regulated manner. To reliably separate EryP and definitive erythroid cells at stages when both lineages are present in the blood, we have used transgenic mouse lines in which expression of green fluorescent protein (GFP) is used to specifically mark EryP. We have also generated mouse ES cell lines in which GFP is expressed exclusively within EryP. We have profiled gene expression at each stage of EryP maturation. We find that genes encoding a number of well known cell surface proteins are expressed on EryP at E7.5-8.5 (yolk sac stages, when progenitor activity is still present) but are gradually or abruptly downregulated at later stages. Progenitor activity is found in the GFP-positive population of E7.5-8.5 embryos and in day 5-6 EBs. The GFP+ (EryP) cells that form in ES cell-derived EBs have a morphology equivalent to EryP erythroblasts from mouse embryos at ~E7.5-8.5 but do not mature beyond that stage. However, the cells have the potential to mature: when replated under conditions that promote progenitor cell proliferation/differentiation, the resulting colonies contain EryP that resemble those from ~E12.5 mouse embryos. We plan to use this system as a starting point to identify factors that can stimulate the immature EryP blasts that form in EBs to mature further and, perhaps, to enucleate. This work is expected to lead to a better understanding of the mechanisms by which stem cells differentiate to red blood cells. This information will be essential for thoughtful approaches to directing the differentiation of ES cells for efficient production of pure populations of red blood cells or their progenitors for therapeutic purposes.

IDENTIFYING EPIGENOMIC DETERMINANTS OF HEMATOPOIETIC STEM CELL COMMITMENT

Britta Will¹, Yonkai Mo², Laura Barreyro¹, Yiting Yu², Amit Verma², Ulrich Steidl¹

¹*Department of Cell Biology and* ²*Department of Medicine and Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, NY 10461*

The balance of self-renewal, lineage commitment, and differentiation, as well as malignant transformation of hematopoietic stem cells (HSC) depends on tightly regulated transcriptional networks. Many key transcriptional regulators of stem cell fate are in turn controlled epigenetically. Dynamic histone modifications regulate rapidly inducible expression and repression of genes during the commitment and differentiation of HSCs. Since cytosine methylation is an equally important component of the epigenetic machinery, we hypothesize that changes in methylation are also critical regulators of HSC commitment and self-renewal.

Our study aims to identify new genes coordinating the self-renewal and lineage commitment of HSCs by characterizing dynamic changes in transcriptional programs in parallel with epigenetic alterations. We have therefore isolated rigorously defined human hematopoietic stem and myeloid progenitor cells derived from healthy donors using high-speed multi-parameter fluorescence-activated cell sorting (FACS). In the first part of the supported project we have optimized a new, high-sensitivity HpaII-tiny fragment enrichment by ligation-mediated PCR (HELP) assay, which we now use to analyze dynamic epigenetic changes in these highly purified cell populations. Transcriptional expression profiles of each cell type were simultaneously characterized by microarray analysis. In the first set of samples we found significant changes of methylation of DNA loci in promoter and gene body regions throughout the different stages of hematopoietic differentiation. Among them were several genes already known to regulate stem cell lineage commitment and differentiation, which also show differentiation stage-specific mRNA transcription. We are currently focusing on the identification of novel genes regulating lineage commitment of HSCs by investigating regions with altered methylation but lacking evident changes in their expression.

This study will enhance our knowledge of epigenetic processes and regulation at the early stem cell level, and identify genes critical for stem cell function that could not be identified by classical transcriptional analysis alone.

REDOX REGULATION OF DROSOPHILA AND MAMMALIAN STEM CELLS MEDIATED BY NRF2 TRANSCRIPTION FACTORS

Ibro Ambeskovic¹, M. Cecelia Barone¹, Dirk Bohmann¹, Christine Hochmut², Henri Jasper², Mark Noble¹
¹*Department of Biomedical Genetics, University of Rochester Medical Center, Rochester, NY and*
²*Biology Department, University of Rochester, Rochester, NY*

The intracellular redox state can modulate signal responses and impact cell metabolism, differentiation and proliferation. In stem and progenitor cells, redox state emerges as an important determinant of pluripotency, self renewal and differentiation. The leucine zipper transcription factor Nrf2 controls redox levels through its target genes that encode redox regulators, such as enzymes involved in glutathione metabolism, and antioxidant enzymes, such as peroxidases. Based on preliminary data generated independently in research on oligodendrocyte precursor (OPC) populations isolated from the mammalian brain and on *Drosophila* intestinal stem cells (ISCs), we proposed that Nrf2 might influence stem cell maintenance, and differentiation. To test this hypothesis experiments in OPC and in *Drosophila* were conducted. Appropriate lenti virus vectors were constructed to specifically manipulate the activity of Nrf2 in OPCs. Increasing Nrf2 using such vectors caused promoted self-renewal and resistance to differentiation, as predicted by our model. Conceptually similar experiments in the *Drosophila* intestine showed that genetic activation of Nrf2 activity prevented stem cells from responding to tissue damage with a regeneration program which, in the case of ISCs, is marked by increased proliferative activity.

Our data support the model that Nrf2-dependent gene expression keeps stem and progenitor cells in a reduced state. At such Nrf2-induced low redox levels these cells are protected from genotoxic stress, but prevented from entering a regeneration or differentiation program. In order for stem and progenitor cells to leave pluripotency and differentiate (as seen in OPCs), or to become hyperproliferative (as seen in *Drosophila* ISCs), Nrf2 activity has to be down regulated. Nrf2 regulation (for example by its inhibitor Keap1) thus serves an evolutionarily conserved role in the preservation of stem and progenitor cell function and in the control of regenerative processes. Further experiments are in progress to corroborate this model.

If verified, the function of Nrf2 as a conserved regulator of stem and progenitor cell function may offer an opportunity for controlling stem cell behavior by small molecule activators of Nrf2.

THE REGULATION OF DNA REPLICATION INITIATION IN MOUSE AND HUMAN EMBRYONIC STEM CELLS

Jeannine Gerhardt¹, Sherri Schultz¹, Inna Lipchina², Zeqiang Guan¹, Lorenz Studer², Carl L. Schildkraut¹
¹*Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY 10461 and* ²*Laboratory of Stem Cell and Tumor Biology, Sloan-Kettering Institute, New York, NY 10021*

It is very important to develop additional methods for identifying characteristics that define the pluripotent state in embryonic stem cells (ESCs); particularly those that detect incompletely reprogrammed induced pluripotent stem cells (iPSCs). One significant and defining epigenetic characteristic of ESCs is their DNA replication program. The DNA replication program specifies the sites along the DNA molecule at which replication initiates and when in the S phase these sites are activated. If ESCs do not initially have the correct replication program, it is possible that some developmental pathways will be affected. We applied a sensitive, high-resolution approach (Single Molecule Analysis of Replicated DNA; SMARD) for fluorescent visualization of the DNA replication program across DNA segments. We captured images of replication intermediates in a population of single DNA molecules and assembled them to represent a time-lapse picture of replication *in vivo*. Our previous studies examined the region surrounding the 350 kb DNA segment containing the *POU5F1* (*OCT4*) gene in three independent hESC lines, hESC-derived multipotent neural rosette cells (R-NSCs) and differentiated microvascular endothelial cells. The location of preferred initiation regions and the direction of replication fork progression through the *POU5F1* gene were conserved in hESC lines but differed in R-NSCs and the endothelial cells studied.

When we examine the region surrounding the *POU5F1* (*OCT4*) gene in a 350 kb DNA segment in a hiPSC cell line (C14), the positions of initiation sites differ significantly from hESCs. We will present studies to determine the degree to which the DNA replication program could be an important tool for characterizing pluripotent cells.

DECIPHERING POLYCOMB-MEDIATED EPIGENETIC REGULATION DURING EMBRYONIC STEM CELL DIFFERENTIATION

Emily Bernstein^{1,2}

¹Department of Oncological Sciences and ²Department of Dermatology, Mount Sinai School of Medicine, New York, NY 10029

Epigenetic regulation underlies the commitment of a genomic locus or chromosome (e.g. inactive X) to a particular transcriptional state throughout differentiation and development. This regulation is mediated by various intersecting mechanisms including, but not limited to, histone post-translational modifications (PTMs) and the proteins that create, remove and 'read' these marks, DNA methylation, and non-coding RNAs. Our work is focused on one of the five mammalian Polycomb homologs, known as Cbx7, in embryonic stem (ES) cells. Our previous work in ES cells has demonstrated that Cbx7 association with chromatin is dependent on RNA and we are currently investigating the nature of these RNA species. It is currently unclear how Polycomb complexes are recruited to their sites of action in the genome ('reader' of H3K27me3), and we are currently investigating: 1) novel protein interactions of Cbx7; 2) Cbx7 PTMs; and 3) RNA interactions that direct recruitment specificity of these complexes to chromatin. These studies, as well as additional studies related to chromatin changes that take place during stem cell differentiation and disease, will be discussed.

ROLE OF NUCLEAR PORE COMPOSITION IN EMBRYONIC AND ADULT STEM CELL DIFFERENTIATION

Floria Lupu¹, Valérie Doye², Elizabeth Lacy¹

¹Developmental Biology Program, Sloan-Kettering Institute, New York, NY 10065 and ²Institut Jacques Monod, Université Paris France

Our research on a gastrulation stage mouse mutant, *mermaid* (*merm*) identified a previously unrecognized molecular player in the regulation of stem/progenitor cell differentiation, the nuclear pore complex – NPC. The *merm* allele encodes a null mutation in Nup133, a nucleoporin subunit of the conserved Nup107-160 subcomplex, an essential structural unit of the NPC. Unexpectedly for a component of the Nup107-160 subcomplex, Nup133 exhibits cell-type and developmental stage-specific expression that is restricted predominantly to dividing progenitors. Lack of Nup133 impairs differentiation of pluripotent epiblast cells, as well as of ES cells, into neural progenitors competent to generate post-mitotic neurons. The Nup133-deficient neural progenitors abnormally maintain features of the transcriptional program and cell cycle of pluripotent early epiblast/ES cells. Since our studies on Nup133 deficient ES cells and embryos detected no global defects in functions normally associated with components of the NPC, nucleo-cytoplasmic transport and stability of chromosome number, Nup133 regulates stem/progenitor cell differentiation by an as yet unknown mechanism. Ongoing experiments to elucidate this mechanism are guided by a working model positing that rapidly proliferating embryonic progenitor cells, as well as adult transit-amplifying progenitors, require Nup133-NPCs to facilitate the alterations in gene activity and/or cell cycle parameters that direct the restriction of a cell's differentiation potential as it commits to a lineage, sub-lineage, or terminal differentiation pathway. Testable corollaries of this model are that NPCs in terminally differentiated cells and in adult tissue stem cells will lack Nup133, possibly to protect and stabilize their gene expression profiles. To examine the requirement for Nup133 in distinct embryonic and adult cell lineages we have developed a line of mice carrying a conditional allele of *Nup133*.

DIRECTED DIFFERENTIATION OF EXTRAEMBRYONIC ENDODERMAL STEM CELLS

Jerome Artus and Anna-Katerina Hadjantonakis

Developmental Biology Program, Sloan-Kettering Institute, New York, NY

Mammalian embryonic development begins with a period mainly devoted to the generation of extraembryonic tissues, the trophectoderm (TE) and the primitive endoderm (PrE), that have an essential role in supplying nutrients to and patterning the embryo. The PrE emerges from the inner cell mass of the blastocyst and is evident as an epithelial layer in contact with the blastocoel. It gives rise to two major tissue types: the parietal endoderm (PE) and the visceral endoderm (VE). The VE lies in close contact with the pluripotent epiblast (EPI) where it serves not only a nutritive function but also an essential role in patterning the embryo. The isolation and characterization of stem cells representative to the first three lineages provides *ex vivo* models to study the mechanisms underlying lineage specification, maintenance and expansion.

We are using extraembryonic Endoderm (XEN) stem cells representing the PrE lineage to study its maintenance and differentiation. Controlling XEN cell differentiation towards the VE lineage may represent an improved method of directing ESC differentiation. Here we report that among the superfamily of TGF β secreted factors, exogenous addition of bone morphogenetic protein (BMP) directs the differentiation of XEN cells into VE. This is demonstrated by the dramatic change in cell morphology as XEN cells form a fully polarized epithelium and by the upregulation of various VE markers. Interestingly, this effect is reversible but stable as cells can be propagated for extended periods of time in the polarized epithelial state, suggesting that they have not entered a committed program of differentiation. Furthermore, using fluorescent reporters and live imaging techniques, our preliminary data suggest that XEN cells, which have been previously reported to represent a heterogeneous population, might oscillate between different states.

CHINMO IS A FUNCTIONAL EFFECTOR OF THE JAK/STAT PATHWAY THAT REGULATES EYE DEVELOPMENT, TUMOR FORMATION AND STEM CELL SELF-RENEWAL IN DROSOPHILA

Maria Sol Flaherty¹, Pauline Salis^{1§}, Cory J. Evans^{3§}, Laura A. Ekas^{1§}, Amine Marouf¹, Jiri Zavadil², Utpal Banerjee³⁻⁶, Erika A. Bach¹

¹Pharmacology Department, New York University School of Medicine, ²Department of Pathology, NYU Cancer Institute and Center for Health Informatics and Bioinformatics, NYU Langone Medical Center, New York, NY 10016-6402, ³Department of Molecular, Cell and Developmental Biology, ⁴Department of Biological Chemistry, ⁵Molecular Biology Institute and ⁶Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, UCLA, Los Angeles, CA, 90095-1606

§These authors contributed equally to this study.

The *Drosophila* STAT transcription factor Stat92E regulates diverse functions, including organ development and stem cell self-renewal. However, the Stat92E functional effectors that mediate these processes are largely unknown. Here we show that *chinmo* is a cell-autonomous, downstream mediator of Stat92E that shares numerous functions with this protein. Loss of either gene results in malformed eyes and head capsules due to defects in eye progenitor cells. Hyperactivation of Stat92E or misexpression of Chinmo results in blood cell tumors. Both proteins are expressed in germline (GSCs) and cyst stem cells (CySCs) in the testis. While *Stat92E* is required for the self-renewal of both populations, *chinmo* is only required in CySCs, indicating that Stat92E regulates self-renewal in different stem cells through independent effectors. Like hyperactivated Stat92E, Chinmo misexpression in CySCs is sufficient to maintain GSCs non-autonomously. Chinmo is therefore a key effector of JAK/STAT signaling in a variety of developmental and pathological contexts.

HAIR FOLLICLE MESENCHYMAL STEM CELLS AS A SOURCE OF SMOOTH MUSCLE CELLS FOR ENGINEERING MECHANICALLY ROBUST AND VASOREACTIVE VASCULAR MEDIA

Hao-Fan Peng¹, Jinyu Liu¹, Daniel D. Swartz^{2,3*}, Stelios T. Andreadis^{1,3*}

¹*Department of Chemical and Biological Engineering,* ²*Department of Pediatrics, Women and Children's Hospital of Buffalo and* ³*Center of Excellence in Bioinformatics and Life Sciences, University at Buffalo, State University of New York, Amherst, NY 14260-4200*

We investigated the potential of hair follicle cells for multi-lineage differentiation and as a source of functional smooth muscle cells. We report that human hair follicle stem cells (HFC) isolated from individual follicles expressed markers of mesenchymal stem cells. HFC can also differentiate towards adipocytes, chondrocytes, osteoblasts or smooth muscle cells. We further isolated smooth muscle progenitor cells (HF-SMC) using a tissue-specific promoter and fluorescence-activated cell sorting. HF-SMC expressed several markers of vascular smooth muscle cells and exhibited vascular contractile function. They also demonstrated high proliferation and clonogenic potential. In addition, we aimed at engineering a vascular media using HF-SMC and a biomaterial, namely small intestine submucosa (SIS). Engineering functional vascular constructs required application of unidirectional mechanical strain to direct cell alignment which was necessary for development of receptor and non-receptor mediated contractility as soon as 24 hr after cell seeding. Within two weeks in culture, the cells migrated into SIS and secreted collagen and elastin, the two major extracellular matrix components of the vessel wall. Notably, vascular reactivity increased significantly and for some agonists approached that of native arteries. The mechanical strength and elasticity were also similar to native blood vessels. Taken together, our data demonstrate that hair follicle is an excellent source of contractile SMC for engineering mechanically strong, biologically functional vascular media with potential for arterial implantation.

EXPANSION OF HUMAN STEM CELLS IN A MICROCARRIER BIOREACTOR AND THEIR DIRECTED DIFFERENTIATION TOWARD PANCREATIC ISLET CELLS

Lye T. Lock¹ and Emmanuel (Manolis) S. Tzanakakis^{1,2}

¹*Department of Chemical & Biological Engineering, State University of New York, Buffalo, NY 14260 and*

²*New York State Center of Excellence in Bioinformatics and Life Sciences, Buffalo, NY 14203*

Human embryonic stem cells (hESCs) hold great promises as sources of therapeutics for a wide range of maladies such as Parkinson disease, myocardial infarction and diabetes mellitus. Of major importance for hESC-based therapies to become a clinical reality is the development of bioprocesses for the expansion of stem cells and their derivatives to large quantities.

Stirred-suspension bioreactors are scalable and allow for continuous monitoring and control of the culture conditions. To that end, microcarrier systems are characterized by a high surface area-to-volume ratio. We demonstrated the use of a microcarrier stirred-suspension culture system for the propagation of pluripotent hESCs. Stem cells seeded on microcarriers and cultivated for ~1 week in a stirred-suspension bioreactor remained viable (>85%) and increased by 32-fold. The cells maintained their expression of pluripotency markers OCT4, NANOG, TRA-1-81 and SSEA4 as revealed by quantitative PCR (qPCR) and antibody staining.

Besides the scalable propagation of pluripotent stem cells, we developed differentiation strategies for directing the stem cell fate along pancreatic islet cell lineages in both static and bioreactor cultures. Human ESCs and induced pluripotent (hiPSCs) were exposed in a stepwise manner to physiologically relevant factors, which are involved in the development of the embryonic pancreas. The differentiating cells transitioned through definitive endoderm (DE), primitive gut tube (PGT), and posterior foregut (PFG) cells with concomitant morphological and biochemical changes. Cells emerged which were positive for markers including PDX1, HNF6, and HBLX9. Markers expression was assessed by qPCR, immunocytochemistry and flow cytometry. We provide a first account of the expansion of hESCs and directed differentiation into pancreatic progeny in scalable microcarrier bioreactor. Current work focusing on further differentiation into pancreatic islet (PI) cells will also be presented. Our findings warrant further development of scalable bioprocesses for producing therapeutically useful cells from stem cells in clinically relevant quantities.

PROTEOGLYCOMICS OF HUMAN EMBRYONIC STEM CELLS

Robert J. Linhardt,^{1,4} Jonathan S. Dordick,^{1,3} Kelley Moreman,⁵ Stephen Dalton,⁵ Alison Nairn,⁵ Leyla Gasimli,¹ Boyangzi Li,⁴ Luciana Meli,² Hope E. Stansfield³

¹Department of Biology, ²Department of Chemical and Biological Engineering, ³Department of Biochemistry and Biophysics, ⁴Department of Chemistry, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY, ⁵Complex Carbohydrate Research Center, University of Georgia, Athens, GA

Many of the important signaling molecules in embryonic stem cells (ESCs) rely on the involvement the glycosaminoglycan (GAG) of proteoglycans (PGs). For example, Kraushaar *et al.*, recently showed that the heparan sulfate PG is required for ESCs to exit from self-renewal (*J Biol Chem* 285:5907, 2010). Our laboratory has developed an approach to sensitively analyze the disaccharide composition of GAG chains of murine ESCs (Nairn *et al.*, *J Proteome Res* 6: 4374, 2007). The GAGs are recovered from 10³-10⁶ cells and sequentially depolymerized with chondroitin lyases and heparin lyases to afford the constitutive disaccharides. The disaccharides are then analyzed using liquid chromatography (LC)—mass spectrometry (MS). Transcript levels of biosynthetic enzymes are matched to the GAG composition and cell-based microarray technology is being used to follow differentiation markers. This process has been used with success with pluripotent human embryonic stem cells, BG01, BG02, H7 and H9 and with human pluripotent embryonal carcinoma cells, NCCIT. A better understanding of the cellular signaling pathways involved in differentiation and expansion may result from tracking the temporal changes in GAG disaccharide content of these pluripotent cells.

ACTIVATION OF HH PATHWAY ENHANCES OSTEOGENIC AND CHONDROGENIC DIFFERENTIATION OF PERIOSTEUM-DERIVED MESENCHYMAL STEM CELLS AND IS INVOLVED IN POSTNATAL BONE REPAIR

Qun Wang, Chunlan Huang, Ming Xue, Xinping Zhang

Center for Musculoskeletal Research, University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642

While the essential role of periosteum in cortical bone repair and regeneration has been well established, the molecular pathways that control the early osteogenic and chondrogenic differentiation of periosteal stem/progenitor cells during repair processes remain superficially understood. Utilizing a segmental bone graft transplantation model in mice, we isolated a population of early periosteum-callus-derived mesenchymal stem cells (PCDSCs) from the healing autograft periosteum. These cells express typical mesenchymal stem cell markers (Sca-1, CD105, SSEA-4, CD29 and CD140) and are capable of differentiating into osteoblasts, adipocytes, and chondrocytes. They demonstrate significant proliferation advantage over MSCs from bone marrow (BMSCs) and adipose tissue (ADSCs) in culture. Characterization of these cells demonstrated that activation of the hedgehog (Hh) pathway effectively promoted osteogenic and chondrogenic differentiation of PCDSCs *in vitro*. When implanted subcutaneously in athymic nude mice, these hedgehog-overexpressing cells markedly induced bone formation. To further determine the role of Hh pathway in adult bone repair, we utilized a genetic approach to delete Smoothed (Smo), the receptor that transduces all Hh signaling at the onset of bone autograft repair via a Tamoxifen-inducible RosaCreER mouse model. We found that deletion of Smo markedly reduced osteogenic differentiation of isolated PCDSCs, as evidenced by reduced alkaline phosphatase staining and marked reduction of the osteoblastic transcriptional factor OSX gene expression. MicroCT and histomorphometric analyses further showed a near 50% reduction of periosteal bone callus formation at the cortical bone junction in bone autografts lacking the expression of Smo. Taken together, these data suggest that Hh pathway plays an important role in adult bone repair via enhancing differentiation of periosteal progenitors. The robust bone formation induced by activation of Hh pathway in periosteum-derived MSCs further implicates the potential utility of Hh-activated MSCs for repair and reconstruction of bone defect.

ISOLATION, EXPANSION IN CULTURE AND CHARACTERIZATION OF STEM CELLS FROM SKELETAL MUSCLE (SATELLITE CELLS)

Natalia Tyhovich, Whitney Ijem, Yoo Shin, KeunHee Park, Todd Rubin, David N. Levy, Louis Terracio
New York University, College of Dentistry, New York, NY

The long-term objective of our laboratory is to engineer an implant derived from a patient's skeletal muscle stem cells (satellite cells) that can be used to restore the structure and function of compromised skeletal muscle. We have proposed to use a pig model as a transition toward our long-range goal in humans.

Using small pieces of muscle that would not be debilitating if removed from a human (about the size of a quarter); we have developed procedures and have successfully isolated pig satellite cells. We have also adapted a tissue culture medium that has allowed us to expand the small number of cells isolated for the muscle (<1,000) to several million cells (>5 million) in two weeks. We have adapted a tissue culture medium for our use that appears to slow down the growth of fibroblasts and stimulates the growth of the satellite cells. We have used Flow Cytometry to characterize the satellite cells and will in the future separate them from the contaminant fibroblasts. We have found a commercial source of an antibody against integrin alpha 7 that is labeled with a fluorescent compound. Alpha 7 integrin is located on satellite cells and not on fibroblasts and has been useful in Flow Cytometry. Our next step is to purify the satellite cells and then re-expand them in culture so that we can have a large number of cells to study in our tissue engineering model. We will transfer this experience and knowledge to the isolation of Human satellite cells.

Impact: We are on target to within three years determine if the proposed approach is feasible as a method to replace lost muscle in humans.

GENERATION OF iPS CELLS FROM PARKINSON'S DISEASE PATIENTS WITH PARKIN MUTATIONS

Houbo Jiang¹, Kazuhiro Nakaso², Jian Feng¹

¹*Department of Physiology and Biophysics, State University of New York at Buffalo, Buffalo, NY and*

²*Department of Neurology, Tottori University, Yonago, Japan*

Parkinson's disease (PD) is characterized by the degeneration of dopaminergic neurons in substantia nigra. A variety of genetic and environmental factors contribute to the pathogenesis of Parkinson's disease. Mutations of parkin represent the most frequent cause of recessive forms of familial Parkinson's disease. To understand how parkin mutations cause the degeneration of nigral dopaminergic neurons and ensuing Parkinson's disease, we are generating iPS cells from dermal fibroblasts of PD patients with parkin mutations. Primary dermal fibroblast cultures were derived from skin biopsies of a PD patient with homozygous exon 3 deletion of parkin and another PD patient with compound heterozygous deletions of exon 3 and exon 5 of parkin. Both lines of fibroblasts were reprogrammed to iPS cells with tetracycline-inducible lentiviruses expressing Oct4, Sox2, Klf4, c-Myc and Nanog. Several iPS clones were obtained for each fibroblast line. All the iPS clones exhibited morphology resembling human embryonic stem (hES) cells and could be passaged indefinitely on MEF feeders or matrigel. Pluripotency markers such as Tra-1-60, Tra-1-81, SSEA3, SSEA4, Oct4, Sox2, Nanog, AP, etc. were expressed at levels similar to H9 hES cells. Most viral transgenes were strongly silenced in the absence of doxycycline. We are in the process of removing the viral transgenes by cre-mediated excision. After virus-free secondary iPS cells are generated, we will differentiate them in vitro to midbrain dopaminergic neurons using a chemically defined, directed differentiation protocol. Functional studies will be performed to assess the impact of parkin mutations on the survival of midbrain dopaminergic neurons.

IDENTIFICATION OF A NOVEL REGULATOR OF HUMAN EMBRYONIC STEM CELL PLURIPOTENCY

Xin Zhang¹, Safak Yalcin¹, Marion Kennedy², Rani Sellers³, Markus Landthaler⁴, Tom Tuschl⁴, Gordon Keller², Saghi Ghaffari^{1,5-7}

¹*Department of Gene and Cell Medicine, Mount Sinai School of Medicine, New York, NY 10029,*

²*McEwen Center for Regenerative Medicine, University Health Network, Toronto, Ontario, Canada,*

³*Department of Pathology, Albert Einstein College of Medicine, New York, NY 10461, ⁴Howard Hughes Medical Institute, Laboratory for RNA Molecular Biology, The Rockefeller University, New York, NY 10065, ⁵Black Family Stem Cell Institute, ⁶Department of Regenerative & Developmental Biology and ⁷Department of Medicine Division of Hematology, Oncology, Mount Sinai School of Medicine, New York, NY 10029*

Forkhead Box O (FoxO) of Forkhead family are evolutionary conserved transcription factors downstream of PI3-kinase/AKT signaling pathway whose function is inhibited by AKT phosphorylation. FoxOs are involved in the regulation of longevity, are essential for stress response and exert critical functions in adult stem cells and in many different tissues, however whether they are involved in the regulation of embryonic stem (ES) cells is not known. FoxO1, FoxO3, FoxO4 are widely expressed while FoxO6 is specific to neuronal tissues. Here we show that in undifferentiated self-renewing ES cells and during lineage specification FoxO protein expression is differentially regulated. In addition, using loss and gain of function approaches in both H1 and HES2 lines, we demonstrate that FoxO1, but not other FoxOs, is critical for maintenance of human ES (hES) cell pluripotency. Doxycycline Tet-On inducible shRNA knockdown of FoxO1 delivered by lentiviruses resulted in 90% reduction in expression of FoxO1 protein associated with rapid and significant reduction in expression of pluripotency markers Oct4, Sox2 and Nanog as well as TRA-1 81 and TRA-1 60 and SSEA4, and inhibition of teratoma formation in mice. Consistent with this, knock down of FoxO1 led to the erroneous induction of mesoderm and endoderm differentiation under undifferentiation self-renewal conditions of hES cells. Similar results were similar for three distinct FoxO1 shRNAs. These striking findings were not the result of loss of a generic function of FoxO1 on cell proliferation, survival or oxidative stress conditions. In addition, ectopic lentiviral expression of FoxO1 specifically induced significant upregulation of Oct4 and Sox2 expression. We show that FoxO1 but not FoxO3 regulates Oct4 and Sox2 by direct binding to and activation of their promoters. These findings support a non-redundant function for FoxO1 in the regulation of hES cell fate and have critical implications for stem cells, development and reprogramming.

IMPROVEMENT OF REPROGRAMMING VIA CHEMICAL AND GENETIC APPROACHES

Claudia A. Doege, Ryouusuke Fujita, William B. Vanti, Skylar Travis, Liang Qiang, Asa Abeliovich
Dept. of Pathology, Columbia University Medical Center, New York, NY

Background: Intriguingly, somatic cells can be reprogrammed into induced pluripotent stem (iPS) cells by ectopic expression of only 4 transcription factors, Oct4, Sox2, Klf4, and Myc. iPS cells hold great promise for regenerative medicine. However, their oncogenic potential and low generation efficiency hinder future clinical application in humans. Thus, development of new strategies with enhanced efficiency and safety are central to reprogramming research.

Reprogramming is thought to be a multi-step process and intracellular signaling is likely a very important component. But, which intracellular signaling cascades are downstream targets of reprogramming factors at the various stages, has yet to be determined. Rapid kinetics via phosphorylation and other protein modifications, switch-like regulation, and the availability of well established inhibitors (chemical compounds) make signaling cascades an exciting target for manipulations to improve iPS cell generation. Indeed, controlled, short-lived pharmacological manipulation of signaling pathways might efficiently generate iPS cells without oncogenic potential.

Results: The PI3K/GSK3 β pathway is important for proliferation, growth, and survival. In fact, we found that in response to ectopic expression of the pluripotency genes this pathway is activated as measured by an increased phosphorylation on Akt473. Furthermore, the GSK3 β inhibitor 6-bromindirubin-3'-oxime (BIO) increases the efficiency of iPS cell generation.

Future Directions and Impact: We are using chemical and genetic approaches targeting different intracellular pathways to replace single, or more factors. This will further our understanding of involved pathways and their importance and function in reprogramming.

COMPARISON OF MOTONEURON DIFFERENTIATION EFFICIENCY BETWEEN hES AND hiPS CELL LINES DERIVED FROM ALS PATIENTS AND CONTROLS

Gist Croft^{1,2}, Derek Oakley^{1,2}, Evangelos Kiskinis^{3,4}, Gabriella Boulting^{3,4}, Mackenzie Weygandt¹, Kevin Egan^{3,4}, Christopher E. Henderson^{1,2}, Hynek Wichterle^{1,2}

¹*Project ALS, Jennifer Estess Laboratory for Stem Cell Research, New York, NY*, ²*Columbia University College of Physicians & Surgeons, Pathology Department, New York, NY*, ³*Harvard Stem Cell Institute, Cambridge, MA* and ⁴*Harvard University Department of Molecular Biology, Cambridge, MA*

The goal of this project is to develop criteria to evaluate the quality of induced pluripotent stem (iPS) cell lines and their applicability to study neurodegenerative diseases. We derived a series of iPS lines from patients with Lou Gehrig's disease (ALS) and controls. The 12 lines included in the study allow us to determine whether the method of iPS cell derivation (3 reprogramming factors vs. 4) or individual donor-specific genetic background influences the behavior of the lines; and whether patient-derived cells exhibit disease-related differentiation phenotypes. In addition, four human ES cell lines were characterized to examine whether iPS cells differ in a significant manner from well characterized ES cells. We confirmed the pluripotency of all iPS and ES cell lines by Tra-1-60 and SSEA3 expression and performed differentiation of all cell lines into motoneurons. Individually, all cell lines except one showed similar expression of cell-surface pluripotency markers and differentiated into motoneurons with similar efficiency. The poorly differentiating line could be identified prospectively by higher and more uniform expression of a pluripotency marker Tra-1-60. Several lines failed to silence reprogramming transgenes in both pluripotent and differentiated cells. However, this was not associated with abnormal differentiation phenotypes. Our results show that these two classes of anomalies occur in iPS cell lines at a manageable rate. We observed no systematic differences between 3 and 4 factor lines or ALS and control iPS lines. We did however, observe subtle donor-specific differences in the efficiency of motor neuron generation. Our results suggest that iPS cell lines and neurons derived from them will be useful as cellular models of neurodegenerative diseases.

THERAPEUTIC β -GLOBIN EXPRESSION IN THALASSEMIA PATIENT INDUCED PLURIPOTENT STEM CELLS FROM GENOMIC SAFE HARBORS

Eirini P. Papapetrou^{1,2}, Gabsang Lee³, Nirav Malani⁴, Isabelle Rivière^{1,2}, Frederic D. Bushman⁴, Lorenz Studer^{1,3}, Michel Sadelain^{1,2}

¹Center for Cell Engineering, ²Molecular Pharmacology and Chemistry Program, ³Developmental Biology Program, Memorial Sloan-Kettering Cancer Center, New York, NY and ⁴Department of Microbiology, University of Pennsylvania, Philadelphia, PA

The advent of human induced pluripotent stem (iPS) cells enables for the first time the derivation of unlimited numbers of patient-specific stem cells and holds great promise for disease modeling and regenerative medicine. However, realizing the full potential of iPS cells requires robust, precise and safe strategies for their genetic modification. Safe human iPS cell engineering is especially needed for therapeutic applications, as stem cell-based therapies that rely on randomly integrated transgenes pose oncogenic risks. Here we describe a strategy to genetically modify iPS cells from patients with β -thalassemia, in a potentially clinically relevant manner. Our approach hinges on the identification of “safe harbors” for transgene expression in the human genome. We show that thalassemia patient iPS cell clones harboring a single copy of a β -globin transgene cis-linked to locus control region (LCR) elements and an excisable Neo-eGFP transcription unit, can be isolated and screened according to chromosomal position. We next demonstrate that iPS cell clones that meet our safe harbor criteria resist silencing and allow for therapeutic levels of globin expression upon erythroid differentiation. Combined bioinformatics and functional analyses thus provide a robust and dependable approach for achieving controllable levels of transgene expression from selected chromosomal loci. This approach may be broadly applicable to introducing therapeutic or suicide genes into patient-specific iPS cells for use in cell therapy.

MODELING OF NEURAL CREST DISEASE IN PATIENT-SPECIFIC HUMAN iPSC AND PGD-hESC LINES FOR FAMILIAL DYSAUTONOMIA AND CHARCOT-MARIE-TOOTH 1A

Gabsang Lee^{1,2}, Hyesoo Kim^{1,2}, Ben Lannon², Christina Ramirez¹, Hakim Djaballah¹, Kevin Eggan³, Lorenz Studer^{1,2}

¹Sloan-Kettering Institute, ²Center for Stem Cell Biology, New York, NY, USA, ³Division of Reproductive Endocrinology & Infertility, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA and ⁴Department of Stem Cell and Regenerative Biology, Harvard Stem Cell Institute, Boston, MA

The ability to generate patient-specific hiPSCs offers great promise for a new era in disease modeling and drug discovery. Here we discuss our current efforts on hiPSC based modeling of Familial Dysautonomia (FD) and of Charcot-Marie-Tooth 1A (CMT1A), genetic NC disorders affecting peripheral neurons and Schwann cells respectively.

As shown in our previous publication, we derived and characterized multiple independent iPSC lines from fibroblasts of FD, a genetic disorder affecting peripheral neurons and caused by mutation/splicing defect in IKBKAP. We observed defects in neuronal differentiation and in cell motility and were able to test the effect of several known candidate chemicals on reversing these phenotypes. One (kinetin) of candidate compounds was able to partially rescue the phenotype in FD-iPSC-neural crest cells (NCCs).

To find novel and more effective drug, we have established a FD-iPSC based high-throughput screening assays using a chemical compound library (over 5000 FDA-approved drugs and natural products). The primary screen measures the impact of these chemicals using 384-well-format qRT-PCR for wild-type IKBKAP expression. While assay development and compound screening is ongoing, our current conditions yield a decent Z'-factor (> 0.5).

CMT1A is caused by the duplication of PMP22-containing genomic region and represents the most common genetic disease in PNS. We have successfully reprogrammed CMT1A fibroblasts into CMT1A-iPSCs and also have derived CMT1A-hESC from PGD embryos to compare potential disease phenotypes. Both CMT1A-iPSC/PGD-hESC lines show a normal karyotype and exhibit appropriate pluripotency marker profiles. NCCs from patient specific lines and control lines have been isolated and differentiated using a novel Schwann cell differentiation protocol.

In conclusion, our studies demonstrate the potential for modeling FD-NC disease and performing high throughput drug discovery in patient-specific and disease-relevant cells, and address the comparative potential of hiPSC and PGD-hESCs in modeling CMT1A disease.

(1) NEURAL PROGENITOR CELLS IN THE DENTATE GYRUS INCREASE IN HUMANS WITH ANTIDEPRESSANT TREATMENT

Maura Boldrini^{1,5,7}, Mark D. Underwood^{1,5}, Kelly M. Burke⁵, Gorazd B. Rosoklija^{1,5,8}, Andrew J. Dwork^{1,3,5}, J. John Mann^{1,5}, Rene' Hen^{1,2,4,6}, Victoria Arango^{1,5}

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

Antidepressants increase the number of neural progenitor cells (NPCs) and mitotic cells in the adult dentate gyrus (DG) in mammals and in patients with Major Depression (MDD). We sought to determine whether NPCs increase in the human DG of subjects with MDD treated with selective serotonin reuptake inhibitors (MDD*SSRIs) or tricyclic antidepressants (MDD*TCAs) compared with untreated MDDs and control subjects with no evidence of psychiatric illness.

The right hippocampi from MDDs (N=10), MDD*SSRIs (N=4), MDD*TCAs (N=5) and matched controls (N=10) were fixed, sectioned at 50 µm and immunostained with antibodies to nestin. The total number of nestin-immunoreactive (-IR) cells in the DG was estimated by stereology. All cases were characterized psychiatrically by psychological autopsy and underwent neuropathological and toxicological screens. We used ANOVA with Tukey *post-hoc* test for statistical analyses.

There were more nestin-IR NPCs in the MDD*SSRIs group (F=3.687; df=3,25; p=0.025) compared with untreated MDDs (p=0.046) and controls (p=0.039). The observed increase in cell number was more pronounced in the most anterior 7mm of the dentate gyrus.

This NYSTEM-supported stem cell research project has provided a confocal microscope (Olympus FV1000) to be shared by several laboratories. In the upcoming year we plan to perform double-labeling experiments to assess the phenotype of replicating cells in the human DG and to use markers for later stages of maturation. This will allow us to determine whether there is a difference in numbers of neuroblasts or mature neurons in the DG of treated MDDs, compared with untreated MDDs and controls.

This project provides the first evidence of more NPCs in the DG of MDD subjects treated with SSRIs, compared with untreated MDDs and controls. If replication of NPCs in the DG is a mechanism of antidepressant action, treatments affecting cell replication and survival might be key to improving the management of MDD.

(2) INTERACTIONS OF GENES AND EARLY LIFE STRESS ON HIPPOCAMPAL NEUROGENESIS IN ADULT MONKEYS

William Blair¹, Tarique D. Perera^{1,2}, Claire E. Sexton³, Yelena Nemirovskaya^{1,4}, Anna V. Rozenboym^{1,4}, Charles B. Nemeroff⁵, Michael Owens⁵, Joel Gelenter⁶, Joan Kauffman⁶, Andrew J. Dwork^{7,8}, Jeremy D. Coplan^{1,4}

¹Geriatrics, New York State Psychiatric Institute, New York, NY, ²Psychiatry, Columbia University, New York, NY, ³Psychiatry, Oxford University, Oxford, UK, ⁴Psychiatry, SUNY Downstate Medical Center, Brooklyn, NY, ⁵Psychiatry, Emory University School of Medicine, Atlanta, GA, ⁶Psychiatry, Yale University School of Medicine, New Haven, CT, ⁷Neuroscience, New York State Psychiatric Institute, New York, NY and ⁸Pathology and Cell Biology, Columbia University, New York, NY

The SUNY primate colony has an extensively characterized model of early life stress. Exposing mothers of infant bonnet macaques to alternating weeks of variable foraging demand (VFD) generates negligent caretaking of infant offspring, resulting in bio-behavioral abnormalities that persist into adulthood, including increased cerebrospinal corticotropin releasing factor (CRF) (Coplan *et al.*, 1996), abnormal reaction to acute intruder stress (Rosenblum *et al.*, 2001), subordination, despair, and clinging behavior (Coplan *et al.*, 1996). We examined whether VFD-rearing is modulated by the presence of short allele of the serotonin transporter-related gene (5-HTTLPR) because this gene interacts with early life stress to predispose monkeys (Barr *et al.*, 2004) and humans (Caspi *et al.*, 2003) to depression and anxiety later in life. We found that VFD-reared subjects with (one or two copies) the short allele of the 5-HTTLPR genotype (n=4) experience a greater loss of maternal attachment during infancy than VFD homozygous for the long allele (n=4). As juveniles and adults, these same VFD with the short allele had greater increments of CRF and more timidity during acute intruder stress than VFD and normal reared (LFD, n=4) subjects homozygous for the long allele. Voxel Based Morphometry (VBM) analyses of 3D MRI hippocampal images showed a significant decrease in left hippocampal volume in VFD subjects compared to normal reared LFDs independent of genotype. In a separate group of adult bonnets, we found that VFD-rearing decreased rates of stem cell proliferation, neurogenesis rates, and expression of the neuroprotective gene BCL2 in the hippocampus (n=4) compared to normal reared (LFD) controls (n=4). Neurogenesis rates negatively correlated with CRF levels independent of rearing status.

(3) DIFFERENTIATION OF HUMAN RETINAL PIGMENT EPITHELIUM CELLS INTO MULTI-LINEAGES

Enrique Salero and Sally Temple

New York Neural Stem Cell Institute, Rensselaer, NY

The retinal pigment epithelium (RPE) forms a single layer of highly specialized cells that is required for maintenance of the retina and remains non-proliferative throughout life. In amphibians and in embryonic chick and mouse, RPE cells can produce other retinal and even lens tissues, suggesting an inherent plasticity. In humans, RPE cells can be activated and proliferate when they are removed from their normal environment. Retinal stem cells have been identified in the ciliary epithelium and iris pigmented epithelium of adult rodents and human that can self-renew in vitro and differentiate into retinal neurons and glia. However, until now, there has been no evidence for a stem-like cell from the adult RPE. The ability of stem cells to self-renew, to form cell lines and differentiate into many cell types led us to hypothesize that the adult RPE retains dormant properties of early neuroepithelial cells that can be activated if it is removed from its normal environment. We demonstrate that adult human RPE cells grown in tissue culture under appropriate culture conditions are highly proliferative and have the ability to produce a wide repertoire of progeny including bone, cartilage, muscle, adipocytes and neural retina progenitors. RPE derived progenitor cells can self-renew and express genes associated with pluripotency, such as SOX2, c-MYC and KLF4. RPE progenitor cells can grow into clones and differentiate into multi-lineages. This study establishes the human RPE to be a unique source of multipotent cells for the study of cell fate choice, cell replacement therapy and disease modeling in the central nervous system. We will continue our studies on the patient-derived RPE cells to demonstrate that these cells are multipotent and test the gene expression changes that accompany these differentiation events. Patient-matched stem cell lines derived from RPE biopsies could be a unique source of multipotent stem cells for the study of cell fate choice and could be used to generate specific cell types for cell replacement therapy.

(4) TRANSCRIPTIONAL CONTROL OF CANCER STEM CELLS IN ACUTE MYELOID LEUKEMIA

Laura Barreyro, Yiting Yu, Britta Will, Cristina Montagna, Amit Verma, Ulrich Steidl

Albert Einstein College of Medicine, Bronx, NY 10461

In acute myeloid leukemia (AML) a pool of rare cancer/leukemia stem cells (LSC) give rise to a hierarchy of functionally heterogeneous bulk tumor cells with limited self-renewal capacity. Thus, defining the characteristics of LSC development is critical to understand the genesis of leukemia and to develop strategies by which these cells can be eradicated. In order to identify functionally critical LSC pathways, fundamentally novel experimental approaches other than the examination of bulk tumor cells need to be established. Within the supported project we are analyzing highly fractionated hematopoietic stem and progenitor cells of patients with AML. So far, we have analyzed samples from patients carrying deletions or complete loss of chromosome 7 (N=6), a frequent AML subtype with particularly bad prognosis. Bone marrow samples from healthy control persons serve as controls (N=6). Cells are stained with antibodies against lineage-antigens, CD34, CD38, CD90, IL3R α and CD45RA. We then isolated long-term hematopoietic stem cells (HSC), short-term HSC, megakaryocyte-erythrocyte progenitors, common myeloid progenitors and granulocyte-monocyte progenitors by means of high-speed multi-parameter fluorescence-activated cell sorting (FACS). Fluorescence in-situ hybridization (FISH) of sorted cells showed that chromosome 7 aberrations were already detectable in the earliest HSCs suggesting that those are part of the leukemia-initiating clone. Total RNA was extracted for each sorted population, linear amplified utilizing single primer isothermal amplification (SPIA), and hybridized to Affymetrix Human Gene 1.0 ST arrays. Raw data were processed using Expression Console software (Affymetrix). Statistical analysis was performed using CLC Genomics Workbench (CLC Bio). We find significant gene expression changes in HSCs of AML patients and healthy controls suggesting that transcriptional deregulation in AML occurs as early as the HSC compartment. We are currently confirming and screening candidate genes for further functional examination, and to test their potential usefulness as targets for LSC-directed therapeutic approaches.

(5) HTLV INFECTION OF HUMAN HEMATOPOIETIC STEM CELLS INDUCES ATLL IN HU-SCID MICE: ROLE OF “INFECTED” CANCER STEM CELLS

Prabal Banerjee^{1,2}, Lindsey Crawford¹, Elizabeth Samuelson¹, Michelle Sieburg^{1,2}, Mark Woodford^{1,2}, Gerold Feuer^{1,2}

¹Department of Microbiology and Immunology and ²Humanized SCID Mouse Center and Stem Cell Processing Laboratory, SUNY Upstate Medical University, Syracuse, NY 13210

Human T cell leukemia/lymphoma virus type 1 (HTLV-1) is a human retrovirus which is intimately linked to the development of adult T cell leukemia/lymphoma (ATLL), a monoclonal malignancy of CD4+/CD25+ T cells. The understanding of how this human retrovirus initiates leukemia at the molecular level has been hampered by the lack of an animal model which recapitulates disease. We have determined that human CD34+ hematopoietic progenitor and stem cells (HP/HSCs), which reside and are enriched in the human bone marrow serve as a reservoir for HTLV-1 infection *in vivo* and that the virus initiates leukemogenesis following infection of stem cells. HTLV-1 infection of CD34+ HP/HSCs and inoculation of NOD/SCID mice results in reproducible induction of CD4+CD25+ T lymphomas, similar to ATLL. Lentivirus transduction of the HTLV-1 Tax oncoprotein (Tax1) also induces T cell lymphomas in HU-SCID mice. This novel animal model now provides the opportunity to characterize molecular events which predispose stem cells to become leukemic and to evaluate the role of accessory HTLV-1 genes on viral replication *in vivo* and in the manifestation of leukemia. We speculate that HTLV-1 infection of CD34+ HP/HSCs induces pre-leukemic events in the bone marrow and establishes an “infected leukemia stem cell” (ILSC) which ultimately gives rise to the mature leukemia. We intend to characterize the pathobiology of HTLV-1 and HTLV-2 infection in the HU-SCID mouse and will characterize the individual roles of the viral genes Tax, p30^{II} and HBZ as determinants of pathogenesis *in vivo*. Characterization of HTLV-1 infection of CD34+ HP/HSCs may establish a new paradigm of oncoviral infections in humans and will provide insight into the extended clinical latency (~20-30 years) displayed by patients who develop ATLL.

(6) HUMAN PROSTATE CELLS ISOLATED BASED ON THE SIDE POPULATION PHENOTYPE DEMONSTRATE MORE STEM CELL PROPERTIES COMPARED TO CELLS ISOLATED BASED ON THE ALDEFLUOR[®] ASSAY

Kalyan J. Gangavarapu¹, Wiam Bshara², Lili Tian³, A. Latif Kazim⁴, Barbara A. Foster¹, Wendy J. Huss^{1,5}
¹Dept. Pharmacology and Therapeutics, ²Dept. of Pathology, ³Dept. Biostatistics University at Buffalo SUNY, Buffalo, NY; ⁴Dept. of Cell Stress Biology and ⁵Dept. of Urology Roswell Park Cancer Institute, Buffalo, NY

Prostate cancer represents a unique model to analyze cancer stem cells because of the high recurrence rate following androgen deprivation therapy and the ability to demonstrate stem cell properties in tissue recombination with rat urogenital mesenchyme (rUGM) that induces prostate tissue formation when implanted under the renal capsule. Benign and cancer stem cell populations can be enriched using functional assays based on the phenotypes of side population (SP) defined by the ATP binding cassette (ABC) mediated efflux of Hoechst or DyeCycle Violet fluorescent substrates, or aldehyde dehydrogenase (ALDH) mediated oxidation of aldehydes, ALDEFLUOR[®]. Both functional assays are based upon mechanisms that protect cells from environmental insult and contribute to a long lived stem cell population. We have initially analyzed and isolated cells based on SP and ALDH activity digested from more than four clinical prostate cancer specimens. Prostate stem cell properties were tested by serial recombination with rUGM and recombinants containing 50-2,000 sorted SP cells demonstrated human ductal growth more frequently >50% compared to <20% recombinants containing equal numbers of ALDH^{br} cells. We designed multiplex primers for 15 different genes and controls associated with stem cell biology and prostate differentiation using PrimerPlex 2.0. Gene expression is detected in single cells isolated based upon stem cell functions of SP or ALDEFLUOR[®] assay by sorting single cells onto hydrophilic circles on AmpliGRID slides. Reverse-transcription and multiplex PCR with fluorescent tagged primers is performed in an individual reaction with an AmpliSPEED cycler and analyzed by fluorescent capillary electrophoresis. A stem cell profile will be developed that can be tested in recombination with rUGM to test stem cell properties. Therefore, these studies seek to demonstrate the stem cell properties of cells isolated by functional assays and will lead to future therapeutic targets against cancer stem cells.

(7) USE OF THE PATIENT PANCREATIC TUMOR/SCID MOUSE XENOGRFT MODEL TO EVALUATE THE ROLE OF CANCER STEM CELLS IN THE RESPONSE OF A TUMOR TO NOVEL THERAPEUTICS

Bonnie L Hylander¹, Thomas Mace¹, Rohit Sharma², John F Gibbs², Scott Abrams¹, Elizabeth A Repasky¹

¹Dept. of Immunology and ²Dept. of Surgery, Roswell Park Cancer Institute, Buffalo, NY

Using a patient tumor/SCID mouse xenograft model, we have observed, as is often seen in the clinic, that even when a tumor undergoes regression in response to treatment, tumors begin to regrow when treatment is ended. It has been shown in preclinical models that cancer stem cells can be particularly resistant to chemo- and radiation therapy. Therefore, it is possible that CSCs survive and repopulate a recurring tumor. This is a critical issue because the role of cancer stem cells in the clinical treatment of malignancies is, as yet, unknown. Using the patient tumor/ SCID mouse model, we are examining whether cancer stem cells (CSCs) respond differently to therapies than the non-stem cells and whether their response dictates the ultimate response of the tumor. We have isolated pancreatic cancer stem cells (Li et al, *Canc. Res.*, 2007) from a patient xenograft which we had previously identified as sensitive to anti- DR5 targeted therapy. Two groups of mice were injected with 10^4 CSCs/ mouse. Mice in the control group received saline and were monitored for tumor growth; 4/5 animals developed tumors in 7 weeks. The other group received weekly treatment with anti-DR5 antibody until Group 1 developed tumors. At that time, no mice in the treated group had developed tumors, confirming the anti-tumor efficacy of the treatment. Treatment was stopped and mice were monitored for 30 weeks post-treatment; no tumors developed in the treated mice. These results suggest that anti-DR5 treatment was effective against both the bulk tumor cells and the CSCs. Results of these results will improve our understanding of the role stem cells play in the response of patients' tumors to novel therapeutics.

(8) IDENTIFYING CANCER STEM CELLS IN MALIGNANT MELANOMA

Asif Karim, Sonali Mohanty, Shweta Tiwary, Xuan Li, Brad Martin, Lei Xu

Department of Biomedical Genetics, University of Rochester Medical Center, Rochester, NY

Malignant melanoma is the most aggressive type of skin cancer and it is highly resistant to current cancer therapies. The cells that initiate melanoma, i.e. the melanoma stem cells, are thought to be responsible for the resistance and targeting these cells is predicted to increase the efficacy of melanoma treatment. However, melanoma stem cells are poorly characterized and no consistent markers exist for their proper identification. We proposed a new strategy of discovering melanoma stem cell markers. We reasoned that metastatic cells are presumed stem cells and therefore highly metastatic melanoma cells must be enriched for stem cells. We have previously derived a series of highly metastatic human melanoma cells from a pool of poorly metastatic parental line, using an experimental metastasis model. Bioinformatic analyses uncovered a list of 44 genes up-regulated in the highly metastatic derivatives relative to the parental line and they correlated with the aggressiveness of human melanoma metastases. We hypothesized that these 44 genes contain markers for melanoma stem cells and we planned to test four of them as a prove of principle. These four genes encode BMPR1A, EDNRB, ERBB3, and FZD7 (BEEF). We have successfully knocked down each of them by RNA-mediated interference in one of the highly metastatic derivatives, MA-2 cells. The knockdown cells showed decreased metastatic abilities relative to controls. Whether they also have decreased stem cell properties is under investigation both *in vitro* and *in vivo*. The results will potentially establish a new method to identify markers for cancer stem cells and provide novel therapeutic opportunities against cancers including malignant melanoma.

(9) USE OF iPS CELLS TO MODEL THE CONTRIBUTION AND SPECIFICITY OF REPLICATION ERRORS IN HEMATOPOIETIC CANCER

Michael E. Rusiniak, Ph.D., Anthony B. Csoka, Ph.D., Dimiter Kunnev, Ph.D., Angela Kudla, Amy Freeland, Gillian K. Cady, Steven C. Pruitt, Ph.D.*

Department of Molecular and Cellular Biology, Roswell Park Cancer Institute, Buffalo, NY 14263

Recent studies by this laboratory and others have provided support for the long standing assumption that replication related genetic damage plays a role in the etiology of both cancer and in age related dysfunction of somatic stem cells. Specifically, these studies have shown that deficiency for components of the DNA replication licensing complex (mini-chromosome maintenance proteins; MCMs) result in use of reduced numbers or origins *in vitro* (Ge *et al.*, 2007) and stem cell deficiency and cancers *in vivo* (Pruitt *et al.*, 2007; Shima *et al.*, 2007). Further, the specific phenotype (e.g. tumor type) resulting from Mcm deficiency in mice is critically dependent on genetic background. Based on these studies, we have hypothesized that the frequency with which genomic rearrangements occur at a given chromosomal location may be dependent on polymorphisms that affect replication origin usage. Here we seek to determine if inefficient replication origin usage at a frequent site of chromosome breakage and translocation on chromosome 11q23 in humans, and which is known to result in mixed-lineage leukemia (MLL), is a consequence of a genetic polymorphism resulting in inefficient origin usage at this site. To test this possibility requires a stable source of untransformed cells prior to undergoing the genetic rearrangement leading to the tumor. iPS cells derived from bone marrow of MLL patients who are in remission, and in which translocations are undetectable, are being generated for this purpose using lenti-viral mediated transduction of 3 or 4 reprogramming factors. Once established, replication origin usage and the frequency of translocation will be determined for each of 6 patient samples and compared with data from iPS cells derived from control donors. The effects of suppressing Mcm levels using siRNA and differentiation of iPS cells towards the hematopoietic lineage *in vitro* will also be determined.

(10) SUBLETHAL WHOLE BODY IRRADIATION CAUSES PROFOUND LATE DYSFUNCTION IN HEMATOPOIETIC STEM CELLS

James Palis¹, Olga Bromberg², Anne D. Koniski¹, Benjamin J. Frisch², Jacqueline Williams³, Paul D. Kingsley¹, Laura M. Calvi²

¹*Center for Pediatric Biomedical Research, Department of Pediatrics,* ²*Endocrine Division, Department of Medicine and* ³*Department of Radiation Oncology, University of Rochester School of Medicine, Rochester, NY*

Hematopoietic stem cells (HSC) are multipotent cells capable of extensive self-renewal that maintain all circulating blood cells throughout the life of an individual. While stem cells in general are considered radiation-resistant because of their quiescence, evidence indicates that HSC are sensitive to even low doses of radiation exposure, particularly in the short term. However, much less is known about the long-term effects of sublethal radiation on HSC. To better define the long-term effects of sublethal whole body irradiation (WBI) on the HSC compartment, adult C57BL/6 mice were treated with a single dose of 6Gy WBI. HSC were quantified by flow cytometry, and their function was tested by competitive repopulation transplantation assays. At 12 weeks after radiation, HSC-enriched lineage^{-negative}/Sca-1⁺/c-Kit⁺ (LSK) cells as well as their long-term (LT-HSC) and short-term (ST-HSC) subsets were dramatically decreased. Surprisingly, there was no evidence of long-term reconstitution from bone marrow cells from irradiated donors, suggesting that sublethal irradiation causes profound late functional defects of the HSC compartment, more severe than suggested by the phenotypic analysis. At 24 weeks, HSC numbers and function were similarly decreased, indicating that sublethal irradiation causes prolonged HSC damage. Despite this persistent devastating effect of sublethal radiation on HSC function, mice 24 weeks post-irradiation had average survival, minimal long-term changes in peripheral blood counts and marrow hematopoietic progenitor numbers, suggesting that radiation-injured HSC function better *in vivo* than when exposed to a recipient environment. One potential explanation for these unexpected results is that sublethal irradiation alters the bone marrow microenvironment to better support damaged HSC. If so, the marrow microenvironment may serve as an important therapeutic target to enhance both short-term and long-term hematopoietic recovery following sublethal clastogenic injury.

(11) MOLECULAR ANTIVIRAL TARGETING FOR CELL AND GENE THERAPY IN HEPATITIS C WITH STEM/PROGENITOR CELLS

Mukesh Kumar¹, Scott Garforth¹, Charles M. Rice² Vinayaka Prasad¹, Sanjeev Gupta¹

¹Albert Einstein College of Medicine of Yeshiva University, Bronx, NY, and ²Rockefeller University, New York, NY

Hepatitis C virus (HCV) is a major contributor to cirrhosis, liver failure or cancer, and new therapeutic approaches are needed. Our consideration is blocking HCV replication or infection, particularly in cells capable of cryopreservation, expansion *in vitro*, and engraftment in the liver after transplantation, will advance cell therapy in chronic hepatitis. Aptamers derived from oligonucleotides bind nucleic acid targets and may be selected from sequence pools by repeated rounds of systematic evolution of ligands by exponential enrichment (SELEX). To demonstrate aptamer targeting of HCV, we constructed anti-HCV aptamers from literature: aptamers 1 and 2 for RNA polymerase, aptamer 4 for NS3 protease and helicase, aptamer 5 for NS3 protease, and aptamer 6 for a loop in a domain of internal ribosomal entry site. We cloned aptamers in lentivirus vectors and transduced Huh-7.5 cells, permissive for replication of HCV substrains. Northern blot analysis of total RNA from transduced Huh-7.5 cells confirmed all five aptamers were correctly expressed. We then generated stable clones of Huh-7.5 cells and verified continued expression of various aptamers. Subsequently, cells were infected with J6/JFH1 chimeric virus (HCVcc). After exposure to HCVcc, Huh-7.5 cells showed HCV infection/replication with NS5A HCV protein expression, whereas immunocytochemistry and FACS or western blotting for NS5A protein was reduced or negative for HCV infection/replication in cells transduced by 3 of 5 anti-HCV aptamers.

Conclusions: Cell/gene therapy approaches can be developed by antiviral aptamers to confer resistance against HCV infection and/or replication. Incorporation of integrating lentivirus vectors will permit permanent gene transfer in candidate stem/progenitor cells. These considerations will allow studies of new aptamers and of other therapeutic constructs, including combinatorial approaches to examine antiviral efficacy against HCV without emergence of resistant quasi-species. In turn, this will permit studies of hepatocytes derived from stem/progenitor cells to address their liver repopulation potential.

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(12) ERYTHROID POTENTIAL WITH EXTENSIVE IN VITRO SELF-RENEWAL CAPACITY EMERGES FROM THE YOLK SAC

Samantha E. England, Jenna Frame, Kathleen E. McGrath, James Palis

Dept. of Pediatrics and Center for Pediatric Biomedical Research, University of Rochester Medical Center, Rochester, NY

Adult-derived erythroid "progenitors" are capable of limited (<10⁶-fold) proliferation when cultured *in vitro* in the presence of erythropoietin, stem cell factor, and glucocorticoids. Here, we report that immature definitive erythroid precursors derived from the mouse yolk sac and early fetal liver are capable not only of limited, but also of extensive (>10³⁰ fold) proliferation *in vitro*. These cultures are composed of cells that divide daily and resemble proerythroblasts by morphology and c-kit/ter-119 immunophenotype. Both limited and extensive proliferation requires the continual presence of all three exogenous factors. Despite prolonged *in vitro* culture, these cells preserve their potential to mature over 3 days into enucleated erythrocytes, indicating that this proliferation represents self-renewal cell divisions. The maturation of extensively self-renewing erythroblasts (ESRE) into reticulocytes results in a 10-fold increase in total cell numbers, consistent with the proerythroblast identity of ESREs. Cell maturation is accompanied by the loss of c-kit and the upregulation of ter-119 on the cell surface. Analysis of globin expression confirms that ESRE are definitive, and not primitive, in nature despite their generation from embryonic yolk sac. Within the hematopoietic hierarchy, self-renewal has only been associated with stem cells. Thus, our findings of extensive self-renewal potential in a nearly terminal, lineage-committed erythroid cell are surprising. We hypothesize that the ability of immature erythroid precursors to self-renew may serve as a novel mechanism to acutely expand the output of the erythron both during embryonic development and in response to acute anemia.

(13) FUNCTIONAL IDENTIFICATION OF TRANSCRIPTIONAL DETERMINANTS OF THE ES CELL STATE AND EARLY LINEAGE COMMITMENT

Yatong Wang, Mahesh Yaragatti, Claudio Basilico, [Lisa Dailey](#)
 Department of Microbiology, NYU School of Medicine, New York, NY, 10016

The ES cell state is 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. ES cell transcription factors (TFs) facilitate the establishment of this chromatin landscape by targeting chromatin-modifying complexes to specific genomic loci. Upon differentiation, lineage-specific TFs are activated while stem-specific TFs are down-regulated, leading to radical changes in chromatin structure and gene expression programs. Thus the distinguishing properties of ES cells and their derivatives are largely directed by distinct sets of core TFs, and a full understanding of these cell states, and the transitions between them, requires the precise identification of the target DNA elements and TFs comprising their transcriptional circuitries. These data may also facilitate efforts for the direct reprogramming and programming of cells toward specific lineages and cell types.

We are applying a new high throughput method for the unbiased, *functional* identification of transcriptional regulatory elements that are *active regulators of endogenous genes* in ES cells, or *become activated* as the cells transit along distinct differentiation pathways. We have isolated DNA fragment populations that are enriched for cis-regulatory elements in ES cells using a method that we have developed for extracting DNA from nucleosome-free regions (NFRs) within chromatin, and have created libraries of constructs whose expression of a GFP reporter gene is driven by these NFR-derived DNAs. Following their stable genomic integration in ES cells, different treatments and sorting strategies (FACS) are being used to isolate stage-specific regulatory elements that are *only active in undifferentiated* ES cells, or only *activated after ES cell differentiation*. High-throughput sequencing, and bioinformatic and experimental approaches will be used to identify the TF binding motifs and TFs that determine the stage-specific activation properties of the selected regulatory DNA elements. Our progress in this effort will be presented.

(14) WNT-MEDIATED REGULATION OF MOUSE MAMMARY STEM CELLS IN MAMMOSPHERES

[Alexander M. Many](#) and Anthony M. C. Brown
 Department of Cell & Developmental Biology, Weill Cornell Medical College, New York, NY

Canonical Wnt signaling is a common regulator of cell fate decisions in development, stem cell renewal, and tissue maintenance. Hyperactivation of the Wnt pathway contributes to tumorigenesis in experimental systems and is observed in many human cancers, including breast cancer. It is possible that the oncogenic effects of Wnt signaling result primarily from effects on the behavior of mammary stem cells and/or cancer stem cells. If so, it will be important to determine whether Wnt signaling regulates the abundance of such stem cells, their self-renewal and differentiation properties, or both.

We are investigating the role of Wnt signaling in mammary stem cells using transgenic mice expressing Wnt1 from a mouse mammary tumor virus (MMTV) promoter, and a combination of *in vivo* and *ex vivo* approaches. We have established a non-adherent mammosphere cell culture system, in which mammosphere-forming cells exhibit some of the known properties of mammary stem cells, such as ability to self renew and differentiate into mammary cell lineages.

In these sphere cultures, the percentage of cells capable of initiating secondary spheres is approximately 1% when derived from wild-type mammary tissue. However, we have observed significant increases in the number of mammosphere initiating units in epithelial cultures derived from MMTV Wnt-1 mice. We are continuing to exploit this system by manipulating Wnt signaling levels *in vitro* and monitoring the expression of mammary stem cell markers in conjunction with functional assays.

Our studies will aid in elucidating the biological mechanisms by which stem cell alterations lead to cancer, and the roles of Wnt signaling in at different stages in that process. This may provide critical information for devising novel methods of clinical intervention against stem-like cells in cancer.

(15) MORPHOGENETIC MECHANISMS OF STEM CELL NICHE ASSEMBLY IN THE *C. ELEGANS* EMBRYO

Daisuke Chihara¹ and Jeremy Nance¹

¹The Helen L. and Martin S. Kimmel Center for Biology and Medicine at the Skirball Institute of Biomolecular Medicine, NYU School of Medicine, New York, NY

Stem cells receive essential regulatory signals from surrounding cells within the niche. We are using the *C. elegans* primordial gonad as a simple model to understand how stem cells assemble into a niche during development. The *C. elegans* primordial gonad contains two somatic gonad precursor cells (SGPs) and two primordial germ cells (PGCs). The primordial gonad assembles during embryogenesis when PGCs and SGPs come together adjacent the intestine.

As a first step in understanding niche assembly, we investigated how PGCs move to the site where the primordial gonad forms. PGCs and many somatic cells move into the interior during gastrulation. Because somatic cells require transcription to ingress and PGCs are transcriptionally quiescent, we hypothesized that other cells might push or pull the PGCs into the embryo. We used timelapse microscopy to identify cells that contact the ingressing PGCs, and used laser killing to determine if the contacting cells are required for PGC ingression. Killing or altering the fate of one group of contacting cells – the intestinal precursor cells – prevented PGC ingression but not ingression of other somatic cells. The intestinal precursor cells ingress into the embryo an hour before the PGCs and lie beneath the PGCs. Using membrane markers, we showed that PGCs and intestinal precursor cells maintain intimate contact throughout gastrulation. PGCs expressed high levels of E-cadherin relative to other cells, and knocking down E-cadherin disrupted PGC ingression and frequently caused the PGCs to detach from intestinal cells. We propose that PGCs upregulate E-cadherin to maintain contact with intestinal precursor cells, which pull the PGCs into the embryo and position them at the site of primordial gonad assembly. Our results add to mounting evidence highlighting the importance of germline stem cell – gut interactions during development and of E-cadherin-mediated adhesion in niche formation or function.

(16) ADAPTING *IN VITRO* EMBRYONIC STEM CELL DIFFERENTIATION TO THE STUDY OF GENE REGULATION DURING T CELL DEVELOPMENT

Armin Lahiji¹, Martina Kucerová-Levisohn¹, Stefan Knirr¹, Roxanne Holmes², Juan-Carlos Zúñiga-Pflücker², Benjamin D. Ortiz¹

¹Department of Biological Sciences, City University of New York, Hunter College, New York, NY and

²Sunnybrook Research Institute, University of Toronto, Toronto, Ontario, Canada

Stem cells are of high potential use to gene therapy strategies for long-term correction of genetic deficiencies and prevention/treatment/cure of various diseases. We focus on the T cells of the immune system. We have been studying a DNA element called a locus control region (LCR) from the T cell receptor (TCR)-a gene locus. This LCR's powerful activities have the potential to improve stem cell gene therapy constructs for use in T cells. However, the activity of this LCR after its introduction into stem cells that are subsequently induced to produce T cells is still unknown. This project has the aim of: 1) testing the ability of embryonic stem cells (ESC) to support full LCR activity after their differentiation to T cells in cell culture. It further aims to: 2) create a "mini-LCR" that reproduces full TCRA LCR activity in the minimum DNA sequence. This latter aim is critically important for the eventual "translation" of TCRA LCR activity to the design of gene therapy vectors that have limited space for extra pieces of DNA. Thus far, we have established the ability to genetically engineer embryonic stem cells, induce their differentiation to T cells *in vitro* and detect the activity of a reporter gene product in those cells. We have also constructed and are preliminarily testing promising mini-LCRs in an established T cell line. We are testing reporter gene constructs containing TCRA LCR DNA in differentiating embryonic stem cells to assess LCR activity in this system, and quantifying the activity of the mini-LCRs in the T cell line. These mini-LCRs will be tested in the embryonic stem cell differentiation assay. Successful completion of this project will establish a novel and rapid system for the adaptation of LCR activity to gene therapy vectors.

(17) DIRECTED DIFFERENTIATION OF HUMAN AND MOUSE EMBRYONIC STEM CELLS INTO THYMIC EPITHELIAL CELLS

Michael Green, Valentina Fossati, Ying Zhou, Hans-Willem Snoeck
Department of Gene and Cell Medicine and Black Family Stem Cell Institute, Mount Sinai School of Medicine, New York, NY

The thymus is essential for T cell development, and thus for the generation of a diverse and self-tolerant adaptive immune system. Thymic epithelial cells (TECs), the predominant stromal elements composing the thymus, are required for T cell maturation. The thymus involutes with age. As a consequence, reconstitution of the T cell repertoire after allogeneic bone marrow transplantation in adults is problematic. The derivation of human TECs will allow for improved immune reconstitution following allogeneic bone marrow transplants in adults. Furthermore, ES or iPS-derived TECs would provide cell therapy for congenital absence of thymus, such as in DiGeorge and nude/SCID syndrome. Finally, the availability of iPS-derived TECs would allow development of improved humanized mice that better model human immune responses and capture genetic diversity in immunoregulation in human populations.

Using both human and mouse embryonic stem cells, we are attempting to derive functional TECs or TEC progenitors by following developmental cues. As the thymus is entirely of endodermal origin (3rd pharyngeal pouch), we first aimed at generating anterior (pharyngeal) endoderm. Using human ES cells, where endoderm generation is more efficient than in mouse ES cells, a strategy was developed for the induction of definitive endoderm, followed by anteriorization of this endoderm so that pharyngeal endoderm was obtained where hindgut and midgut signals were depleted. In the mouse, we have generated iPS cells from Foxn1:GFP reporter mice. Within the endoderm, Foxn1 is specific for TECs. These cells are used to screen conditions for the generation of TECs from pharyngeal endoderm using high-throughput flow cytometry. Finally, we are examining whether regulated expression of FOXN1 in ES-derived pharyngeal endoderm will drive a TEC progenitor fate.

(18) MODULATING THE MODULATOR: EPIGENETIC CONTROL BEYOND THE HISTONE CODE

Kyoko L. Yap¹, Side Li², Martin J. Walsh^{1,2}, Ming-Ming Zhou¹
¹*Department of Structural and Chemical Biology and* ²*Department of Pediatrics, Mount Sinai School of Medicine, New York, NY 10029*

A balance between self-renewal and lineage commitment of a stem cell lies at the heart of how its chromatin structure enacts different transcriptional programs to instruct pluripotent cell behavior. In accordance with this paradigm is the notion that self-renewal engages in the process of silencing of genes that otherwise upon expressed would instruct cells toward lineage commitment. Polycomb repressive complexes (PRCs) are responsible for epigenetic control of gene silencing in eukaryotic cells. PRC-directed gene silencing is directed by histone H3 methylation at lysine 27 (H3K27me) at target loci in chromatin initiated by the lysine methyltransferase EZH2 of PRC2. Methylation of H3K27 is then followed by association with PRC1 through binding to chromobox (CBX) proteins such as CBX7, resulting in chromatin structural condensation and target gene silencing. Recent studies including ours reveal that in addition to histone modifications, non-coding RNA functions in concert with PRCs in epigenetic gene silencing. However, the underlying molecular mechanism remains elusive. In this presentation, I will present our new structural and mechanistic insights into the repression of the *INK4/ARF* locus that plays a fundamental role in determining stem cell pluripotency and cellular lifespan. I will also discuss functional implications of the molecular interplay between non-coding RNA and histone lysine methylation in gene repression in human biology and disease.

(19) MONITORING REAL-TIME GENE EXPRESSION DURING DIFFERENTIATION OF MESENCHYMAL STEM CELLS USING HIGH THROUGHPUT LIVE CELL ARRAYS

Jun Tian¹, Pedro Lei¹, Roshan Padmashali¹, Peng Xu¹, Stelios T. Andreadis^{1,2}

¹Bioengineering Laboratory, Department of Chemical and Biological Engineering, University at Buffalo, State University of New York, Amherst, NY and ²Center of Excellence in Bioinformatics and Life Sciences, Buffalo, NY

Sequencing of the human genome led to a rapid cataloguing of genes and expressed sequence tags of higher organisms, including humans. However, the challenge still remains to understand the function of gene products in different biological contexts including differentiation of adult or embryonic stem cells. Mesenchymal stem cell (MSC) differentiation involves precisely programmed gene expression that transforms stem cells to tissue specific cell types such as fat, bone cartilage and muscle. Identification of temporal patterns of gene expression would potentially help uncover the regulatory mechanisms of mesenchymal stem cell (MSC) differentiation and would ultimately provide information to guide efficient differentiation of stem cells for cell-based therapies. To this end, we developed scalable live-cell microarrays to measure gene expression dynamics in real-time and in a high-throughput manner. First, we generated dual-promoter lentiviral vectors harboring a transcriptional regulatory element encoding for green fluorescence protein to monitor cell activation in response to exogenous stimuli and a constitutive promoter driving red fluorescence protein for internal signal normalization. Second, we generated a library of lentiviral vectors encoding promoters or response elements of genes that are potentially involved in MSC differentiation towards the myogenic or osteogenic lineages. Recombinant lentiviruses were then immobilized in a microarray format to monitor and automated fluorescence microscopy was used to monitor gene expression in real time as MSC differentiated toward smooth muscles or osteoblasts. Data normalization by red fluorescence intensity eliminated errors due to spot-to-spot variability in transduction efficiency or changes in cell proliferation. In addition, this high-throughput methodology is currently applied in evaluating various biochemical or physical stimuli on MSC differentiation in a high-throughput manner. In contrast to standard methods, our studies provide rich dynamic information of gene expression over a period of several days as MSCs undergo differentiation thereby providing real-time data that maybe useful in deciphering gene regulatory networks in stem cell differentiation.

(20) GLYCOSYLATION CHANGES IN EMBRYONIC STEM CELLS AND ITS EFFECT ON CELL ADHESION

Shilpa A. Patil¹, Abhirath Parikh¹, E. S. Tzanakakis¹, Sriram Neelamegham¹

¹Department of Chemical and Biological Engineering, State University of New York at Buffalo, NY

Glycosylation is an important post-translational modification that regulates the formation of carbohydrate structures on protein and lipid scaffolds. This in turn regulates diverse processes including cell adhesion, bacterial/viral interaction with host cell, and cancer metastasis. Glycan structures are formed by the catalytic action of a family of enzymes called glycosyltransferases. The levels of these enzymes in the Golgi are a key feature regulating glycan structures. We have developed a method to monitor these enzyme activities in a simple and rapid manner. Particular emphasis is placed on studies of enzymes mediating the formation of O-glycans with focus on members of the sialyltransferase, fucosyltransferase and galactosyltransferase families. Reactions are performed in small volumes with an array of carbohydrate substrates, followed by rapid, tandem separation of product from unreacted radioactive sugar-nucleotide. Using this strategy, we quantify and compare different enzyme levels in a given cell system. In the case of mouse embryonic stem cells (mESCs), we observed quantitative differences as the stem cells differentiate towards beating cardiomyocytes. As the cells differentiated over a 16-day time course, •(1,3)fucosyltransferases were down-regulated while •(2,3)sialyltransferases were upregulated. During inflammation, selectins expressed on the vascular endothelium bind leukocytes via sialyl Lewis-X (sLe^x) type structures that are borne on the leukocyte cell-surface glycoprotein, P-Selectin Glycoprotein Ligand-1(PSGL-1). Based on the measured glycosyltransferase activities and requirements for selectin-mediated adhesion, we hypothesize that mESCs that are transduced to stably express PSGL-1 may bind selectins during the course of cellular differentiation to cardiomyocytes. Alternatively, additional glycan engineering strategies may be employed on the surface of mESCs to engineer functional PSGL-1 that express the sLe^x epitope. Our presentation will highlight current progress that addresses this hypothesis. Successful engineering of selectin-ligands on stem cells can enable delivery of ESCs to sites of inflammation that are typically targets for the field of regenerative medicine.

(21) ABSOLUTE QUANTITATION BY MASS SPECTROMETRY PROVIDES UNIQUE SYSTEMS BIOLOGICAL VIEW OF SOMATIC HUMAN STEM CELL PROTEINS DURING DEVELOPMENTAL CHALLENGES

Lewis M. Brown¹, Elizabeth S. Oswald², Alexandr Gornstein¹, J. Chloë Bulinski¹, Clark T. Hung²
¹Department of Biological Sciences and ²Department of Biomedical Engineering, Columbia University, New York, NY

In this study, the efficacy of an absolute quantification (label-free) proteomics technique was demonstrated in an analysis of adipose-derived stem cells. An internal standard protein added to every liquid chromatography/mass spectrometry (LC/MS) sample enhanced this technique. The internal standard peptides were then used to accurately quantify protein amount: the area under the intensity plot for each peptide was normalized to the intensities of the internal standard peptides (previous work has demonstrated that the intensities are a linear function of protein amount, regardless of protein sequence). We also added an additional normalization technique that takes into account the total measured nanograms of protein in each run. Thus, our approach provides a systems biology tool that allows comparison among different cells and tissues and provides concentrations measured as (fmol)/(μg of total cell protein). These values can be readily expressed as moles per cell. This technique paves the way towards better understanding *in vivo* reaction kinetics, and energy allocation.

In the current experiment, multipotential adipose-derived stem cells were subjected to a physiologically-relevant hyperosmotic culture environment in an effort to trigger development of chondrocyte characteristics. This cell type presents an advantageous population for allogenic and autologous cartilage tissue engineering, due to the accessibility and abundance of adipose tissue waste from elective cosmetic surgery procedures. For each osmotic treatment three independent biological replicates were analyzed (three separate cultures of somatic stem cells; proteins extracted separately from each culture). For each of the three replicates, separate 120 min LC/MS runs were done, thus providing nine replicate analyses for each treatment. This level of replication allowed accurate detection of many potential protein markers of stem cell differentiation at a high level of confidence. For the 15 proteins that differed the most in expression, approximately half were stem cell- or differentiation-related; many others were associated with osmoregulation.

(22) HUMAN iPS CELL-BASED MODELS FOR NEURODEGENERATION

Claudia A. Doege, Natsumi Watanabe, William B. Vanti, Skylar Travis, Liang Qiang, Asa Abeliovich
 Dept. of Pathology, Columbia University Medical Center, New York, NY

Introduction/Background: The ultimate goal of this proposal is to generate accurate disease models for Alzheimer's and Parkinson's disease for the development of effective therapeutics. Currently, no therapeutics alter the course of these major diseases of aging, and a significant hurdle has been the lack of cell models that recapitulate critical aspects of the disease process. Here we are developing iPS cell-based models of Alzheimer's and Parkinson's.

Progress Toward Specific Aims: We have succeeded in generating iPS cell models of familial forms of Alzheimer's disease, including mutant iPS cells with Presenilin 1 and Presenilin 2 mutations, as well as normal controls. Also, we have generated iPS cells from patients with sporadic forms of Alzheimer's and Parkinson's, and in the process of generating additional cell lines. These lines have been validated using a number of standard criteria, and neurons can be generated from them.

Future Directions: Additional studies in the coming year are focused on dissecting the phenotype of neurons derived from the patient and control fibroblasts. Emphasis is placed on identifying disease-related aspects.

Impact: We emphasize that to our knowledge there are no published description of such lines and analyses, so the work is novel and potentially of high impact. There is a critical need for cell models of AD and PD for therapy development.

(23) GENERATION OF HUMAN iPS LINES TO STUDY THE GENETIC RESISTANCE TO AGING-RELATED DISEASES

Ritu Kumar¹, Nir Barzilai², Todd Evans¹

¹Weill Cornell Medical College, New York, NY and ²Albert Einstein College of Medicine, Bronx, NY

The determinants that regulate healthy human lifespan (healthspan) are largely unknown, but clearly involve resistance to aging-related diseases, including diabetes, heart disease, cancer, and neurodegenerative disorders. Genetic studies using invertebrate and rodent models associate long life with modulation of metabolic pathways related to insulin/IGF-1 signaling or lipoprotein levels. However, the effect of human aging on healthspan has features that are not well modeled in small animals with relatively short lives. It is clear that there are genetic determinants. Individuals exhibiting the inherited trait of exceptional longevity, or EL (centenarians and their families) provide a unique opportunity to study mechanisms that modulate human healthspan. We hypothesize that humans with inherited EL possess enhanced stem cell activity, for example, an increased ability to maintain healthy and functioning stem or progenitor cell populations over long times (many decades) with relatively suppressed rates of stem cell aging, enhanced regenerative capacity, or more stable differentiation potential. Until recently it was not possible to test this hypothesis. With the advent of iPS cell technology, it is now possible to generate pluripotent ES-like cells from such individuals and to compare functional stem and progenitor activities with those derived from unrelated individuals lacking the EL trait. We are generating iPS lines from this rare cohort of fortunate individuals and control donors, comparing lines derived using several distinct cell types. In particular, to facilitate the project we are optimizing iPS generation from granulocytes obtained from a simple 10 ml draw of circulating blood. Our goal is to identify genes and pathways that provide individuals with resistance to aging-related disease, in order to identify new therapeutic targets to enhance human healthspan.

(24) CULTURE, AND ANALYSIS OF HUMAN EMBRYONIC STEM CELLS AND EMBRYONAL CARCINOMA CELLS USING THREE-DIMENSIONAL CELL MICROARRAYS

Leyla Gasimli¹, Luciana Meli², Hope E. Stansfield³, Janet L. Paluh¹, Jonathan S. Dordick² and Robert J. Linhardt⁴

¹Department of Biology, ²Department of Chemical and Biological Engineering, ³Department of Biochemistry and Biophysics, ⁴Department of Chemistry, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY

We have developed pluripotent cell-culture microarrays in a three-dimensional format, containing clusters of ~ 100 cells, that closely replicate the *in vivo* conditions of the developing embryo. Human embryonic stem cells (hESC) or embryonal carcinoma cells are encapsulated in 30 or 60 nL 1% alginate hydrogels and deposited on spots of poly-L-lysine that are surrounded by a hydrophobic polystyrene surface. This affords spots of ~500 μ m in diameter that remain discrete due to the hydrophobicity of the surface. This can be used for high-throughput screening of molecules and growth conditions. A cell-based chip that can be integrated into a miniaturized PDMS well array is also being developed, which allows individual culturing of spots while simultaneously screening compounds of interest in high throughput. Such screening should be useful in understanding mechanisms and principles of fate determination of pluripotent cells. This will especially be important for revealing the role of intracellular and extracellular molecules, such as glycans, which drive differentiation and proliferation. This platform has been used to probe a variety of metabolic agents for protein expression in other cell types but is particularly well suited for use with pluripotent cells.

LIST OF PARTICIPANTS

- Abeliovich, Asa**
Columbia University
aa900@columbia.edu
- Abrate, Christian**
Cornell University
ca258@cornell.edu
- Aguirre-Ghiso, Julio**
Mount Sinai School of Medicine
Julio A. Aguirre-Ghiso@mssm.edu
- Ahmad, Faizzan**
The New York Stem Cell Foundation
fahmad@nyscf.org
- Alexeeva, Vera**
Mount Sinai School of Medicine
vera.alexeeva@mssm.edu
- Anders, David**
Wadsworth Center
anders@wadsworth.org
- Anderson, Stewart**
Weill Cornell Medical College
saa2007@med.cornell.edu
- Andreadis, Stelios**
State University of New York at Buffalo
sandread@buffalo.edu
- Arango, Victoria**
Columbia University
va19@columbia.edu
- Artus, Jerome**
Sloan-Kettering Institute
artusj@mskcc.org
- Au, Edmund**
NYU Medical Center
aue03@nyumc.org
- Bach, Erika**
NYU School of Medicine
Erika.bach@nyu.edu
- Bahou, Wadie**
Stony Brook University Medical Center
wbahou@notes.cc.sunysb.edu
- Bare, Christopher**
The Rockefeller University
cbare@rockefeller.edu
- Baron, Margaret**
Mount Sinai School of Medicine
margaret.baron@mssm.edu
- Bauer, Lauren**
The New York Stem Cell Foundation
lbauer@nyscf.org
- Becht, Jennifer**
The New York Stem Cell Foundation
jbecht@nyscf.org
- Bernstein, Emily**
Mount Sinai School of Medicine
emily.bernstein@mssm.edu
- Bishop, David**
Mount Sinai School of Medicine
david.bishop@mssm.edu
- Bohmann, Dirk**
University of Rochester Medical Center
dirk_bohmann@urmc.rochester.edu
- Boldrini, Maura**
Columbia University
mb928@columbia.edu
- Bouhassira, Eric**
Albert Einstein College of Medicine
bouhassi@aecom.yu.edu
- Bragado, Paloma**
Mount Sinai School of Medicine
paloma.brag@mssm.edu
- Brautigam, Bonnie**
Wadsworth Center
bjb08@health.state.ny.us
- Brown, Anthony**
Weill Cornell Medical College
amcbrown@med.cornell.edu
- Bulinski, J. Chlo.**
Columbia University
jcb4@columbia.edu
- Chambers, Stuart**
Sloan-Kettering Institute
chambers@mskcc.org
- Chaudhry, Hina**
Mount Sinai School of Medicine
hina.chaudry@mssm.edu
- Chaurasia, Pratima**
Mount Sinai School of Medicine
pratima.chaurasia@mssm.edu
- Chen, Yong**
Albert Einstein College of Medicine
yong.chen@einstein.yu.edu
- Chou, Kathy**
Wadsworth Center
yxc10@health.state.ny.us
- Christiano, Angela M.**
Columbia University
amc65@columbia.edu
- Cohen, Ira**
SUNY at Stony Brook
ira.cohen@stonybrook.edu
- Croft, Gist**
Columbia University
gfc2102@columbia.edu
- Dailey, Lisa**
NYU School of Medicine
lisa.dailey@nyumc.org
- Daines, Richard F.**
New York State Department of Health
rfd02@health.state.ny.us
- Ding, Jianqiang**
Albert Einstein College of Medicine
jianqiang.ding@einstein.yu.edu
- Ding, Ye**
Wadsworth Center
yding@wadsworth.org
- Doege, Claudia**
Columbia University
cad2114@columbia.edu
- Doeschate, Judy**
Wadsworth Center
jld05@health.state.ny.us
- Doetsch, Fiona**
Columbia University
fkd2101@columbia.edu
- D'Souza, Sunita**
Mount Sinai School of Medicine
dsou2501@mssm.edu
- Effgen, Gwen**
Columbia University
ge2121@columbia.edu

Egli, Dieter
The New York Stem Cell Foundation
degli@nyscf.org

Elliot, Robin
Parkinson's Disease Foundation
relliot@pdf.org

Enikolopov, Grigori
Cold Spring Harbor Laboratory
enikolop@cshl.edu

Evans, Todd
Weill Cornell Medical College
tre2003.med.cornell.edu

Fasano, Christopher
New York Neural Stem Cell Institute
chrisfasano@nynsci.org

Feng, Jian
State University of New York at
Buffalo
jianfeng@buffalo.edu

Feuer, Gerold
SUNY Upstate Medical University
feurg.upstate.edu

Flatto, Olivia
The New York Stem Cell Foundation
oflatto@nyscf.org

Fortier, Lisa
Cornell University College of
Veterinary Medicine
laf4@cornell.edu

Gasimli, Leyla
Rensselaer Polytechnic Institute
gasiml@rpi.edu

Ghaffari, Saghi
Mount Sinai School of Medicine
saghi.ghaffari@mssm.edu

Ghazizadeh, Soosan
SUNY Stony Brook
soosan.ghazizadeh@sunysb.edu

Gincel, Dan
Maryland Stem Cell Research Fund
dgincel@marylandtedco.org

Goff, Stephen P.
Columbia University
spg1@columbia.edu

Goldman, Steven
University of Rochester Medical
Center
Steven_Goldman@urmc.rochester.edu

Gornstein, Alexandr
Columbia University
lb2425@columbia.edu

Greve, Line
c/o: Zeta Phi Fraternity
lineg@dsr.life.ku.dk

Gronostajski, Richard
State University of New York at
Buffalo
rgron@buffalo.edu

Gupta, Sanjeev
Albert Einstein College of Medicine
sangupta@aecom.yu.edu

Hackett, Catherine
Cornell University
chr23@cornell.edu

Hansis, Chris
NYU School of Medicine
chris.hansis@med.nyu.edu

Hazelrigg, Tulle
Columbia University
tih1@columbia.edu

Hen, René
New York State Psychiatric Institute
rh95@columbia.edu

Hill, Alexis S.
New York State Psychiatric Institute
ash2146@columbia.edu

Hohn, David
Roswell Park Cancer Institute
david.hohn@roswellpark.org

Houshmand, Bahar
Stony Brook University
baharhoushmand@yahoo.com

Huang, Li Ting
SUNY Stony Brook
liting.huang@sunysb.edu

Huss, Wendy
Roswell Park Cancer Institute
wendy.huss@roswellpark.org

Hylander, Bonnie
Roswell Park Cancer Institute
bonnie.hylander@roswellpark.org

Jacobs, Christopher
Columbia University
crj2111@columbia.edu

Jadali, Azadeh
Stony Brook University
ajadali@notes.cc.sunysb.edu

Jesudoss, Michael
Masonic Research Laboratory
xavier@mmrl.edu

Kahler, David
The New York Stem Cell Foundation
dkahler@nyscf.org

Kholmanskikh, Stanislav
Weill Cornell Medical College
stu2005@med.cornell.edu

Kloss, Christopher
Weill Cornell Medical College
cck2001@med.cornell.edu

Kohn, Matthew
Wadsworth Center
mjk09@health.state.ny.us

Kottman, Andreas
Columbia University
ak139@columbia.edu

Lacy, Elizabeth
Sloan-Kettering Institute
e-lacy@mskcc.org

Lailier, Nathalie
Albert Einstein College of Medicine
nathalie.lailier.einstein.yu.edu

Lajugie, Julien
Albert Einstein College of Medicine
jlajugie@aecom.yu.edu

Laufer, Edward
Columbia University
elaufer@columbia.edu

Lee, Gabsang
Sloan-Kettering Institute
gabsang@gmail.com

Lehmann, Ruth
NYU Medical Center
Ruth.Lehmann@nyumc.org

Lemischka, Ihor
Mount Sinai School of Medicine
Ihor.lemischka@mssm.edu

Liang, Dong
Mount Sinai School of Medicine
liang.dong@mssm.edu

Lin, Reigh-Yi
Mount Sinai School of Medicine
reigh-yi.lin@mssm.edu

Linhardt, Robert
Rensselaer Polytechnic Institute
linhar@rpi.edu

Lock, Lye Theng
State University of New York at
Buffalo
lyelock@buffalo.edu

Loomis, Mario
Loomis Plastic Surgery
Doctor@drloomis.com

Lopez, Maria
Wadsworth Center
mcl05@health.state.ny.us

Lu, Helen
Columbia University
hl2052@columbia.edu

Lu, Jonathan
Columbia University
jl3365@columbia.edu

Major, Tamara
Sloan-Kettering Institute
majort@mskcc.org

Many, Alexander
Weill Cornell Medical College
alm2037@med.cornell.edu

Marshall, Caroline
The New York Stem Cell Foundation
cmarshall@nyscf.org

Maynard-Reid, H. Hugh
North Brooklyn Health Center
maynard@nychhc.org

Mazel, Svetlana
The Rockefeller University
mazel@rockefeller.edu

McHugh, Marti
Wadsworth Center
mgm08@health.state.ny.us

McKeon, David
The New York Stem Cell Foundation
dmckeon@nyscf.org

Meli, Luciana
Rensselaer Polytechnic Institute
melil@rpi.edu

Morse, Randall
Wadsworth Center
randall.morse@wadsworth.org

Murtha, Matthew
New York University
murtha.matthew@gmail.com

Nance, Jeremy
Skirball Institute at NYU School of
Medicine
jeremy.nance@med.nyu.edu

Narayan, Santosh
Memorial Sloan-Kettering Cancer
Center
narayans@mskcc.org

Neelamegham, Sriram
University at Buffalo
neel@buffalo.edu

Noggle, Scott
The New York Stem Cell Foundation
snoggle@nyscf.org

Novakofski, Kira
Cornell University
kdn22@cornell.edu

Oakley, Derek
Columbia University
dho2101@columbia.edu

Ortiz, Benjamin
CUNY, Hunter College
ortiz@genectr.hunter.cuny.edu

Oswald, Elizabeth
Columbia University
lizoswald@gmail.com

Packer, Samuel
North Shore-Long Island Jewish
Health System
eyeethics@aol.com

Palis, James
University of Rochester Medical
Center
James_palis@urmc.rochester.edu

Papapetrou, Eirini
Sloan-Kettering Institute
papapete@mskcc.org

Paul, Alex
Columbia University
ajp2142@columbia.edu

Paul, Jeremy
NYU Langone Medical Center
jeremy.paul@med.nyu.edu

Perera, Tarique
Columbia University
tp119@columbia.edu

Pruitt, Steven
Roswell Park Cancer Institute
steven.pruitt@roswellpark.org

Rendl, Michael
Mount Sinai School of Medicine
michael.rendl@mssm.edu

Reyes, Virginia
Wadsworth Center
vmr02@health.state.ny.us

Rogler, Charles
Albert Einstein College of Medicine
charles.rogler@einstein.yu.edu

Rogler, Leslie
Albert Einstein College of Medicine
leslie.rogler@einstein.yu.edu

Rosen, Michael
Columbia University
mrr1@columbia.edu

Ross, M. Elizabeth
Weill Cornell Medical College
mer2005@med.cornell.edu

Roy-Chowdhury, Jayanta
Albert Einstein College of Medicine
jayanta.roy-chowdhury@einstein.yu.edu

Roy-Chowdhury, Namita
Albert Einstein College of Medicine
namita.roychowdhury@einstein.yu.edu

Rusiniak, Michael
Roswell Park Cancer Institute
michael.rusiniak@roswellpark.org

Salero, Enrique
New York Neural Stem Cell Institute
enriquesalero@nymsci.org

Schildkraut, Carl
Albert Einstein College of Medicine
carl.schildkraut@einstein.yu.edu

Schimenti, John
Cornell University
jcs92@cornell.edu

Schnabel, Lauren
Cornell University
lvs3@cornell.edu

Shen, Qin
New York Neural Stem Cell Institute
qinshen@nynsci.org

Snoeck, Hans-Willem
Mount Sinai School of Medicine
hans.snoeck@mssm.edu

Solomon, Susan
The New York Stem Cell Foundation
ssolomon@nyscf.org

Sproul, Andrew
The New York Stem Cell Foundation
asproul@nyscf.org

Stansfield, Hope
Rensselaer Polytechnic Institute
stansh@rpi.edu

Steidl, Ulrich
Albert Einstein College of Medicine
ulrich.steidl@einstein.yu.edu

Sturman, Lawrence
Wadsworth Center
lss02@health.state.ny.us

Sundman, Emily
Cornell University
Eas282@cornell.edu

Tall, Gregory
University of Rochester Medical
Center
gregory_tall@urmc.rochester.edu

Temple, Sally
New York Neural Stem Cell Institute
sallytemple@nynsci.org

Terracio, Louis
New York University
louis.terraccio@nyu.edu

Themeli, Maria
Sloan-Kettering Institute
themelim@mskcc.org

Tomishima, Mark
Sloan-Kettering Institute
tomishima@mskcc.org

Tripoli, Linda
Wadsworth Center
lmt01@health.state.ny.us

Tumbar, Tudorita
Cornell University
tt252@cornell.edu

Vijayakumar, Sapna
Mount Sinai School of Medicine
sapna.vijayakumar@mssm.edu

Vunjak-Novakovic, Gordana
Columbia University
gv2131@columbia.edu

Wang, Jianlong
Mount Sinai School of Medicine
jianlong.wang@mssm.edu

Weygandt, Mackenzie
Columbia University
mw2578@columbia.edu

Wichertle, Hynek
Columbia University
hw350@columbia.edu

Wils, Madelyn
New York City Economic
Development Corporation
mwils@nycedc.com

Xu, Lei
University of Rochester Medical
Center
lei_xu@urmc.rochester.edu

Zhang, Xinping
University of Rochester Medical
Center
xinping_zhang@urmc.rochester.edu

Zhou, Ming-Ming
Mount Sinai School of Medicine
Ming-ming.zhou@mssm.edu

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PROGRAM-AT-A-GLANCE

MAY 26 – Elebash Recital Hall

- 8:00 AM Registration Opens and Continental Breakfast – Recital Hall Lobby
- 9:00 AM Shared Equipment and Facilities Workshop – Part 1
- 10:15 AM Break
- 10:30 AM Shared Equipment and Facilities Workshop – Part 2
- 12:00 PM Lunch – Concourse Lobby (one floor down)
- 1:20 PM Opening Remarks
- 1:30 PM Session I: Neural Stem Cells
- 3:00 PM Break
- 3:30 PM Session II: Stem Cells and Cancer
- 5:00 PM Reception/Poster Session – Concourse Lobby (one floor down)

MAY 27 – Elebash Recital Hall

- 8:00 AM Registration Opens and Continental Breakfast – Recital Hall Lobby
- 9:00 AM Session III: Stem Cells in Diseases
- 10:30 AM Break
- 11:00 AM Session IV: Stem Cell Biology
- 12:30 PM Lunch – Ninth Floor Conference Room
- 1:30 PM Session V: Tissue Engineering and Technology
- 3:00 PM Break
- 3:30 PM Session VI: Pluripotency and Reprogramming
- 5:00 PM Adjourn

