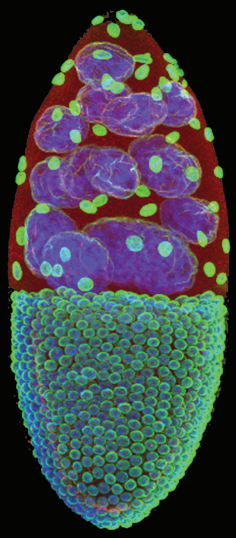
NYSTEM 2011



May 24 & 25, 2011

CUNY Graduate Center, 365 Fifth Avenue, New York, NY

Science Accelerating Therapies

NEW YORK STATE STEM CELL SCIENCE

Wadsworth Center
NEW YORK STATE DEPARTMENT OF HEALTH

NYSTEM NEW YORK STATE STEM CELL SCIENCE

Science Accelerating Therapies

CUNY Graduate Center

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Welcome to NYSTEM 2011: *Science Accelerating Therapies*, our third annual symposium. It has been an eventful year. Stem cell science has continued to progress at a remarkable pace, with important advances both in our understanding of stem cells and translation of that knowledge to clinical applications. NYSTEM continues to foster the growth of scientific knowledge about stem cell biology, and the development of new therapies within New York's stem cell research community. This symposium is a showcase of that effort. We anticipate an exceptional meeting that reflects the strength and diversity of New York's research community. NYSTEM supported scientists have made many important contributions this year, some of which we will learn more about in the next two days. Participants represent more than thirty New York institutions, from Long Island to Buffalo. They bring the wide array of scientific disciplines, technological expertise, and clinical translational experience that are essential to continue breaking down the barriers that lie between the science of today and future therapies.

We extend thanks to the Program Committee members for their sustained and thoughtful efforts to develop this program. We received nearly 80 abstracts – almost twice the number as last year. The Committee's task of selecting speakers was truly a challenge. Of necessity, many outstanding abstracts will be presented as posters only. Please take every opportunity to look at these exciting presentations. Finally, in addition to the plenary and poster sessions, this year's symposium includes two new gatherings: an Education Workshop convened by the Curriculum Development and Summer Internship awardees to exchange information about their innovative programs; and a Translation Panel featuring participants from academia, the FDA and industry, who will provide an informative discussion of the challenges and opportunities they face.

We have done our best to include sufficient time for discussions. Enjoy the meeting, contribute to the discussion, and share your ideas! Thank you for your participation.

Sincerely,

Lawrence S. Sturman

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

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GENERAL INFORMATION

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

Lunches
Lunches will be served in the Concourse Lobby. Meal service will require your registration badge.

Posters
The poster session (5:15-7:00 PM Tuesday) is in the breakout rooms across the Concourse Lobby from the auditorium. Posters should be left up until after lunch on Wednesday and will be available for viewing throughout the meeting. They must be removed by 5:00 PM Wednesday.

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

PROGRAM SCHEDULE

TUESDAY, MAY 24, 2011

7:30 AM Registration Opens and Continental Breakfast

CONCURRENT SESSIONS

SESSION A – EDUCATION INITIATIVES WORKSHOP (Breakout Room)

8:30 – 10:30 Chair: **Laurel Southard**, *Cornell University*
Speakers: Katayoun Chamany, *The New School for Liberal Arts* (Curriculum)
Daniel Kalderon, *Columbia University* (Curriculum)
Dina Markowitz, *University of Rochester* (Curriculum)
John Russell, *Syracuse University* (Curriculum)
Robert Van Buskirk, *Binghamton University* (Curriculum)
David Bynum, *Stony Brook University* (Summer Undergraduate Experience)
Alice Heicklen, *Columbia University* (Summer Undergraduate Experience)
Laurel Southard, *Cornell University* (Summer Undergraduate Experience)

SESSION B – SHARED FACILITIES (Proshansky Auditorium)

9:00 – 10:40 Chair: **Sally Temple**, *Neural Stem Cell Institute*

9:00 – 9:20 **Wadie Bahou**, *Stony Brook University*
Creation of the Stony Brook University Stem Cell Facilities Center

9:20 – 9:40 **Chi Yun**, *New York University School of Medicine*
Developing an Integrated Platform for siRNA and shRNA-Based Genome-Scale Screens in Eukaryotic Stem Cells

9:40 – 10:00 **Stephen Dewhurst**, *University of Rochester*
Upstate Stem Cell cGMP Facility

10:00 – 10:20 **Richard Gronostajski**, *University at Buffalo*
Establishment of the Western New York Stem Cell Culture and Analysis Center (WNSTEM)

10:20 – 10:40 **Christopher Henderson**, *Columbia University Medical Center*
High-Throughput Screening and Chemistry Shared Facility

10:40 – 11:00 **BREAK**

11:00 – 12:30 **PLENARY I – Stem Cells in Cancer and Other Disease**
Chair: **Eva Hernando**, *New York University Medical Center*

11:00 – 11:30 **Viviane Tabar**, *Memorial Sloan-Kettering Cancer Center*
A Novel Role for Cancer Stem Cells in Tumor Angiogenesis

11:30 – 11:50 **Gen Suzuki**, *University at Buffalo*
Intracoronary Injection of Cardiosphere-Derived Cells (icCDCs) Improves Regional Function by Recruiting Endogenous Cell Repair Mechanisms in Chronically Ischemic Myocardium

11:50 – 12:10 **Zhu Wang**, *Columbia University Medical Center*
Lineage-Tracing Analysis of Prostate Epithelial Basal Cells in Regeneration and Cancer

12:10 – 12:30 **Mark Noble**, *University of Rochester*
Metabolic and Molecular Mechanisms Regulating Precursor Cell Function and Cell Signaling in Normal Development and Disease

12:30 – 1:30 **LUNCH AND POSTERS**

1:30 – 1:45 **WELCOME REMARKS, INTRODUCTION OF KEYNOTE SPEAKER**

1:45 – 2:35 **KEYNOTE:** Stem Cells of the Skin: Their Biology and Clinical Promise
Elaine Fuchs, *The Rockefeller University*

2:35 – 3:15 **PLENARY II – Stem Cell Biology**
Chair: **Ruth Lehmann**, *New York University Medical Center*

2:35 – 2:55 **Hans-Willem Snoeck**, *Mount Sinai School of Medicine*
Generation of Anterior Foregut Endoderm from Human Embryonic and Induced Pluripotent Stem Cells

2:55 – 3:15 **David Hoey**, *Columbia University*
Primary Cilia Mediate the Upregulation of Cox2 and Bmp2 in Response to Oscillatory Fluid Flow in Human Mesenchymal Stem Cells

3:15 – 3:45 **BREAK AND POSTERS**

3:45 – 5:15 **PLENARY III – Tissue Engineering and Regenerative Medicine**
Chair: **Emmanuel S. Tzanakakis**, *University at Buffalo*

3:45 – 4:15 **Gordana Vunjak-Novakovic**, *Columbia University*
Bioengineering Strategies for Stem Cell Research and Regenerative Medicine

4:15 – 4:35 **Daniela Battista**, *Memorial Sloan-Kettering Cancer Center*
Induced PSA Expression in ES-Derived Dopaminergic Neurons Improves Graft/Host Integration and Behavioral Recovery in PD Animals

4:35 – 4:55 **Su Wang**, *University of Rochester Medical Center*
High Efficiency Myelination of the Hypomyelinated Shiverer Mouse Brain Using Human iPS Cell-Derived Glial Progenitor Cells

4:55 – 5:15 **Helen Lu**, *Columbia University*
Engineering Tissue-to-Tissue Interface via Nanofiber Scaffold Design and Controlled Stem Cell Differentiation

5:15 – 7:00 **POSTER SESSION/RECEPTION**

WEDNESDAY, MAY 25, 2011

8:00 AM Registration Opens and Continental Breakfast

9:00 – 12:00 **TRANSLATION PANEL DISCUSSION**
Moderator: Steven Goldman, *University of Rochester Medical Center*
Participants: 9:00 – 9:25 Stephen Chang, *NYSCF*
9:25 – 9:50 Bruce Schneider, *FDA CBER*
9:50 – 10:15 Karl Kieburtz, *University of Rochester Medical Center*
10:15 – 10:40 BREAK
10:40 – 11:00 Edmund Mickunas, *Advanced Cell Technology*
11:00 – 11:20 Jason Kolbert, *NeoStem*
11:20 – 11:40 Sridaran Natesan, *Sanofi-Aventis*
11:40 – 12:00 Panel Discussion

12:00 – 1:00 **LUNCH AND POSTERS**

1:00 – 2:30 **PLENARY IV – Disease Models and Therapeutic Approaches**
Chair: **Craig Jordan**, *University of Rochester*

1:00 – 1:30 **Shahin Rafii**, *Weill Cornell Medical College*
Contribution of the Vascular Niche to Organ Regeneration

1:30 – 1:50 **Cecile Terrenoire**, *Columbia University Medical Center*
Functional Comparison of Long QT-Associated Cardiac Ion Channels (I_{Na}, I_{Kr}, I_{Ks}) in Human Embryonic Stem Cells and Human Induced Pluripotent Stem Cells

1:50 – 2:10 **Abdellatif Benraiss**, *University of Rochester*
Sustained Ependymal Over-Expression of BDNF and Noggin via AAV4 Triggers Functional Medium Spiny Neurogenesis and Delays Disease Progression in a Mouse Model of Huntington's Disease

2:10 – 2:30 **J. Mario Wolosin**, *Mount Sinai School of Medicine*
Ex Vivo, *In Situ* Expansion of the Limbal Epithelial Stem Cell Pool

2:30 – 3:00 **BREAK AND POSTERS**

3:00 – 4:30 **PLENARY V – Pluripotency, Reprogramming and Directed Differentiation**
Chair: **Lorenz Studer**, *Sloan-Kettering Institute*

3:00 – 3:20 **Angela Christiano**, *Columbia University Medical Center*
Generation of Patient-Specific Induced Pluripotent Stem Cells from Patients with Recessive Dystrophic Epidermolysis Bullosa and their Differentiation into Keratinocytes

3:20 – 3:40 **Vladislav Sandler**, *Albert Einstein College of Medicine*
Identification of a Set of Transcription Factors for Direct Reprogramming of Human Somatic Cells into Hematopoietic Stem Cells/Progenitors

3:40 – 4:00 **Yvonne Gruber-Mica**, *Sloan-Kettering Institute*
The Derivation of Melanocytes from Human Pluripotent Stem Cells for Disease Modeling

4:00 – 4:30 **Ihor Lemischka**, *Mount Sinai School of Medicine*
Pursuing Pluripotency

4:30 – **CLOSING REMARKS**

EDUCATION INITIATIVES WORKSHOP

[Breakout Rooms]

THE DEVELOPMENT, IMPLEMENTATION, AND ASSESSMENT OF AN INTERDISCIPLINARY STEM CELL CURRICULUM FOR NON-MAJORS

Katayoun Chamany[1], Warren Balinsky[1], Michael Pettinger[1], Lisa Rubin[1], Ann Snitow[1], Julia Wargaski[1], Wendy Newstetter[2], Adrienne Asch[3]
[1]The New School for Liberal Arts, New York, NY; [2]Georgia Institute of Technology, Atlanta, GA; [3]Yeshiva University, Albert Einstein College of Medicine, New York, NY

A team comprised of eight faculty and five students spanning the natural sciences, social sciences, and design have worked collaboratively to develop a semester-length non-majors interdisciplinary course titled “Stem Cells and Social Justice” using a case-based approach and a social justice framework. The curriculum is referred to as “Stem Cells across the Curriculum” (SCAC), as it contains modules that can be used together, or separately, in courses that span the liberal arts curriculum. The content of the modules include biological topics such as cloning, chimeras and nuclear reprogramming, and social themes such as competing values and equity, access to stem cell science and technology, and human subjects research standards. A backwards design approach was used to first identify learning outcomes and to then develop modules that meet these learning outcomes. The SCAC team participated in faculty development workshops to familiarize themselves with interdisciplinary education, curriculum development, and assessment, and collaborated on module development in biweekly meetings. Class observations by members of the SCAC team informed the refinement of modules and visual representations of biological and social concepts. Three capstone learning activities that involve role play through case based pedagogy have been developed around the following topics that all connect to human subject research: HeLa Case Study, Oocyte Payment Case Study, and Hope and Hype/ FDA injunction Case Study. Portions of the draft curriculum were piloted on The New School campus: a one-day module in the Higher Education Opportunity Program Summer bridge course designed for incoming freshman; a two-day module in an introductory level university lecture course titled “Feminist Thought and Action”; and a one-day module in an undergraduate science course focused on cell biology. A Science for Civic Engagement and Responsibilities assessment instrument (SENCER SALG) was customized to measure student learning and affect for the spring 2011 semester-length course “Stem Cells and Social Justice.” In addition, a collection of 150 resources including scholarly articles, books, films, and websites have been annotated in a Refworks bibliographic database accessible to educators interested in adapting the resources for a particular course or course unit. Student Outreach Projects in the “Stem Cells and Social Justice” course will also inform the curriculum development.

IMPLEMENTATION OF A NEW UNDERGRADUATE COURSE, "STEM CELLS: BIOLOGY, APPLICATIONS AND ETHICS" AT COLUMBIA UNIVERSITY

Daniel Kalderon, Ph.D.
Columbia University, New York, NY

A new undergraduate course will be offered to Columbia University students suitably qualified by a year of college introductory biology and one advanced biology class. The fundamental science of pluripotent and multipotent stem cells will be the central theme of the course, which will also explicitly address and integrate ideas about the ethical, economic, regulatory and societal implications of stem cell research, as well as its potential medical applications. The stem cell theme provides an exciting and critical focus for the course instructors to integrate their diverse expertise in pure science, its medical applications and bioethics. Stem Cell science is progressing rapidly, opening up entirely new landscapes for further understanding and treatment of a large variety of medical conditions. These new possibilities, and the attendant experimental or medical procedures, have unprecedented societal impact and must therefore be evaluated carefully from ethical, legal and economic, as well as scientific perspectives. The course aims to educate students in the basic science of stem cells and to use that knowledge to inform discussions about societal implications. Toward scientific understanding, the course will emphasize the logic of scientific deductions, limitations in our understanding and the realities of research progress, by including primary research papers, laboratory visits and public research seminars as critical supplements to a progressive core of lectures. To develop skills in integrating ethical and other societal concerns with biomedical science, the course will include case studies and extensive small group discussions. The course will be distinguished by a strong science component, qualifying it as a course for science majors but leaving it accessible to non-science majors as well. A full, integrated exploration of the interface of science with our lives will render the course uniquely valuable in guiding students' future participation and leadership in public discourse pertaining to science and society.

THE SCIENCE AND ETHICS OF STEM CELLS: A CASE STUDY-BASED COURSE FOR UNDERGRADUATES

Dina Markowitz, Ph.D.
University of Rochester, Rochester, NY

The rapid advances in stem cell research require that the general public have an understanding of the science and its related ethical, legal and social implications. This need is especially important for college-age students, as these young adults are our future workforce, decision-makers, health care providers, and patients. The goal of this proposal is to create an undergraduate stem cell course for non-biology majors that will provide students with an overview of stem cell science and an opportunity to explore related ethical legal and social implications (ELSI). We will convene a multidisciplinary curriculum development and instructional team of faculty, including biology curriculum experts, a philosopher with expertise in ELSI and medical/research ethics and a stem cell researcher. Together, this group will create an undergraduate course using real-life case study curriculum modules and instructional strategies, then teach the course at the University of Rochester and at Monroe Community College. Finally, we will collect and analyze evaluation data to make appropriate revisions to the course. From review of the case studies, students will learn how to gather and critically evaluate current information on stem cell science and ELSI, and they will develop decision-making skills that will be useful for their future roles as active and informed citizens. Each case study module will include learning objectives that cover scientific concepts as well as ELSI issues of stem cell science. This project will also provide other undergraduate faculty, who will be able to adopt these curriculum modules and adapt them for use in their own courses, with models for presenting information on the science and ELSI of stem cells.

DEVELOPMENT OF AN INTERDISCIPLINARY PORTABLE COURSE ON STEM CELLS

John Russell, Ph.D.
Syracuse University, Syracuse, NY

The promise of stem cells for use in regenerative medicine is a matter of great public interest, but public understanding of relevant issues lags behind. As with any emerging technology, there are uncertainties and legitimate differences of opinion about the associated technologies and proposed uses. The fact that, at least until recently, the source of human stem cells with the greatest medical potential was human embryos, has raised important ethical and legal issues, issues that are often technical and always complicated. How are responsible citizens to judge the merits of claims and counterclaims coming from groups whose vested interests are sometimes clear and other times subtle? An interdisciplinary Steering Committee was created with faculty from Syracuse University and the State University of New York-Upstate Medical University to develop a one-semester, three-credit undergraduate course at Syracuse University that will broadly inform students about the science, ethical, religious, social and legal issues involved and help students develop the analytical skills necessary to address such complex issues. The course will also cover the role of the news media and the ways in which interested groups use the media to build their particular cases with the public. The proposed course will be led by an interdisciplinary group of faculty using lectures, assigned readings and small group discussions to cover this material in a highly integrated manner with respectful treatment of a broad range of ethical and religious viewpoints. We will develop complex problems and cases to present to our students, who will organize into small teams to develop their responses to the cases. Importantly, we will also develop ways to make these cases and all other course materials available to instructors at other institutions who do not have access to the breadth of expertise found in Syracuse.

THE BUSINESS AND BIOLOGY OF STEM CELLS IN CELL THERAPY

Robert Van Buskirk, Ph.D.
Binghamton University, Binghamton, NY

This proposal will fund two new stem cell courses at Binghamton University. The Fall 2010 course, "The Biotechnology of Stem Cells," will be taught by Professor Robert Van Buskirk, a cell biologist/tissue engineer who developed EpiDerm, a commercially successful stem cell-derived, human skin used worldwide for scar management. This course, limited to junior and senior biology majors, will include oral presentations of stem cell companies' history, underlying science, products and market potential, and a requirement to write an NIH SBIR (small business) grant focused on stem cells. The companion Fall 2011 course, "The Commercialization of Stem Cells", will be taught by Professor John Baust, a cell biologist/cryobiologist who developed commercially successful stem cell transport solutions now used internationally for shipping human stem cells for cell therapy. This course, offered to both undergraduate business and science (non-biology) majors, will include a tutorial on cell biology and stem cells, oral presentations on stem cell companies and a requirement to write a business plan focused on developing new stem cell companies. Dr. Alfred Cioffi, a well-known bioethicist, will present a guest lecture, and lead a discussion on embryonic stem cell bioethics. A retreat at a biotechnology company will be held at the end of the semesters where students will develop a slate of stem cell bioethical principles based on the course experiences. Each Fall course will alternate every other year and an optional Spring semester laboratory research internship will be available at a local biotechnology company. The overall intent of this project is to cross-fertilize both biology majors and non-biology science/business majors so that the former feel academically confident to pursue an entrepreneurial career in the stem cell sciences whereas the latter group may be similarly encouraged to enter stem cell patent law and other business disciplines that demand a solid grounding in stem cell biology.

SUMMER UNDERGRADUATE EXPERIENCE IN STEM CELL RESEARCH

David Bynum, Ph.D.
Stony Brook University, Stony Brook, NY

Advances in stem cell research are rapidly progressing, providing potential for future treatments for many diseases and injuries. In this proposal we will make available undergraduate research experiences in Stony Brook research laboratories to eight outstanding students from around the nation. This will be done by: 1) obtaining commitment from our research community to bring undergraduate students into their laboratories; 2) recruiting students nationally through our website, contacts and posters; 3) providing these students with financial support, housing, career counseling, mentoring, tours and guest lectures that supplement their research experience; 4) offering students travel support to attend local and national meetings to learn from other researchers and present their work; 5) tracking these students through their subsequent education and research careers to assess program impact. This proposal will be led by the director of CESAME (Center for Science and Mathematics Education) and the Vice Dean for Scientific Affairs in the School of Medicine at Stony Brook. The CESAME director has fifteen years experience in directing similar programs and has received local, state and national recognition for his work, including a visit to the White House where he received the Presidential Award for Excellence in Science, Mathematics and Engineering Mentoring. The Vice Dean for Scientific Affairs targets emerging opportunities in external funding sources to areas of research interest and expertise. He is a recent recipient of a NYSTEM grant, Therapy of Hemophilia A using Megakaryocyte-targeted Stem Cell Delivery.

SUMMER UNDERGRADUATE RESEARCH EXPERIENCE IN STEM CELL SCIENCE

Alice Heicklen, Ph.D.
Columbia University, New York, NY

Columbia University will offer Stem Cell Fellowships to a select group of motivated undergraduate students who will benefit from the opportunity for hands-on stem cell laboratory research. This fellowship aims to teach the next generation of scientists the tricks of the trade to performing stem cell research. This involves hands on experience in premier stem cell labs, weekly meetings within the laboratories, the development of the tools to explain the research carried out to individuals outside of the specific laboratory, developing stem cell curricula with secondary school teachers and exposure to research carried out in the broader stem cell field.

Students will work full-time on independent study projects under the guidance of a research scientist. In addition to the research performed in the laboratories, the program begins with an orientation to laboratory safety, graduate student career panel, ethics discussions and lectures by Columbia University and non-Columbia-University faculty on the research they are performing in their labs. The second component is a module where students meet weekly with other undergraduates in the program under the guidance of a graduate student to learn how to describe their research to individuals not in their specific laboratory. This is a skill that is frequently neglected but needs to be taught if we are going to improve the general public understanding of science. Each student will present orally to their peers, present orally to scientists in stem cell laboratories and write an original research article based on their research.

CORNELL UNDERGRADUATE STEM CELL SCIENCE PROGRAM

Laurel Southard, A.B.D.
Cornell University, Ithaca, NY

This proposal is a partnership between the Cornell University Undergraduate Biology major and stem cell scientists at Cornell. The Cornell Undergraduate Stem Cell Science Program (CUSSP) will have several goals. First, we will provide eight students with an intensive 10-week summer research opportunity in the lab of one of our accomplished Cornell mentors. These students will learn techniques and skills that are routinely used in modern research, and after initial training, will be involved in investigatory research. In addition, the summer program will introduce them to the literature of stem-cell research through a directed journal club. Students will meet weekly to read, present and discuss scientific papers in a structured, mentored environment. In addition, we will provide seminars and panels that will introduce the students to career and graduate school opportunities, ethical dilemmas in stem cell science and to potential role models in the Cornell stem cell community. Several of our mentors are clinician scientists who will bring a medical emphasis to the program. The students will also gain valuable experience in writing and speaking about their research through a series of presentation opportunities, including presenting their work at a national professional or undergraduate meeting. Our overall goal is to provide an enticing look at research in hopes that the majority of these students will choose careers in science. This project will be focused on stem cells, but will provide the students a wide perspective on current research in the field.

SHARED FACILITIES SESSION

CREATION OF THE STONY BROOK UNIVERSITY STEM CELL FACILITIES CENTER

Wadie F. Bahou
Stony Brook University, Stony Brook, NY

Stony Brook has well-documented interests in stem cell research, as exemplified by successful competition in many of the developmental initiatives put forth by the Empire State Stem Cell Board. Our success has been evident at both research and educational levels, with submission of >50 NYSTEM grants coming from 11 departments and four distinct colleges/schools throughout the University. To created value-added synergies for stem cell research, we will create a multi-user facility that will support 25 funded investigators and 8 investigators seeking funding, as a single-point-of-entry resource to broadly provide scientific and educational support in stem cell manipulation and analysis. Specifically, we propose to leverage pre-existing University facilities to create three distinct but overlapping core facilities, focusing on (1) Stem Cell Processing and Education, (2) Stem Cell Gene Transfer, and (3) Stem Cell Analysis. Generation of these facilities requires one-time renovation and equipment costs to create state-of-the-art refurbished laboratories, followed by operational costs for facilities support in subsequent years. The ability to leverage pre-existing University resources will create a value-added infrastructure that will synergistically enhance the quality and breadth of stem cell research and education facilities for the local and regional Long Island biomedical research community. The creation of symposia and pilot/feasibility grants will provide for exchange of ideas, and will serve to attract new users into the facility. Innovative aspects of the Stony Brook Stem Cell Center include application of unique expertise and resources in proteomics and genomics to characterize stem cells; and development of a unique educational resource for students, teachers, and the local community, thereby providing hands-on instructional venues to train and support a specialized workforce pursuing commercialization of nascent technologies related to stem cell research.

DEVELOPING AN INTEGRATED PLATFORM FOR siRNA AND shRNA-BASED GENOME-SCALE SCREENS IN EUKARYOTIC STEM CELLS

Chi Y. Yun, Shauna Katz, Ramanuj DasGupta
New York University School of Medicine, New York, NY

The primary objective of this proposal is to establish a collaborative, multi-institutional, state-of-the-art, RNA-interference (RNAi)-based high-throughput screening platform. This shared resource will be used for the systematic and comprehensive query of gene function using genome-scale short interfering RNA (siRNA) and/or short hairpin RNA (shRNA) screens in the regulation of self-renewal versus differentiation in a variety of adult and embryonic stem cells (SCs) and induced pluripotent cells (iPSCs). A key feature of this proposal is to integrate the complementary expertise, informatics, and technologies available through the RNAi-screening facility at NYU School of Medicine (for siRNA-based high-throughput screening platforms in cultured cells) and the proposed shRNA Core and High-Content Screening Shared Resource Facility at Mount Sinai School of Medicine (for lentivirus-based shRNA screens in specific (stem) cell types). The creation of this unique resource, in addition to the availability of human stem cell lines from the hESC-SRF at Mount Sinai will provide unprecedented access to state-of-the-art screening capabilities for stem cell researchers to probe biological pathways using RNAi technologies.

A key feature of this proposal is the commitment to make this integrated platform for siRNA and shRNA-based genome-scale screens fully accessible to the entire scientific community of New York State, which we are confident will promote multi-institutional collaborations. Importantly, a major mandate of the integrated screening resource is to encourage sharing of screening data generated at the facility. The creation of a public screen-database will generate awareness of post-genomic screening technologies and encourage academic researchers in NY State to utilize this unique and unprecedented resource available to them. This open access policy will be essential to realize the true potential of the functional genomic approaches employed to better understand the biology and regulation of stem cells.

UPSTATE STEM CELL cGMP FACILITY

Stephen Dewhurst[1] and Gerhard Bauer[2]
[1]Department of Microbiology & Immunology, and Stem Cell and Regenerative Medicine Institute, University of Rochester, Rochester, NY; [2]Laboratory Director GMP Facility, Stem Cell Program, Institute For Regenerative Cures (IRC), University of California, Davis CA

Current Good Manufacturing Practices (cGMP) are the procedures that biotech companies must follow to ensure that the products they produce meet U.S. Food and Drug Administration (FDA) standards for composition, purity, potency and quality. At present, no cGMP manufacturing facilities are available for human stem cell products in Upstate New York. This creates a roadblock to the development of new stem cell medicines, which the Upstate Stem Cell cGMP Facility is intended to overcome. Users of this facility will include stem cell scientists throughout Upstate New York, and their colleagues/collaborators at other New York institutions. The proposal has three specific objectives:

1. To create and operate a cell-based GMP facility to support stem cell research in Upstate New York. The facility will provide specialized laboratories for the isolation, propagation and distribution of human stem cells and their derivatives.
2. To accelerate stem cell clinical trial activity in Upstate New York, by producing cGMP grade products for human use. The facility will also provide expert guidance on regulatory requirements, and interactions with government regulatory bodies.
3. To facilitate collaboration between stem cell researchers in Upstate New York.

The planned facility will comprise three production suites/facilities:

1. Suite for tissue derived neural stem cells, including both fetal and adult tissue derived cells.
2. Suite for tissue derived autologous mesenchymal stem cells and ES cells.
3. Support and Overflow Suite. This will support reagent preparation, monoclonal antibody (Mab) production, sterile cell sorting, and overflow projects. We plan to produce Mabs under cGMP to facilitate purification of neural stem cells; future goals include integration of a FACS Aria into the facility to permit sterile cell sorts.

ESTABLISHMENT OF THE WESTERN NEW YORK STEM CELL CULTURE AND ANALYSIS CENTER (WNYSTEM)

Richard M. Gronostajski[1,5,6,8,9], Michael J. Buck[1,6,8], Jian Feng[2,5,7-9], Michal K. Stachowiak[3,5], Emmanuel S. Tzanakakis[4,8]

Depts. of [1]Biochemistry, [2]Physiology and Biophysics, [3]Pathology and Anatomical Sciences, [4]Chemical and Biological Engineering and the [5]Neuroscience Program, State University of New York at Buffalo, Buffalo NY; [6]Developmental Genomics Group and [7]Neurodegenerative Disease Group of the [8]New York State Center of Excellence in Bioinformatics and Life Sciences, Buffalo NY; [9]Dept. of Molecular and Cellular Biology, Roswell Park Cancer Institute, Buffalo, NY

Stem Cell Biology and Regenerative Medicine hold the promise of revolutionizing health care and providing therapies for a variety of diseases and conditions including cancer, diabetes, paralysis, neurodegenerative diseases and aging. Shared Stem Cell Facilities provide an important mechanism to promote and support stem cell research. The inter-decanal and inter-institutional NYSTEM-funded Western New York Stem Cell Culture and Analysis Center (WNYSTEM, pronounced "WIN-nee-stem") is a shared facility currently being established at the University at Buffalo with the participation of the Roswell Park Cancer Institute. The goal of WNYSTEM is to promote and facilitate fundamental and translational stem cell research and development throughout Western New York, New York State and the world. WNYSTEM will maintain four core facilities: the Stem Cell Culture, Banking and Training Facility (SCCF, E.S. Tzanakakis, Director), the induced Pluripotent Stem Cell Generation Facility (iPSF, J. Feng, Director), the Stem Cell Engraftment and *in vivo* Analysis Facility (SCEF, M.K. Stachowiak, Director) and the Stem Cell Sequencing/Epigenomics Analysis Facility (SCSF, M.J. Buck, Co-Director). These 4 core facilities will be coordinated and supported by an administrative unit which will also function to promote stem cell research through seminars, small facility-based research grants and educational activities. The administrative unit will be advised and overseen by Internal and External Advisory Committees that will provide important administrative and scientific direction and support. The four core facilities will provide training and/or research materials including cells, media, and reagents to investigators at UB, RPCI and other institutions in New York State. In cooperation with stem cell centers throughout New York State and Southern Ontario, WNYSTEM will provide the equipment, facilities and expertise needed to promote and support cutting-edge research in Stem Cell Biology and Regenerative Medicine.

HIGH-THROUGHPUT SCREENING AND CHEMISTRY SHARED FACILITY

Christopher Henderson[1,*], Andrea Califano[2], Donald Landry[3], Brent Stockwell[4]
[1]Departments of Pathology, Neurology and Neuroscience,Center for Motor Neuron Biology and Disease, Columbia Stem Cell Initiative; [2]Joint Centers for Systems Biology; [3]Department of Medicine; [4]Departments of Chemistry and Biology, Columbia University, New York, NY

Introduction/Background: Stem cells and different cell types made from them can be used in multiple ways to help us understand and treat human diseases. While some approaches focus on the replacement of missing cells in human patients, stem cells can be used model diseases in the culture dish, thereby creating an exciting new tool for drug discovery. Using stem cell derivatives from mice or human patients, one can recreate in culture the abnormal process (e.g. death, degeneration, or proliferation) that leads to disease in humans. Then, one by one, large chemical collections containing more than 100,000 compounds, or collections of shRNA/siRNAs, can be tested for their ability to correct the defect. This is the technique of “high-throughput screening (HTS)”. In the majority of cases, the results of HTS are to generate a greater mechanistic understanding of the cellular process studied, as a means for identifying therapeutic targets. In some cases, however, the compounds identified can be moved forward stepwise for validation as a basis for subsequent drug development process.

Compounds that show activity in the HTS assays need to be further optimized by chemical modification. To provide insights into the molecular mechanism of the disease process, they can be labeled and used for target identification. Others can be modified by medicinal chemistry to make them more drug-like, allowing them to reach affected tissues and exert potent effects without causing toxicity.

Summary of Goals and Objectives: a) Bring high-throughput screening, target identification and medicinal chemistry within reach of stem cell biologists in New York State; b) Establish a NYSTEM High-Throughput Screening and Chemistry Shared Facility (HTSCSF) to provide access to collections of chemical compounds and gene libraries, as well as all necessary specialized robotic equipment and relevant screening and chemical expertise; c) Support pilot projects focused on therapeutic development.

Innovative Elements of the Project: This will be the first statewide facility to allow drug screening on stem cell models for a wide range of currently incurable diseases.

PLENARY I – Stem Cells in Cancer and Other Disease

A NOVEL ROLE FOR CANCER STEM CELLS IN TUMOR ANGIOGENESIS

Viviane Tabar
Department of Neurosurgery, Memorial Sloan-Kettering Cancer Center

Glioblastoma (GBM) is among the most aggressive human tumors. It is characterized by florid neo-vascularization. Thus inhibitors of angiogenesis have been of particular interest leading to the clinical use of VEGF inhibitors such as bevacizumab, a monoclonal antibody that binds VEGFA. Unfortunately, data from several recent trials have shown disappointingly modest progress in tumor control and patient survival. Mechanisms of resistance to VEGF pathway inhibition remain unclear but are not due to the development of mutations within the pathway effectors.

Recent data from our lab (Wang *et al*. Nature 2010: 829-33) demonstrate that glioblastomas comprise a cancer stem-cell like subpopulation that is capable of direct differentiation into tumor vessels. In fact we demonstrate that the majority of endothelial cells within tumor vessels harbor somatic genomic aberrations identical to those seen within the tumor cells, regardless of the tumor genotype or transcriptomal class. In addition, we show that a defined subpopulation of GBM cells can transition to an endothelial lineage, *in vitro* and under single cell clonal conditions, as well as *in vivo*. Interestingly VEGF inhibition via pharmacological or genetic means can control maturation of the tumor-derived vessels, but does not interfere with tumor cell differentiation into endothelial progenitors. This may explain, at least in part, the poor performance of anti-VEGF therapies. However, the suppression of the Notch pathway via gamma-secretase inhibitors or shRNA for Notch1, resulted in significant but incomplete inhibition of GBM cancer stem cell differentiation into endothelial progenitors. These findings have now been reproduced in mouse models of glioma and may represent an important advance in our understanding of tumor biology.

INTRACORONARY INJECTION OF CARDIOSPHERE-DERIVED CELLS (icCDCs) IMPROVES REGIONAL FUNCTION BY RECRUITING ENDOGENOUS CELL REPAIR MECHANISMS IN CHRONICALLY ISCHEMIC MYOCARDIUM

Gen Suzuki[1], Merced M. Leiker[1], Thomas Cimato[1,2], John M. Canty, Jr.[1,2]
[1]University at Buffalo, Buffalo, NY; [2]VA Medical Center, Buffalo, NY

Background: Adult stem cells have been emerged as an alternative approach to treat patients with ischemic heart disease. However, mechanistic understanding of adult stem cell therapy is still unclear.

Results/Progress: CDCs were isolated from heart biopsies and expanded *ex vivo*. Cultured CDCs expressed specific proteins seen in immature cardiac cells (passage1-6) followed by mature cardiac proteins at later phases (>passage7). Early phase CDCs ($40x10^6$ cells) were injected into the major coronary arteries in swine with chronically ischemic and normal myocardium for 4-weeks. Histological data are summarized in the Table. icCDCs improved regional myocardial function (LAD wall thickening: 2.7 ± 0.4 mm to 4.9 ± 0.2 mm, $p<0.01$) and produced myocytes (myocyte nuclear density, Table) in ischemic myocardium with no effect in the normal myocardium. The beneficial effects were associated with increased myocytes in the cell cycle (Ki67) and in mitosis (pospho-HistoneH3) with no effect in normal myocardium. The data support the notion that CDCs facilitate the regeneration of cardiac myocytes associated with the improvement of cardiac function in diseased but not normal myocardium.

Future Directions: We will determine whether newly formed myocytes arise from injected CDCs or from stimulating adult heart tissue to proliferate.

Impact: The successful completion of this preclinical study in an animal model with a heart size that is similar to humans provides critical information supporting the clinical application of CDCs in patients with ischemic heart disease. Since CDCs already express cardiac specific proteins, it overcomes difficulties in directing stem cells to cardiac myocytes.

Table. Quantitative Histology / Immunohistochemistry

	Ischemic Myocardium		Normal Myocardium	
	Untreated (n=6)	icCDCs (n=10)	Untreated (n=6)	icCDCs (n=4)
Myocyte Nuclear Density (nuclei per mm^2)	830 ± 4†	1535 ± 48*†	1212 ± 43	1227 ± 38
$Ki67^+$ myocytes (nuclei per 10^6 myocytes)	410 ± 82†	1129 ± 161*†	284 ± 69	460 ± 137
$pHH3^+$ myocytes (nuclei per 10^6 myocytes	9 ± 5	224 ± 35*†	2 ± 2	10 ± 10

Mean ± SE, *$p<0.05$ *vs*. Untreated, †$p<0.05$ *vs*. Normal

[*Supported by NYSTEM contract C024351*]

LINEAGE-TRACING ANALYSIS OF PROSTATE EPITHELIAL BASAL CELLS IN REGENERATION AND CANCER

Zhu A. Wang and Michael M. Shen
Departments of Medicine and Genetics & Development, Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, New York, NY

The tissue localization of prostate epithelial stem cells is highly relevant for investigating the cell type(s) of origin for prostate cancer. Differences in the cell of origin in the stem cell lineage hierarchy may influence later disease outcomes. Several studies have shown that the prostate basal epithelial compartment contains stem cells that can be transformed to initiate cancers in tissue reconstitution assays. However, previous lineage-tracing studies from our laboratory have identified a luminal stem cell population in mouse that can serve as cells of origin for prostate cancer *in vivo* (X. Wang *et al*., 2009, Nature 461:495-500). Therefore, we have been interested in using lineage-tracing approaches to explore the potential stem cell and cancer-initiating properties of prostate basal cells. In particular, this methodology allows the assay of cancer formation in its native site *in vivo.*

In our studies, we have utilized *CK5-Cre^{ERT2}* transgenic mice for inducible lineage-marking of basal cells, followed by analysis of lineage-marked basal cells during androgen-mediated prostate regeneration and basal cell-specific oncogenic transformation. Our results suggest that normal basal cells may not behave as stem cells that drive prostate regeneration *in vivo*, since they are extremely inefficient in generating luminal cells and therefore lack the property of multipotency. Nonetheless, basal cells can serve as cells of origin for prostate cancer since targeted deletion of the *Pten* tumor suppressor in basal cells results in PIN and luminal differentiation. Our ongoing studies will further elucidate the roles of specific cell types and the molecular pathways involved in prostate cancer initiation and progression.

METABOLIC AND MOLECULAR MECHANISMS REGULATING PRECURSOR CELL FUNCTION AND CELL SIGNALING IN NORMAL DEVELOPMENT AND DISEASE

Mark Noble, Ibro Ambeskovic, Christopher Folts, Brett Stevens, Chris Proschel, Margot Mayer-Proschel
University of Rochester Stem Cell and Regenerative Medicine Institute, University of Rochester, Rochester, NY

Our studies have revealed a central role for intracellular redox status in modulating major progenitor cell functions of survival, self-renewal and differentiation in development and disease. Small (15-20%) changes in redox status can cause >1000% increases in cell survival in response to suboptimal trophic factor exposure, prevent death induced by multiple physiological stressors, modulates the balance between self-renewal and differentiation in dividing precursors and may even be crucial in effective transplantation repair of spinal cord injury. Moreover, signaling molecules that regulate these functions converge on redox state modulation as a necessary component of their activities. Studies on oligodendrocyte progenitor cells (OPCs), ancestors of myelin-forming oligodendrocytes of the CNS, reveal that many of these outcomes are modulated by a novel regulatory pathway coupling oxidative status with receptor tyrosine (RTK) degradation. In this pathway, increased oxidation sequentially activates Fyn kinase and the c-Cbl ubiquitin ligase, causing degradation of RTKs essential for division and survival, including receptors for PDGF, EGF and IGF-I. Active in multiple cell types and activated by multiple disease-related stressors, the redox/Fyn/c-Cbl pathway is a central nexus for regulation of cell function by such critical signaling molecules as thyroid hormone and neurotrophin-3, and for toxicity in diverse disease-related conditions including exposure to environmental toxicants, amyloid-β peptides and in chronic degenerative and pediatric genetic disorders. Still further disease relevance is shown by findings that glioblastomas and breast cancers inactivate the redox/Fyn/c-Cbl pathway and that restoring pathway activity reverses chemotherapy resistance, inhibits cancer stem cell function and prevents tumor growth. Our studies provide new insights into regulation of progenitor cell function that integrate metabolism, signaling function and biological outcomes into a novel mechanistic framework with broad applications for enhancing repair and understanding causes and treatments of a wide range of diseases.

[*Supported by NYSTEM contracts 23056, 24319, 26437 and SCIRB contract 23691*]

Keynote Address by Elaine Fuchs, Ph.D.

STEM CELLS OF THE SKIN: THEIR BIOLOGY AND CLINICAL PROMISE

Adult tissues require stem cells to replenish cells during normal turnover (homeostasis) and in response to injury. How stem cells balance self-renewal and differentiation is of fundamental importance to our understanding of normal tissue maintenance and wound repair. Moreover, increasing evidence suggests that the regulatory circuitry governing this balancing act is at the root of some types of tumors both in mice and in humans. The skin is an excellent model system to understand how stem cells function in normal tissue generation and how this process goes awry in cancer. Using skin as our paradigm, we've identified multi-potent stem cells within the hair follicle. In normal homeostasis, these cells become activated to fuel each new round of hair growth. Upon injury, these cells become activated to migrate upward and replenish the skin epidermis. We've been dissecting how extrinsic signaling to stem cells sets off a cascade of changes in transcription that governs the activation, polarization and migration of stem cells during tissue development, homeostasis, hair cycling and wound repair. We've also been exploring when stem cells that have exited their niche reach a point of no return when they lose their potential for being a stem cell. Finally, we've studied what instructs activated stem cells to return to quiescence and stop making tissue. Our findings have provided us with new insights into our understanding of the process of stem cell dynamics, and in doing so have revealed mechanisms which are also deregulated in a variety of different human cancers.

Elaine Fuchs is the Rebecca C. Lancefield Professor in Mammalian Cell Biology and Development at The Rockefeller University. She is also an Investigator, Howard Hughes Medical Institute. Fuchs has published >250 papers and is internationally known for her research in skin biology and associated human genetic disorders, which include skin cancers and life-threatening genetic syndromes such as blistering skin disorders. Fuchs' current research focuses on the molecular mechanisms that underlie how multipotent stem cells respond to external cues, change their program of gene expression, exit their niche and adopt specific fates to make the epidermis, sebaceous glands and hair follicles of the skin in normal homeostasis and wound repair. She is also interested in how these pathways in normal stem cell biology go awry in squamous cell carcinomas and in aging.

Fuchs received her Ph.D. in Biochemistry from Princeton University, and after her postdoctoral research at the Massachusetts Institute of Technology, she joined the faculty at the University of Chicago. She stayed there until 2002 when she relocated to The Rockefeller University. Fuchs' awards and honors include the Presidential Young Investigator Award, the Richard Lounsbery Award from the National Academy of Sciences, the Novartis-Drew Award for Biomedical Research, the Dickson Prize in Medicine, the FASEB Award for Scientific Excellence, the Beering Award, the National Medal of Science, the L'Oreal-UNESCO Award and the Charlotte Friend Memorial Award from the American Association for Cancer Research. This year, she received the Madison Medal, the Passano Award and the Albany Prize. Fuchs is a member of the National Academy of Sciences, the Institute of Medicine of the National Academy of Sciences, the American Academy of Arts and Sciences and the American Philosophical Society, and she holds honorary doctorates from Mt. Sinai/New York University School of Medicine and from the University of Illinois, Champaign-Urbana. Fuchs is also a past President of the American Society of Cell Biology and is currently President of the International Society for Stem Cell Research.

PLENARY II – Stem Cell Biology

PRIMARY CILIA MEDIATE THE UPREGULATION OF COX2 AND BMP2 IN RESPONSE TO OSCILLATORY FLUID FLOW IN HUMAN MESENCHYMAL STEM CELLS

David A. Hoey[1,2] and Christopher R. Jacobs[1]
[1]Department of Biomedical Engineering, Columbia University, New York, NY; [2]Department of Anatomy, Royal College of Surgeons, Dublin, Ireland

Osteoporosis is a debilitating bone disease which occurs in part when mesenchymal stem cells (MSCs) fail to produce sufficient numbers of osteoblasts to counteract bone resorption by osteoclasts. A key regulator of MSC differentiation is physical loading and understanding how MSCs translate a physical stimulus into a chemical response is an active area of research. Primary cilia are immotile, solitary cellular extensions that protrude from the apical surface of nearly every cell in the human body. Recent studies have revealed the primary cilium to be a multifunctional antenna, sensing both mechanical and chemical changes in the extracellular environment. The application of 2 hrs of oscillatory fluid flow (OFF) to human MSCs (hMSCs) significantly upregulated both COX2 and BMP2 mRNA expression over both no flow and osteogenic differentiation controls ($P<0.05$). This increase in expression was sustained for 2 hrs following flow but returned to no flow levels at later time points. Primary cilia were visualized using immunocytochemistry and were found to exist as rod like structures extending linearly from the perinuclear region of the cell. Primary cilia were found on 71 ± 4% of hMSCs extending 3-6 μm in length. IFT88 (protein required for ciliogenesis) mRNA expression was successfully knocked down using siRNA targeting IFT88 as verified by real-time qRT-PCR. In cells treated with control siRNA, exposure to flow resulted in a significant increase in COX2 and BMP2 expression ($P<0.05$) whereas in cells treated with siRNA targeting Polaris, exposure to flow did not result in a significant increase in either COX2 or BMP2 mRNA expression. In this study we present evidence that the primary cilium acts as a mechanosensor in hMSCs, providing a novel mechanism by which mechanical stimuli translates into osteogenic responses. Our results therefore highlight the primary cilium as a potential therapeutic target for efforts to prevent bone loss during diseases such as osteoporosis.

[*Supported by NYSTEM contract N08G-210 and an IRCSET-Marie Curie International Mobility Fellowship in Science, Engineering and Technology*]

GENERATION OF ANTERIOR FOREGUT ENDODERM FROM HUMAN EMBRYONIC AND INDUCED PLURIPOTENT STEM CELLS

Michael D. Green[1], Antonia Chen[1], Maria-Cristina Nostro[2], Sunita L. d'Souza[1], Christoph Schaniel[1], Ihor R. Lemischka[1], Valerie Gouon-Evans[1], Gordon Keller[2], Hans-Willem Snoeck[1]
[1]Department of Gene and Cell Medicine, Mount Sinai School of Medicine, New York, NY; [2]McEwen Centre for Regenerative Medicine, Ontario Cancer Institute, Toronto, Canada

Directed differentiation of human embryonic stem (hES) cells and human induced pluripotent state (hiPS) cells captures *in vivo* developmental pathways for specifying lineages *in vitro*, thus avoiding perturbation of the genome with exogenous genetic material. Thus far, derivation of endodermal lineages has focused predominantly on hepatocytes, pancreatic endocrine cells and intestine. The ability to differentiate pluripotent cells into anterior foregut derivatives would expand their utility for cell-based therapy and basic research to tissues important for immune function, such as the thymus; for metabolism, such as thyroid and parathyroid; and for respiratory function, such as trachea and lung. We find that dual inhibition of TGF-β and BMP signaling after specification of definitive endoderm from pluripotent cells results in a highly enriched anterior foregut endoderm population that is uniquely competent to subsequently be patterned along dorsoventral and anteroposterior axes. These findings provide a platform for the generation of anterior foregut endoderm derivatives.

PLENARY III – Tissue Engineering and Regenerative Medicine

BIOENGINEERING STRATEGIES FOR STEM CELL RESEARCH

Gordana Vunjak-Novakovic
Department of Biomedical Engineering, Columbia University, New York NY

The overall goal of stem cell bioengineering is to develop new treatment modalities for improving human health, by pursuing interdisciplinary research in three related areas: (1) Tissue engineering of human grafts for application in regenerative medicine, (2) Models of disease, and (3) Controllable systems for biological research. In all cases, experimentation at the interfaces of biology, engineering, and medical sciences is critical for unlocking the full regenerative potential of stem cells.

This talk will focus on advanced technologies that are currently being developed to engineer functional tissue grafts, study the development and disease, and regulate the fate and function of human stem cells. First, we discuss tissue engineering of viable anatomically shaped human bone grafts, by an integrated use of adult human mesenchymal stem cells, biomaterial scaffolds, and perfusion bioreactors. Next, we discuss tissue engineering of a cardiac patch using human adult and embryonic cells, and the use of this patch for heart repair following myocardial infarction. Then, we discuss novel technology platforms for advanced stem cell research, providing spatial and temporal gradients of regulatory factors. Finally, we discuss the significance of stem cell bioengineering for providing functional human tissue grafts, and for the establishment of physiologically relevant models enabling us to study stem cells in native-like three-dimensional contexts of development or disease.

[*Supported in part by NYSTEM contract C026449*]

INDUCED PSA EXPRESSION IN ES-DERIVED DOPAMINERGIC NEURONS IMPROVES GRAFT/HOST INTEGRATION AND BEHAVIORAL RECOVERY IN PD ANIMALS

Daniela Battista, Yosif Ganat, Lorenz Studer, Urs Rutishauser
Cell Biology Program, Sloan-Kettering Institute, New York, NY

Grafted stem cell-derived neurons used as a potential treatment for Parkinson disease (PD) do not integrate well into host brain, as evidenced by their limited ability to migrate and send axons into surrounding brain tissue. To improve graft migration and integration, we engineered polysialic acid (PSA) expression into mouse embryonic stem cells.

We generated a PSA-expressing Nurr::GFP mouse cell line and grafted either 95,000 or 55,000 ES-derived dopaminergic cells into the striatum of lesioned mice. Two months after their injection, about 60% of the cells co-labelled for GFP and either tyrosine hydroxylase (TH), FoxA2 or Girk2, indicating that they maintained a DA fate. The Nurr/PST grafts displayed an increase in cell survival, and gave rise to longer processes. They also displayed an increase in tyrosine hydroxylase (TH) intensity further away form the core of the graft, indicating that they are extending and delivering dopamine at a distance from the transplantation site. The increased TH intensity correlated with improved behavioral recovery, and the Nurr/PST grafts had more and bigger synapse-like varicosities, indicating that they might be forming more connections with host tissue.

Our data suggest that ectopic expression of PSA is altering cell interactions within the graft and its immediate environment, making it possible for the cells to extend processes for longer distances, and subsequently to form connections.

HIGH EFFICIENCY MYELINATION OF THE HYPOMYELINATED SHIVERER MOUSE BRAIN USING HUMAN iPS CELL-DERIVED GLIAL PROGENITOR CELLS

Su Wang[1,2], Janna M. Bates[1,2], Xiaojie Li[1,2], Steven J. Schanz[1,2] , Devin Chandler-Militello[1,2], Lucy E. Gee[1,2], Nimet Maherali[4], Konrad Hochedlinger[4], Lorenz Studer[5], Martha S. Windrem[1,2], Steven A. Goldman[1-3]

[1]Center for Translational Neuromedicine and the Departments of [2]Neurology, [3]Neurosurgery, University of Rochester Medical Center, Rochester, NY; [4]Harvard Stem Cell Institute, Boston, MA; [5]Developmental Biology Program, Sloan-Kettering Institute, New York, NY

We have previously described techniques for the identification, isolation, molecular characterization, and transplantation of glial progenitor cells derived from the fetal human brain. Neonatal engraftment by these cells permits the widespread myelination of congenitally dysmyelinated shiverer mouse brain, and can rescue both the neurological phenotype and lifespan of these animals. Yet the possibility of immune rejection of allogeneic grafts has hindered the clinical development of this approach. To address this issue, we investigated the use of human induced pluripotential stem cells (hiPS cells) as a source from which new glial progenitor cells (GPCs) and their derived oligodendrocytes (OLs) might be generated. We developed a high-efficiency strategy by which to differentiate hiPS cells into GPCs that are in turn able to mature into both astrocytes and myelinogenic OLs, *in vitro* and *in vivo*. Using this protocol we have successfully generated and validated a population of olig2^{+}/PDGFR• $^{+}$/Nxk2.2^{+}/sox10^{+} human GPCs. Using this protocol, we found that the efficiencies of GPC induction from hiPSCs (KØ4 cells, K. Hochedlinger, keratinocyte-derived; C27 cells, L. Studer, fibroblast-derived) were similar to that of human embryonic stem cells (hESCs; WA09), in that their otherwise unsorted yields of olig2^{+}/Nkx2.2^{+}GPC colonies were 63% (KØ4) and 79% (hES WA09), respectively. hiPSC-derived GPCs, as well as their oligodendrocytic and astrocytic progeny, could then be differentially isolated using FACS directed at glial lineage markers that included A2B5, CD140a/ PDGFR• , CD9, O4, and GLT1. In addition, these hiPSC-derived GPCs could be differentiated into mature MBP^{+} OLs and GFAP^{+} astrocytes in culture. Importantly, these hiPS-GPCs readily matured into myelinogenic oligodendrocytes *in vivo* and efficiently myelinated the forebrains of myelin-deficient shiverer mice by 12-13 weeks after neonatal graft. Of note, no tumor formation was noted from these grafts as long as 3 months after transplant. These results demonstrate that high efficiency in vivo oligodendrocytic differentiation and myelination can be achieved from human iPS cells, suggesting the potential utility of iPS-derived autografts in treating acquired disorders of myelin.

[*Supported by the New York State Stem Cell Board (NYSTEM), the National Multiple Sclerosis Society, and NINDS R01NS39559*]

ENGINEERING TISSUE-TO-TISSUE INTERFACE VIA NANOFIBER SCAFFOLD DESIGN AND CONTROLLED STEM CELL DIFFERENTIATION

Helen H. Lu, Kristen L. Moffat, Anne S. Kwei, William N. Levine, Cevat Erisken, Xinzhi Zhang
Department of Biomedical Engineering, Columbia University, New York, NY

Musculoskeletal motion is facilitated by synchronized interactions between multiple tissue types and the seamless integration of bone with soft tissues such as tendons, ligaments or cartilage. Many of these soft tissues transit into bone through a multi-region fibrocartilaginous interface, which serves to minimize the formation of stress concentrations while enabling load transfer between soft and hard tissues. Given its functional significance, re-establishment of the soft tissue-to-bone interface is thus critical for promoting the integrative repair of biological and synthetic soft tissue grafts. To address the challenge of biological graft fixation, our approach centers on mesenchymal stem cell (MSC)-mediated interface tissue engineering, guided by the working hypothesis that this complex multi-tissue transition may be regenerated by controlled differentiation of MSC-derived, interface-relevant cell populations on a stratified scaffold pre-designed with a biomimetic gradient of structural and functional properties. Focusing on the rotator cuff tendon-to-bone insertion site, and inspired by current understandings of the native interface structure-function relationship, *in vitro* and *in vivo* evaluations of biomimetic, nanofiber-based stratified scaffolds for tendon-to-bone interface regeneration will be presented. The novel nanofiber scaffold is optimized *in vitro*, and its functionality further enhanced with select biofactors. It is anticipated that interface tissue engineering will promote the development of a new generation of tendon fixation devices that would enable integrative and functional soft tissue repair. Moreover, by bridging distinct types of tissue, interface tissue engineering will be instrumental for the *ex vivo* development and *in vivo* translation of integrated musculoskeletal tissue systems with biomimetic complexity and functionality.

TRANSLATION PANEL DISCUSSION

Steven A. Goldman, MD, PhD, is the Edward and Alma Vollertsen Rykenboer Professor and Chairman of the Department of Neurology at the University of Rochester, and Neurologist-in-Chief of Strong Memorial Hospital. He is also Chief of the Department's Division of Cell and Gene Therapy, and Co-Director of Rochester's Center for Translational Neuromedicine. Goldman holds additional appointments as a Professor of Neurosurgery, and as the Dean Zutes Chair in Biology of the Aging Brain. Goldman moved to Rochester in 2003 from the Weill Medical College of Cornell University, where he was the Nathan Cummings Professor of Neurology, and Attending Neurologist at New York Presbyterian Hospital. A summa cum laude graduate of the University of Pennsylvania, he obtained his PhD with Fernando Nottebohm at the Rockefeller University in 1983, and his MD from Cornell in 1984. Dr. Goldman interned in Medicine and did his residency in Neurology under Fred Plum at New York Hospital-Cornell, and Jerome Posner at the Memorial Sloan-Kettering Cancer Center, before joining the Cornell faculty. Dr. Goldman has published over 200 papers in his field, over 100 as first or senior author. He is a recipient of the Jacob Javits Neuroscience Investigator Award of the NIH, and has been elected to the Association of American Physicians and American Society for Clinical Investigation, as well as the American Neurological Association. Dr. Goldman remains active clinically, with subspecialty interests in stroke, myelin disease and neuro-oncology, and he is the program director for Rochester's neuro-oncology fellowship.

Stephen Chang, PhD, joined NYSCF in 2010 after serving as Chief Scientific Officer of Stemgent upon the company's founding in 2008. He was previously the CEO of Multicell Technologies and continues as a director of this company. Prior to that, he was the CEO of Astral Immunotherapy which was acquired by Multicell Technologies in 2005. Previously, he was chief science officer (CSO) and vice president of Canji Inc. / Schering Plough Research Institute in San Diego from 1998 to 2004. He has also held senior management positions at Chiron and Viagene. Dr. Chang is president of CURES, a coalition of patient advocates, biotechnology companies, pharmaceutical companies, and venture capitalists dedicated to ensuring the safety, research, and development of innovative lifesaving medications. He is also a board member of Histogen, Inc., a privately held company in regenerative medicine. Dr. Chang earned his doctoral degree in biological chemistry, molecular biology, and biochemistry from the University of California, Irvine.

Dr. Chang's research interest is in the area of translational applications of basic science to pharmaceutical products. He holds over 20 patents and has initiated or been responsible for over nineteen IND involving novel biologic and small molecule compounds. A biochemical geneticist by training, Dr. Chang has been involved in HIV therapeutics, cancer therapeutics, vaccine applications, arthritis, Multiple sclerosis, ophthalmology, hepatitis and stem cell research.

Karl Kieburtz, MD, MPH, is the Director of the Center for Human Experimental Therapeutics and Professor of Neurology, Community and Preventive Medicine and Environmental Medicine at the University of Rochester Medical Center in Rochester, New York. His primary clinical and research interests are neurodegenerative diseases affecting the basal ganglia, particularly Parkinson disease, Huntington disease, and HIV related neurologic disorders. He is the principal investigator for the NINDS sponsored trials of neuroprotective agents for PD (NET-PD) and currently chairs the Executive Committee of the Parkinson Study Group. He also directs the Coordination Center for other NIH and foundation supported multi-center academic consortia.

Edmund Mickunas*,* Vice President of Regulatory**,** is in charge of spearheading Advanced Cell Technology's efforts to translate its cutting edge research into cellular therapies ready for clinical trials. Mr. Mickunas successfully submitted INDs for Stargardt's Macular Dystrophy and Dry Age Related Macular Degeneration programs to the FDA. The trials for these indications will begin in 2011.

Mr. Mickunas brings 30 years of experience from a number of disciplines including biotechnology, medical device and pharmaceuticals. He has worked in preclinical program development, clinical affairs, regulatory affairs/compliance and quality assurance, and has worked closely with all disciplines involved in product development. Prior to joining Advanced Cell, Mr. Mickunas was Executive Director, Regulatory Affairs and Quality Assurance for Applied Genetic Technologies Corporation, a privately held gene therapy and genomics-focused biotech company. He previously held the position of Vice President of Regulatory and Clinical Affairs at Oragenics, where he managed the submissions and clinical trial program for genetically modified bacteria as a therapeutic product. He has also held senior regulatory positions at Bioheart, Inc., Cytomed and LeukoSite. Additionally, he has previously consulted in regulatory affairs, clinical affairs and quality assurance. He received his B.S. and pursued graduate studies in human biochemistry at Fairleigh Dickinson University, as well as a Masters Degree from Fairfield University.

Sridaran Natesan, PhD, Scientific Site Head (R&D) & Head, External Innovation and Partnering (US, Northeast) Sanofi-aventis

Dr. Sridaran Natesan is the Scientific Site Head (R&D) at Sanofi-aventis in Cambridge and also the Head of External Innovation and Partnering for the US Northeast region. He is also a member of the leadership team of the Prospective Strategic Initiative group at Sanofi-aventis. From 2005-2009, he served as the co-leader of the stem cell initiative and Distinguished Scientist at Sanofi-aventis.

Dr. Natesan received his Ph.D degree in Molecular Genetics from the University of Calgary and pursued his post-doctoral work at the Cold Spring Harbor Laboratories in New York. Dr. Natesan joined Ariad Pharmaceuticals in Cambridge, MA in 1994 as a Senior Research Scientist in its Gene Therapy Division. Later, he joined Aventis Pharmaceuticals as a Principal Scientist and led a group focused on cell based screens and stem cell biology. Dr. Natesan is one of the founding members of the Aventis Cambridge Genomics Center and played a key role in the development of the center including as its Head of the Scientific Programs and member of the senior management for several years. He also served as the Head of Molecular Pharmacology at the center during this time.

Dr. Natesan is a member of the Industry Committee of International Society for Stem Cell Research (ISSCR). He is also member of the Outreach committee of the US India Chamber of Commerce. Dr. Natesan is responsible for setting up major academic alliances at Sanofi-aventis including those with MIT and Harvard.

Bruce S. Schneider, MD, is General Medicine Team Leader in the Office of Cellular, Tissue, and Gene Therapies/CBER/FDA. Dr. Schneider regulates the clinical development of numerous cellular and gene therapies for a variety of clinical indications. He has a special interest in pancreatic islet cell transplantation for Type 1 Diabetes and has been involved in both research and policy issues related to design of clinical islet transplant trials.

Dr. Schneider also serves on numerous FDA and interagency committees, including the NIH-FDA Interagency Artificial Pancreas Working Group and the Metabolic Diseases Steering Committee of the Biomarkers Consortium. Both are FDA Critical Path initiatives. He is also FDA representative to the Scientific Advisory Board of the Collaborative Islet Transplant Registry.

Dr. Schneider received his MD degree from Harvard Medical School and clinical training at Mount Sinai Medical Center in New York City. Prior to joining FDA, he was a member of the full-time faculty of Rockefeller University, Chief of Endocrinology and Metabolism at Long Island Jewish Medical Center, and Professor of Medicine at the Albert Einstein College of Medicine in New York. He is a past recipient of the Irma T. Hirschl Career Scientist Award and the Elliot Osserman Distinguished Service Award. In 2009, he received FDA's Outstanding Service Award for his work in islet transplantation and endocrinology.

Jason Kolbert, MBA, Vice President of Strategic Business Development

Jason Kolbert is NeoStem's newest addition joining the firm in March of 2011 as Vice President of Strategic Development. As a research analyst on Wall Street, Jason has been following the company for the past year. Prior to joining NeoStem, Jason served as a managing director and the head of research at National Securities where he recently engineered a merger between National Securities and Opus Point Partners to create a new joint venture dedicated to the life science arena. Prior to joining National Securities, Jason spent seven years at Susquehanna International Group where he founded the firm's healthcare effort. This included managing a $1 billion life science fund, the creation of Susquehanna venture capital group in China and managing the department's objectives and resources (12 analysts and support personnel). The role continued to develop into the firm's sell-side research platform where Jason led a dedicated team of analysts. His work was regularly featured in the media with multiples presences on CNBC.

Jason's career began as a chemist in the pharmaceutical industry, during which time he pursued his master's in business administration in finance. As a fluent Japanese speaker with a background in chemistry and a finance degree, he was recruited by Schering-Plough into a corporate finance, marketing and business development role reporting to the President. Upon returning from Japan, Jason joined Salomon Smith Barney (7 years) in investment banking working with industry leaders across multiple sectors in the healthcare space focused on banking and finance in Asia-U.S. companies.

Jason received his undergraduate degree in Chemistry from the State University of New York - New Paltz, where he graduated with honors and holds a Master's Degree in Business with a specialization in finance from the University of New Haven. He is a licensed analyst (series 86, 87, 7, 63) and holds an Honorable Discharge USMC PLC Flight program.

PLENARY IV – Disease Models and Therapeutic Approaches

CONTRIBUTION OF THE VASCULAR NICHE TO ORGAN REGENERATION

Shahin Rafii
Ansary Stem Cell Institute and Department of Genetic Medicine, Howard Hughes Medical Institute, Weill Cornell Medical College, New York, NY

Interaction of stem cells with their niche cells is essential for self-renewal and differentiation of stem and progenitor cells. We have found that endothelial cells (ECs) are not just passive conduits to deliver oxygen and nutrients, but also establish an instructive vascular niche, which by elaboration of paracrine trophogens, known as angiocrine factors, directly promote organogenesis. Activation of Akt-mTOR pathway in the ECs stimulates expression of angiocrine factors, including Notch-ligands, IGFBPs, FGFs and TGF-modulators, that induce expansion of hematopoietic and hepatic stem and progenitor cells. Specifically, angiocrine expression of Notch ligands promotes expansion of authentic long-term repopulating hematopoietic stem cells, while angiocrine expression of Wnt2 and hepatocyte growth factor (HGF) induces liver regeneration. Modulation of specific angiocrine factors in ECs will provide for a therapeutically effective means to stimulate and sustain organogenesis.

FUNCTIONAL COMPARISON OF LONG QT-ASSOCIATED CARDIAC ION CHANNELS (I_{Na}, I_{Kr}, I_{Ks}) IN HUMAN EMBRYONIC STEM CELLS AND HUMAN INDUCED PLURIPOTENT STEM CELLS

Cecile Terrenoire[1], Kai Wang[1], Gordon Keller[2], Darrell N. Kotton[3], Robert S. Kass[1]
[1]Department of Pharmacology, College of Physicians and Surgeons, Columbia University Medical Center, New York, NY; [2]McEwen Centre for Regenerative Medicine, University Health Network, Toronto, Canada; [3]The Pulmonary Center, Boston University School of Medicine, Boston, MA

Human induced Pluripotent Stem cells (hiPS cells) have been developed as an alternative to human Embryonic Stem cells (hES cells) in order to carry out drug screenings and to study diseases such as the Long QT (LQT) syndrome, a heritable cardiac arrhythmia linked to sudden death. To this date, the basic electrophysiological properties of cardiac cells derived from hiPS cells (hiPSC-CMs) have not been fully characterized and it is still not known how they compare with cardiac cells derived from hES cells (hESC-CMs). Here we report investigation of the electrophysiological properties of single hESC-CMs and hiPSC-CMs during the first 30-60 days of cytokine directed differentiation with a focus on cardiac ion channels associated with Long QT syndrome. I_{Na} (LQT3), defined as inward current blocked by TTX (30 µM), had a mid-point of steady-state inactivation at -65.1 ± 2.4 mV (n=6) in hESC-CMs and at -68.9 ± 1.1 mV (n=9) in hiPSC-CMs. The late sodium current, measured at the end of 100 ms-depolarizing pulses to -10 mV (from -90 mV), represented 0.06 ± 0.03% (n=6) and 0.03 ± 0.01% (n=9) of I_{Na} peak current in hESC-CMs and hiPSC-CMs, respectively. I_{Kr} (LQT2), defined as E4031-sensitive outward potassium current during depolarization, was characterized by a bell-shape current-voltage curve when measured during the test pulse, with a maximum current observed at 10 mV in both cell types. I_{Kr} channels had a mid-point of activation at -2.6 ± 2.2 mV (n=11) in hESC-CMs and at 1.7 ± 1.2mV (n=15) in hiPSC-CMs. I_{Ks} (LQT1), defined as Chromanol 293B-sensitive outward potassium current slowly increasing during prolonged depolarization, had a mid-point of activation at 9.1 ± 1.7mV (n=11) in hESC-CMs and at 9.5 ± 3.1 mV (n=8) in hiPSC-CMs. This is the first comprehensive comparison of the biophysical properties of I_{Na}, I_{Kr} and I_{Ks} in hESC-CMs and hiPSC-CMs. Our study suggests that hiPSC-CMs constitute a relevant cellular model for studying ion channels mutations that affect channels biophysical properties.

[*Supported by NYSTEM contract N09G125*]

SUSTAINED EPENDYMAL OVER-EXPRESSION OF BDNF AND NOGGIN VIA AAV4 TRIGGERS FUNCTIONAL MEDIUM SPINY NEUROGENESIS AND DELAYS DISEASE PROGRESSION IN A MOUSE MODEL OF HUNTINGTON'S DISEASE

Abdellatif Benraiss[1,2*], Michael Toner[1], Qiwu Xu[2], Cécile Boyer[1], Elodie Bruel-Jungerman[1], Aris Economides[4], Beverly Davidson[5], Ryoichiro Kageyama[6], Maiken Nedergaard[2,3], Steven A. Goldman[1-3*]
Depts. of [1]Neurology and [2]Neurosurgery, [3]Center for Translational Neuromedicine, University of Rochester, Rochester, NY; [4]Regeneron Pharmaceuticals, Tarrytown, NY; [5]Dept. of Medicine, University of Iowa, Iowa City, IA; [6]Institute for Virus Research, Kyoto University, Shogoin-Kawahara, Sakyo-ku, Kyoto 606-8507, Japan

Huntington's disease (HD) is a hereditary neurodegenerative disease characterized by progressive impairment of motor function, psychiatric disturbance, and dementia with an invariably fatal outcome. Stereotaxic delivery of adenovirus expressing BDNF and noggin into the lateral ventricle is sufficient to induce recruitment of new medium spiny neurons to the adult neostriatum. This strategy was proven to slow disease progression and extend survival in the R6/2 HD mouse model. However, the practical therapeutic value of this strategy of inducing new striatal medium spiny neurons was limited by the transient expression of adenoviral vectors. We therefore used intraventricular injection of adeno-associated virus-4 (AAV4), which provides persistent transgene expression by transduced cells, to elicit the sustained periventricular overexpression of BDNF and noggin. In the present study, we found that a single intracerebral injection of AAV4 vectors expressing BDNF and noggin triggered the sustained and robust recruitment of substantial numbers of new medium spiny neurons in the neostriata of R6/2 HD mice. Newly-generated neurons arose from the subventricular zone and migrated into the neostriatum, wherein they extended fibers to the globus pallidus, and could be retrogradely labeled by intrapallidal injection of Alexa-labeled cholera toxin B. This process could be visualized in trigenic R6/2 x AAV4-BDNF/noggin-treated nestinCreER^{T2} x floxed Rosa26-EFYP mice, in which both neural progenitor cells and their derived new neurons could be specifically reported as YFP^{+} following tamoxifen (TXN) treatment. We found that BDNF and noggin treatment induced substantial numbers of new YFP^{+} medium spiny neurons in this model, in both R6/2 trigenics and wild-type bigenic controls. The new medium spiny fully differentiated into both Substance P-expressing and enkephalin-expressing striopallidal projection neurons. Using whole cell patch clamp in neostriatal slices, we then assessed the newly generated MSNs physiologically. We found that newly generated CTB^{+}/EGFP^{+} striopallidal projection neurons derived from AAV4-BDNF/noggin-treated, TXN-treated nestinCreER^{T2} x floxed Rosa26-EFYP mice exhibited sustained action potentials to depolarization, indicating their functional as well as anatomic integration into the striato-pallidal circuitry. Importantly, AAV4-BDNF and noggin treatment significantly and substantially delayed motor deterioration and increased the life span of these R6/2 mice. These data suggest that the induction of sustained neuronal addition to the HD neostriatum may comprise a potential strategy by which to treat striatal neuronal loss in Huntington Disease as well as in other disorders of striatal neuronal loss.

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EX VIVO, *IN SITU* EXPANSION OF THE LIMBAL EPITHELIAL STEM CELL POOL

Alexander Barash[1] and J. Mario Wolosin[1,2]
[1]Department of Ophthalmology, [2]Black Family Stem Cell Institute, Mount Sinai School of Medicine, New York, NY

Limbal epithelial stem cell deficiency (LSCD) results in partial or full loss of vision. In monocular instances, the corneal surface and vision can be reestablished by transplant of cultures outgrown from an explant of a contra-lateral limbal biopsy. In this approach the originating biopsy is discarded and graft success is a function of the presence of stem/progenitor cells within the outgrowth. We have now tracked the stem/progenitor cell fate/dynamics in this clinical model. ABCG2 transport (generates side population, or SP cells) and feeder cell-supported colony formation efficiency (CFE) was used as phenotype indicators. SP status was determined using a newly identified, non-toxic, bathochromic ABCG2 substratum (JC1), in combination with three ABCG2-specific inhibitors (FTC, Ko 143 and glafenine). Limbal biopsies were explanted on Falcon inserts and sequentially transferred to new inserts every 8-10 days, yielding up to five generations of biopsy-derived epithelial cultures. Unexpectedly, SP (= JC^{low}) percentiles in the outgrowths increased, rather decrease, with successive culture cycles (e.g., from 19.5% to 25.8%; n=5; $p<0.05$ for human cells 1^{st} and 2^{nd} generations and from 27.4%, 32.5% and, 72% for the 1^{st}, 2^{nd} and 4^{th} generations in rabbit). Rabbit SP and non-SP cell CFE were 12.3 and 0.9% (n=4; $p < 0.01$), respectively, consistent with the concept that SPs are highly enriched in stem cells. Independent measurement of SP content in the epithelium remaining over the limbal biopsy reveled that this parameter increased from less than 1% in fresh, un-incubated human limbus (e.g., Budak, JCS; 118: 1715) to 20% after 6 days of culture. The results are consistent with *in situ* conservative duplication of limbal stem cell that, in turn, causes the time-dependent increase in explant outgrowth SP percentile. Current approaches to reconstructive surgery for LSCD may need to be modified accordingly.

[*Supported by NYSTEM contract C026450*]

PLENARY V – Pluripotency, Reprogramming and Directed Differentiation

GENERATION OF PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS FROM PATIENTS WITH RECESSIVE DYSTROPHIC *EPIDERMOLYSIS BULLOSA* AND THEIR DIFFERENTIATION INTO KERATINOCYTES

Munenari Itoh[1] and Angela M. Christiano[1,2]
Departments of [1]Dermatology, [2]Genetics and Development, Columbia University, New York, NY

In recent years, generation of induced pluripotent stem cells (iPSCs) from patients with several human diseases has been reported. Such cells are very useful tools to investigate unknown disease mechanisms and therapeutic testing. Moreover, autologous patient-specific iPSCs (PS-iPSCs) have great potential to provide a source of cells for regenerative therapies for specific human diseases, because they have unlimited proliferative capacity and extensive differentiation capability into required cell types. We focused on recessive dystrophic epidermolysis bullosa (RDEB), an inherited blistering skin disorder caused by mutations in the *COL7A1* gene for developing iPSC-based therapy. In this study, we explored the potential of iPSCs to establish a stem cell source for gene and cell therapy for RDEB. We succeeded in generating iPSCs not only from both fibroblasts as well as keratinocytes of normal individuals, and also from fibroblasts of two patients with RDEB. We showed that both types of iPSCs closely resemble ESCs and reported iPSCs in many respects, including the expression of stem cell markers, methylation status of the *NANOG* promoter region and differentiation capability, among others. Moreover, our study demonstrated that the loss of type VII collagen in RDEB cells had no influence on generation of iPSCs. Furthermore, we developed a directed differentiation protocol by which both normal and RDEB patient-specific iPSCs can be differentiated into fully functional keratinocytes which can be matured and terminally differentiated in 3D skin equivalents. An additional aim of this study is to correct the *COL7A1* mutation in PS-iPSCs. For gene correction by homologous recombination, a targeting vector with neomycin-resistant selection cassette was generated based on BAC recombineering, and nucleofected into PS-iPSCs. We obtained several iPSC lines which appear to be genetically corrected after drug selection. We are simultaneously pursuing a new source of PS-iPSCs from 'revertant mosaic' (spontaneously corrected) keratinocytes in phenotypically normal patches of skin, which are common among EB patients, express detectable type VII collagen protein, and are easily accessible by skin biopsy. Taken together, our aim is to develop PS-iPSC-based cell technology toward clinical utility using both approaches in parallel, with the goal of establishing a curative therapy for RDEB.

IDENTIFICATION OF A SET OF TRANSCRIPTION FACTORS FOR DIRECT REPROGRAMMING OF HUMAN SOMATIC CELLS INTO HEMATOPOIETIC STEM CELLS/PROGENITORS

Vladislav. M. Sandler and Eric E. Bouhassira
Albert Einstein College of Medicine, Bronx, NY

Modern methods of treatment of numerous malignant (e.g. leukemia and lymphoma), and genetic (e.g. immunodeficiencies, hemoglobinopathies, and Fanconi's Anemia) blood diseases rely on hematopoietic stem cell (HSC) transplantation. While it is possible to prospectively isolate HSCs/ progenitors from patient's blood and bone marrow to use for transplantations, they neither can be reliably maintained and expanded in undifferentiated state in culture nor manipulated to eliminate genetic cause of a disease. In order to overcome this problem we have attempted to rationally identify a set of transcription factors necessary for reprogramming human somatic cells into HSCs. During development the first definitive HSCs are found in the dorsal aorta within the aorta-gonad-mesonephros (AGM) region, in two arteries that connect the dorsal aorta with the yolk sac, and the vitelline and umbilical arteries. Mouse as well as human placentas and placental vasculature are considered to be potent hematopoietic sites from midgestation to birth. We have compared a transcription profile of human HSCs and human umbilical arterial endothelial cells (HUAECs). We have identified a set of transcription factors and micro RNAs that are differentially expressed in these two cell type. Over-expression of a subset of these factors in human embryonic fibroblasts converted them into CD45+ cells. In a methyl-cellulose colony forming assay CD45+ fibroblasts were able to generate CD11b+, CD16+, and CD71+ cells.

THE DERIVATION OF MELANOCYTES FROM HUMAN PLURIPOTENT STEM CELLS FOR DISEASE MODELING

Yvonne Gruber Mica[1], Gabsang Lee[2], Stuart M. Chambers[2], Mark Tomishima[2], Lorenz Studer
[1]Gerstner Sloan-Kettering Graduate School of Biomedical Sciences; [2]Developmental Biology Program, Sloan-Kettering Institute, New York, NY

Melanocytes are pigment-producing cells found predominantly in the epidermis where they establish a photo-protective barrier against UV-irradiation induced DNA damage. Defects in melanocyte biology are associated with numerous pigmentation disorders including albinism, vitiligo, and piebaldism. Yet, while the developmental biology of melanocytes has been well studied in avian and murine models, the processes underlying melanocyte development in the human system remain poorly understood. We hypothesized that basic developmental insights can be applied to model the progressive and selective specification of human pluripotent cell lines along the melanocytic lineage.

Melanocytes are known to arise from the neural crest (NC), a transient, migratory population of cells unique to vertebrates. With the goal of establishing a rapid and defined protocol for the induction of neural crest *in vitro*, we adapted a recently developed protocol that supports the highly efficient differentiation of human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) into both central nervous system and neural crest precursors. Using a *Sox10::GFP* BAC hESC reporter line, we have found that optimization of Wnt, BMP, and TGF-β signaling within the context of the dual SMAD inhibition protocol greatly enhances NC induction to 65% of the population. Interestingly, a narrow window of Wnt signaling appears to govern neural crest induction within the context of this protocol. We further found that melanocyte-competent progenitors within the NC population could be prospectively identified and enriched based on their co-expression of *Sox10::GFP* and c-kit. A brief period of additional maturation was sufficient to establish a pure and expandable population of pigmented melanocytes.

Our studies not only offer a novel framework for exploring the molecular mechanisms underlying melanocyte development but in combination with patient-derived iPSCs will also allow for the generation of melanocyte-related models of human disease that will represent a powerful platform for future applications in drug discovery.

PURSUING PLURIPOTENCY

Ihor R. Lemischka
Black Family Stem Cell Institute, Lillian and Henry M. Stratton Professor, Department of Gene and Cell Medicine and Developmental and Regenerative Biology, Mount Sinai School of Medicine, New York, NY

Embryonic stem (ES) cells represent an attractive model system to elucidate the molecular and cellular mechanisms responsible for cell fate decisions. These cells also hold great promise for the future of regenerative medicine. Much progress has been made in recent years to elucidate the mechanisms that control ES cell pluripotency, as well as lineage-specific commitment. Much remains to be elucidated, and there is currently no systems level “picture’ of how cell fate decisions occur in response to defined input signal, and as a function of time. We have embarked on efforts to first: identify most, if not all, regulatory components that mediate cell fate decisions in murine ES cells, and second: to develop methodologies to analyze a cell fate decision as it occurs over time following a defined stimulus, and at multiple biochemical/molecular levels. The experimental platform that underlies most of our studies is controlled, short hairpin (sh)RNA-mediated down regulation of important cell fate regulators. We have analyzed a cell fate decision process at the levels of changes in chromatin architecture, transcriptional activity, steady-state mRNA populations, and the entire nuclear protein population. In addition, we have performed these analyses as a function of time. Numerous interesting observations have been obtained, and a synthesis of our numerous observations represents a first systems level “picture” of a change in stem cell fate. We have also embarked on similar studies in the human ES cell system, as well as in ES-like cells derived from human fibroblasts using recently reported reprogramming approaches.

(1) CBX7 SMALL MOLECULE MODULATORS PROVOKE EMBRYONIC STEM CELL DIFFERENTIATION

Francesca Aguilo[1], SiDe Li[1], Kyoko Yap[1], Jesus Gil[2], Ming-Ming Zhou[1], Martin J. Walsh[1]
[1]Mount Sinai School of Medicine, New York, NY; [2]Clinical Sciences Centre, Hammersmith Hospital Campus, Imperial College Faculty of Medicine, London, UK

The chromobox (CBX) family of proteins consists as a group of conserved nuclear proteins that share a single chromodomain that bind specifically to methylated lysine residues on histone H3. The chromobox family proteins exist within distinguishable subgroups that identify CBX proteins to preferentially recognize either histone H3K9 methylation or H3K27 methylation. These proteins are further identified by the presence of either a chromo shadow (CS) domain or *Polycomb* (Pc). As a chromobox protein within the Polycomb repressive complex 1, CBX7 shares a Pc domain and binds to H3K27 methylation of both di- and tri-methylated states of histone H3 in chromatin. Our studies reveal the CBX7 is among the most highly–expressed of the chromobox proteins in ES cells. Using small molecules ligands directed at the aromatic cage of the chromodomain these small molecule compounds specifically disrupt the interaction between CBX7 and H3K27 methylation. We tested the role of this small molecule inhibitor to CBX7 (CBX7i) in ES cells and identified CBX7 inhibition through CBX7i as favoring neuroectoderm lineage patterning through activation of key neuroectoderm lineage–specific markers and by the induction of critical *Hox* genes. Consistent with the role of CBX7 to maintain the state of ES cell pluripotency, perturbation of CBX7 through the chromodomain by small molecules help define the role of CBX7 in restricting lineage fate commitment.

(2) INCREASED EFFICIENCY OF REPROGRAMMING ADULT SKIN FIBROBLASTS THROUGH FLUORESCENCE ACTIVATED CELL SORTING

Faizzan S. Ahmad[1], Haiqing Hua[2], Sai Theja[1], Andrew A. Sproul[1], Dorota Moroziewicz[1], Lauren A. Bauer[1], Alexandra S. Bercow[1], Zaaqhir Z. Imam[1], Dietrich M. Egli[1,2], Kevin Eggan[3-5], Scott A. Noggle[1], David J. Kahler[1]
[1]The New York Stem Cell Foundation, New York, NY; [2]Department of Pediatrics and Naomi Berrie Diabetes Center, Columbia University, New York, NY; [3]Stowers Medical Institute, Harvard University, Cambridge, MA; [4]Howard Hughes Medical Institute, [5]Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA

Induced Pluripotent Stem Cell (iPS) Technology has generated potential tools for disease modeling and regenerative medicine. Therefore, methods that increase the efficiency of iPS generation will greatly facilitate the application of this technology. Here we describe the utility of Fluorescence Activated Cell Sorting (FACS) in generating iPSCs from primary human adult skin fibroblasts. It has been previously described that when human fibroblasts are reprogrammed by forced expression of four transcription factors (Oct4, Klf4, Sox2, cMyc) (via retroviral infection) pluripotent surface markers (e.g. SSEA4, Tra1-60) are upregulated while fibroblast surface markers (e.g. CD13) are downregulated. Employing these sorting parameters between days 7-14 (days post infection), we have isolated enriched populations of $CD13^{NEG}SSEA4^{POS}Tra\text{-}1\text{-}60^{POS}$ cells, decreasing the time required to manually pick and expand colonies, and improving the efficiency of getting fully reprogrammed iPSCs. Based on our analysis FACS sorted iPS cells are morphologically indistinguishable from those generated by more traditional methods. They express pluripotent marker genes (e.g. Nanog) and are able to give rise to the three germ layers *in vitro*. Minimizing the time to establish iPS lines and increasing their purity of will aid in establishing methods for high throughput production of iPS cell lines and screening campaigns. Ergo our studies suggest that FACS enriches reprogrammed fibroblasts thereby improving kinetics and efficiency of reprogramming.

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

Faizzan Ahmad[1], David Kahler[1], Lauren Vensand[1], Anita Ritz[1], Sai Theja[1], Dorota Moroziewicz[1], Jennifer Becht[1], Dieter Egli[1,2], Haiqing Hua[2], Linshan Sheng[1], Hector Martinez[1], Andrew Sproul[1], Samson Jacob[1], Matthew Zimmer[1], Keren Weiss[1], Kevin Eggan[3-5], Scott Noggle[1]
[1]The New York Stem Cell Foundation, New York, NY; [2]Department of Pediatrics and Naomi Berrie Diabetes Center, Columbia University, New York, NY; [3]Stowers Medical Institute, Harvard University, Cambridge, MA; [4]Howard Hughes Medical Institute, [5]Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA

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

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(4) PROMOTION OF PLURIPOTENT STEM CELL RESEARCH – GOALS OF THE hESC/iPSC SHARED RESOURCE FACILITY AT MOUNT SINAI

Vera Alexeeva[1], Sandra Ortega[1] and Sunita D'Souza[1]
[1]Department of Gene and Cell Medicine, Black Family Stem Cell Institute, Mount Sinai School of Medicine, New York, NY

The primary objective of the hESC/ iPSC Shared Resource Facility (SRF) is to make available the newest technology in the stem cell arena to Mount Sinai and other New York State scientists. We are currently focusing on generating iPSCs using the excisable loxP flanked STEMCCA cassette as well as modified RNA generated from the four Yamanaka factors. The former method will eliminate the ectopic expression of the inserted stem cell genes while the latter method will aid in the creation of patient-specific iPSCs lines that are completely free of exogenous DNA. These two methods are also being used to create induced pluripotent cancer cells (iPCs) to explore the relationship between cancer and stem cells. We are also working to develop an astrocyte and a cardiac reporter cell lines using lentiviral GFAP-GFP and Tnnt2-RFP reporters respectively. Simultaneously with ongoing R&D, the Core also supplies tested stem cell reagents to over fifty labs at vastly discounted pricing. A web-based administrative system entitled CORES (Core Ordering & Reporting Enterprise System) is currently being set-up to facilitate the supply of stem cell reagents to interested labs in an efficient manner. Lastly, the Core continues to conduct classes to teach iPSCs generation and hESC/iPSC/iPC cell differentiation into the lineage of choice. Taken together, these services will have a three-fold benefit. Firstly, they will alleviate the quality control burden of individual scientists and allow them to concentrate on important scientific questions. Secondly, they will allow collaborative projects involving hESC/iPSC/iPC cell lines to be initiated with multiple laboratories by removing the prohibitive cost and providing the expertise required to establish and sustain this technology and lastly they will provide the Core with a source of revenue. In conclusion, significant progress has been made with the help of NYSTEM funding.

(5) A2B5-DEFINED TUMOR PROGENITORS DERIVED FROM HUMAN GLIOMAS EXHIBIT A CORE SET OF DYSREGULATED GENES AT ALL STAGES OF TUMOR PROGRESSION, IN ADDITION TO GRADE-ASSOCIATED TRANSCRIPTIONAL CHANGES CONSISTENT WITH EPITHELIAL-TO-MESENCHYMAL TRANSITION

Romane Auvergne[1,2,*], Fraser J. Sim[1,2,*], Su Wang[1,2], Devin Chandler-Militello[1,2], Jaclyn Burch[1,2], Xiaojie Li[1,2], Nimish Mohile[1,2], Kevin Walter[3], Mahlon Johnson[4], Pragathi Achanta[5], Alfredo Quinones-Hinojosa[5], Sridaran Natesan[6], Steven A. Goldman[1,2]
[1]Center for Translational Neuromedicine, Departments of [2]Neurology, [3]Neurosurgery, and [4]Pathology, University of Rochester Medical Center, Rochester, NY; [5]Neurosurgery, Johns Hopkins University Medical School; [6]Sanofi-Aventis Pharmaceuticals, Cambridge, MA

Glial progenitor cells (GPCs) of the adult human white matter, which express gangliosides recognized by monoclonal antibody A2B5, are a potential source of glial tumors of the brain. We used A2B5-based sorting to extract progenitor-like cells from a range of human glial tumors (WHO II-IV) that included low-grade and anaplastic oligodendroglioma, oligo-astrocytoma, astrocytoma, and glioblastoma multiforme.

The A2B5$^+$ tumor cells proved tumorigenic upon orthotopic xenograft, and the tumors generated reflected the phenotypes of those from which they derived. Expression profiling revealed that A2B5$^+$ tumor progenitors isolated from both low- (n=10) and high-grade (n=10) gliomas expressed a cohort of genes by which they could be distinguished from A2B5$^+$ GPCs isolated from normal adult white matter (n=8). Most of the genes differentially expressed by glioma-derived A2B5$^+$ cells varied as a function of tumor stage, such that with tumor progression, genes associated with both the TGF-• pathway, and with the epithelial-to-mesenchymal transition (EMT) were up-regulated in A2B5$^+$ tumor progenitors. Nonetheless, a small number of genes were invariably differentially expressed at all stages of gliomagenesis; these transcripts appeared with initial gliomagenesis in WHO stage II-derived A2B5$^+$ cells, and persisted as differentially dysregulated in grade III and IV tumors as well. This conserved set of glioma progenitor-associated genes included CD24, SIX1 and EYA1, which were up-regulated, and MTUS1 and SPOCK3, which were down-regulated, at all stages of tumor progression. qPCR and immunolabeling confirmed the differential expression of these genes in primary gliomas, while pathway analysis permitted their segregation into differentially active signaling pathways.

By comparing the expression patterns of glial tumor progenitors across anaplastic progression to their identically-isolated normal homologues, we have thus identified a discrete set of genes associated with *both* initial glial tumorigenesis *and* later progression, whose selective targeting might influence glioma growth at all stages of tumor progression.

[*Supported by NYSTEM contract C026428*]

(6) TESTOSTERONE INCREASES EXPRESSION OF TRANSIENT OUTWARD CURRENT (I_{to}) IN HUMAN INDUCED PLURIPOTENT STEM CELL (hiPSC)-DERIVED CARDIOMYOCYTES

Hector Barajas-Martinez, Dan Hu, Wu Yuesheng, Jackie Treat, Michael Xavier Doss, Charles Antzelevitch
Cardiac Research Institute, Masonic Medical Research Laboratory, Utica, NY

Background: Recent studies have provided evidence for an effect of testosterone to modulate the expression of the transient outward current I_{to} and thus the electrocardiographic and arrhythmic manifestations of J wave syndromes, including Brugada and early repolarization syndromes. The aim of the present study was to examine the effects of testosterone on the expression of I_{to} in human iPS (hiPS) cardiomyocytes.

Methods and Results: Embryoid bodies (EBs) were made from a human iPS cell line reprogrammed with Oct4, Nanog, Lin28 and Sox2 using a serum free differentiation protocol supplemented with growth factors for selective cardiac differentiation. The beating clusters were micro-dissected from the entire EBs, enzymatically dissociated and plated on fibronectin-coated dishes. The cells were treated with 25 nM testosterone for a period of 4 weeks. Function of I_{to} channels was studied using whole-cell patch clamp techniques. At +40 mV, 4-aminopyridine (4AP)-sensitive potassium current amplitude was significantly greater in testosterone treated human iPS like cardiomyocytes compared with the control (9.12 ± 3.71 vs. 1.50 ± 0.61 pA/pF; n=5-6; $p < 0.001$). Current decay was slower in presence of testosterone (52.7 ± 3.3 ms vs 32.6 ± 1.7 ms; n=5-2; $p < 0.05$).

Conclusions: Testosterone significantly modifies the intensity and characteristics of the transient outward potassium current in human iPS like cardiomyocytes. These results are consistent with the effect of the androgen hormone to alter the gender-dependent expression of I_{to} and thus may explain the marked gender differences observed in the manifestation of life-threatening arrhythmias associated with the J wave syndromes.

[*Supported by NYSTEM contract C026424*]

(7) NEUROGENESIS AND ANGIOGENESIS ARE CORRELATED IN THE ADULT HUMAN DENTATE GYRUS AND BOTH ARE HIGHER WITH SSRIs IN MAJOR DEPRESSION

Kelly M. Burke[1], Laika R. Simeon[1], Mark D. Underwood[1,3], Gorazd B. Rosoklija[1,3,8], Andrew J. Dwork[1,3,5], J. John Mann[1,3], Rene´ Hen[1,2,4,6], Victoria Arango[1,3], Maura Boldrini[1,3,7]
Divisions of [1]Molecular Imaging and Neuropathology and [2]Integrative Neuroscience, New York State Psychiatric Institute, New York, NY; Departments of [3]Psychiatry, [4]Neuroscience, [5]Pathology and Cell Biology, [6]Pharmacology, Columbia University, New York, NY; [7]Department of Neurological and Psychiatric Sciences, University of Florence, Florence, Italy; [8]Macedonian Academy of Sciences & Arts, Republic of Macedonia

Neural progenitor cells (NPCs) grow and differentiate in neurogenic niches, around new capillaries in adult dentate gyrus (DG). Angiogenesis and neurogenesis are co-regulated and both enhanced by growth factors. Vascular endothelial growth factor (VEGF) is required for antidepressant-induced cell proliferation, stimulates BrdU incorporation into immature neural cells and increases cell number in cultures. We hypothesized that antidepressants and lithium increase mitotic cells (Ki-67 immunoreactive), NPCs and new capillaries (nestin immunoreactive) in the adult human DG. Cells and capillaries from matched treated and untreated subjects with mood disorders (MD) and normal controls (n=44 total) were quantified postmortem by stereology.

NPC number and density correlated with nestin-IR capillary area (mm^2, $p < 0.001$ and $p = 0.015$, respectively) and length (mm, $p = 0.001$ and $p < 0.001$), respectively. Mitotic cell number correlated with capillary volume fraction of the DG ($p=0.011$). DG volume correlated with NPC number ($p = 0.003$), capillary area ($p = 0.003$) and volume ($p = 0.005$). We used age as covariate since capillary complexity (nodes per vessel, $p = 0.034$) is lower and NPCs are fewer with increasing age ($p = 0.05$).

NPC number ($F = 3.189$, $p = .014$) and capillary area ($F = 2.750$, $p = .027$) differ among MD subjects treated with SSRIs, tricyclic antidepressants or lithium, untreated MDs and controls, using age, PMI and pH as covariates. MDs on SSRIs had more NPCs than untreated MDs ($p = .041$) and controls ($p = .032$). Average capillary area was highest in MDs treated with lithium or SSRIs and comparable in untreated MDs and controls.

We present the first evidence from adult human brain that neurogenesis and angiogenesis appear linked in DG and are both are higher in depressed subjects treated with SSRIs. These effects may be important for the antidepressant action of SSRIs.

(8) HIGHLY EFFICIENT DERIVATION OF MOTONEURONS FROM HUMAN EMBRYONIC STEM CELLS USING BAC TRANSGENESIS

Elizabeth L. Calder[1,2], Dimitris G. Placantonakis[3], Mark J. Tomishima[2,4], Viviane Tabar[5], Lorenz Studer[2,5]
[1]Weill Cornell Graduate School, New York, NY; [2]Developmental Biology Program, Sloan-Kettering Institute, New York, NY; [3]Department of Neurosurgery, New York University, New York, NY; [4]SKI Stem Cell Research Facility, [5]Department of Neurosurgery, Sloan-Kettering Institute, New York, NY

Motoneuron diseases, such as Amyotrophic Lateral Sclerosis and Spinal Muscular Atrophy, are characterized by the progressive loss of motoneurons (MNs). Human embryonic (hESCs) and induced pluripotent stem cells (hiPSCs) represent valuable sources for generating MNs and studying their pathophysiology and therapeutic potential in disease. While patient-specific iPSCs hold great promise for drug discovery and potential cell replacement therapies, both strategies will require reproducible access to large numbers of MNs. Current differentiation protocols for human pluripotent cells are inefficient and lengthy, hindering the ability to use this source for large-scale studies.

In this study, we adapted the dual-SMAD inhibition protocol for early neural induction by subsequently patterning and differentiating in the presence of retinoic acid and an activator of sonic signaling. Using a spinal MN specific *HB9::GFP* hESC reporter line, we optimized the activation of different cell signaling pathways as well as the duration and timing of patterning factors to develop a well-defined, small molecule-based protocol. This protocol allows us to generate up to 40% *HB9::GFP*+ cells after 2 weeks of differentiation. GFP+ cells express markers characteristic of MN identity by both immunohistochemistry and qRT-PCR, including the transcription factors HB9, Isl1, and Nkx6.1 as well as choline acetyltransferase. These MNs can be maintained in culture feeder-free for up to 2 weeks post sort. Ongoing work is focused on optimizing purification strategies using a high throughput platform to screen for cell surface markers. In addition, we have established an in vitro transplantation assay for hiPSC-derived MNs into organotypic slices prepared from a rat model of ALS to study human MNs in the disease environment. We have developed a highly efficient, rapid protocol for MN derivation that allows us to generate large numbers of MNs from human pluripotent cells and to establish a human-based framework for disease modeling and mechanistic studies.

(9) TRANSGENE FREE DISEASE DERIVED-iPS DIFFERENTIATION INTO HEMATOPOIETIC AND ERYTHROID CELLS

Chan-Jung Chang and Eric E. Bouhassira.
Hematology, Albert Einstein College of Medicine, Bronx, NY

Human induced pluripotent stem cells (hiPSC) have been shown to have differentiation potential similar to human embryonic stem cells (hESC). In particular, the questions of whether the source and the age of the somatic cells used to produce iPS have an influence on the differentiation potential of the iPS, has not been fully explored. From our previously study shown that co-culture hESC with immortalized fetal hepatocytes (FHB) yield CD34+ cells that can be further expanded in serum-free liquid culture to obtain large number of megaloblastic nucleated erythroblasts. With extending the liquid culture, we found that the hemoglobin composition switching from basophilic erythroblasts expressing Hb Gower I ($\zeta_2\varepsilon_2$) to orthochromatic erythroblast hemoglobin Gower II($\alpha_2\varepsilon_2$). By extending the co-culture of hESC with FHB from 14 day to 35 day, the CD34+ obtained from extending culture follow expanding and differentiating by liquid culture, shown more developmentally mature fetal-liver like erythroblast which expressed HbF ($\alpha_2\gamma_2$).

In the current study, we have used retroviruses expressing Oct4, Sox2, Klf4 and c-Myc to produce iPS from embryonic and fetal mesenchymal cells, from adult skin fibroblasts, and hair keratinocytes, and compared them in details with hESC. Morphology, growth characteristics, surface antigen expression and mRNA expression (Affymetrix Human Gene 1.0 ST Array) were undistinguishable from undifferentiated H1 and H9 hESC. PCR analysis of pluripotency factors revealed that in most

iPS clones the four exogenous reprogramming factors were mostly silenced except in one interesting clone, obtained from fetal liver which had not silenced any of the four factors. Differentiation along the hematopoietic and erythroid lineages yielded numbers of $CD34^+$ cells (2 to 10%) similar to those obtained with hESCs and red blood cells that undergo the same two globin switches as hESCs. Interestingly, the iPS clone that did not silenced the four factors differentiated predominantly into $CD34^+$ cells, yielding 10 to 20 times more $CD34^+$ cells than the other iPS, although these $CD34^+$ cells had very poor erythroid differentiation potential. We conclude that iPS with apparently normal globin switching potential can be obtained from embryonic, fetal or adult somatic cells originating from multiple tissue sources. Aberrantly reprogrammed iPS with interesting differentiation potential are also obtained. Aberrant reprogramming can be associated with poor silencing of the reprogramming factors. In order to avoid the consequences caused by virus insertion, we reprogrammed disease fibroblasts with episome vectors. The iPS cells from alpha thalassemia fibroblast that has four alpha gene deletions (FIL-/SEA-), and the aTiPS expressed typical hESC markers. The aTiPS can form teratomas with injected into immuno-compromise mice, and shown no trasngene insertions in the PCR results. The CD34+ cells obtain from 14 days coculture of aTiPS with FHB, after expend and maturated by liquid culture, express Gower I ($\zeta_2\varepsilon_2$) compare to the erythroblast from the hESC and other iPS from normal cells with the same 14 co-culture protocol that express Gower II ($\alpha_2\varepsilon_2$). However, the erythroblast derived from aTiPS didn't show microcytic or hypochromic features in our 14 days differentiation.

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(10) CHROMATIN MODIFYING AGENTS PROMOTE THE *EX VIVO* PRODUCTION OF FUNCTIONAL HUMAN ERYTHROID PROGENITOR CELLS

Pratima Chaurasia[1], Dmitriy Berenzon[1, 2], Ronald Hoffman[1]
[1]Division of Hematology/Medical Oncology, Department of Medicine, Tisch Cancer Institute, Mount Sinai School of Medicine, New York, NY; [2]Wake Forest University, School of Medicine, Hematology and Oncology, Winston Salem, NC

Presently, blood transfusion products are composed of terminally differentiated cells with a finite life span. We have developed an *ex-vivo* generated TP composed of erythroid progenitor cells (EPC) and precursor cells. Several histone deacetylase inhibitors (HDACIs) including suberolylanilide hydroxamic acid (SAHA), trichostatin A (TSA) and valproic acid (VPA) were used *in vitro* to promote the preferential differentiation of cord blood (CB) CD34+cells to EPC. A combination of cytokines (SCF, TPO, IL3 and Flt3) and VPA *(i)* promoted the greatest degree of EPC expansion, *(ii)* led to the generation of EPC which were capable of differentiating into the various stages of erythroid development, *(iii)* led to epigenetic modifications (increased H3 acetylation) of promoters for erythroid specific genes, which resulted in the acquisition of a gene expression pattern characteristic of primitive erythroid cells, *(iv)* promoted the generation of a transfusion product that when infused into NOD/SCID mice produced mature RBC containing both human adult and fetal globins as well Rh blood group antigen which persisted for 3-weeks and the retention of human EPC and erythroid precursor cells within the bone marrow (BM) of recipient mice. This *ex vivo* generated EPC-TP likely represents a paradigm shift in transfusion medicine due to its potential to continue to generate additional RBC following its infusion.

(11) REGULATION OF HAIR FOLLICLE STEM CELL ACTIVATION AND SELF-RENEWAL *IN-VIVO*

Ting Chen, Nicole Stokes, Elaine Fuchs
Howard Hughes Medical Institute, The Rockefeller University, New York, NY

In the adult skin, hair follicles (HFs) undergo cycles of new hair growth followed by destruction and rest. The process is fueled by follicle stem cells (SCs) that reside in a permanent niche (bulge) and undergo cycles of quiescence and activation. Understanding the signaling pathways and intrinsic factors that control the maintenance, activation and differentiation of SCs is not only relevant to basic SC biology, but also has clinical significance, particularly in human cancers, where these

mechanisms go awry. Our study shows that HF regeneration happens as a two-step process involving molecularly distinct epithelial compartments: bulge and hair germ (HG), where HG cells fuel initial steps in hair regeneration, while the bulge is the engine maintaining the process. During the two-step activation of HF SCs there are changes in crucial intrinsic factors that associate with the cell fate change which is reflected by long-term self-renewal ability and responsiveness to active signals. Our *in vivo* loss of function studies discovered that one of the bulge stem cells specific transcription factors is involved in regulating HF SCs activation both during development and wound healing. This is the first known intrinsic factor involved in regulating HF SCs self-renewal *in vivo*. Our future plan involves dissecting the molecular mechanism of how the function of this protein is regulated during SC self-renewal. And an ongoing shRNA based screen to identify other proteins involved in HF SC self-renewal will give us more information of how this critical cell function is regulated.

(12) CONTROLLING THE SUBTYPE IDENTITY OF HUMAN STEM CELL DERIVED MOTOR NEURONS

Gist F. Croft[1], Mackenzie W. Amoroso[1], Anne Davis[2], Christopher E. Henderson[1], Hynek Wichterle[1]
[1]Project A.L.S./Jenifer Estess Laboratory for Stem Cell Research, Departments of Pathology, Neurology, and Neuroscience, Center for Motor Neuron Biology and Disease (MNC), and Columbia Stem Cell Initiative (CSCI), Columbia University, New York, NY; [2]Department of Obstetrics and Gynecology, Columbia University, New York, NY

If reprogramming somatic cells from patients with neurological disease is to enable studying the affected human cell types *in vitro*, two basic challenges must first be met: to define the appropriate cellular phenotypes *in vivo*, and to selectively generate them *in vitro*. In the motor neuron (MN) degenerative diseases ALS and SMA, spinal MNs innervating different muscles show selective vulnerability or resistance to disease. We therefore set out to define the subtypes of human MNs *in vivo* and to generate these *in vitro*. Here we report that human MNs share molecular markers and organization with mouse. Human MNs expressed the MN marker proteins HB9 and or ISLET (ISL), and could be unambiguously assigned to mouse motor column cognates based on their rostrocaudal and intersegmental anatomical position and their expression of the mouse column markers LHX3 and FOXP1: Medial Motor Column (MMC:$LHX3^{+}$), Lateral Motor Column and Preganglionic Motor Column (LMC and PGC: $FOXP1^{high}$ and $FOXP1^{low}$ respectively), and Hypaxial Motor Column (HMC:$FOXP1^{-}LHX3^{-}$). Human MNs also expressed the hox genes HOXA5, HOXC8, and HOXD9 in a sequential colinear progression which identified specific rostrocaudal MN domains. We then used combinations of these markers to classify motor neuron subtypes derived from human embryonic stem cells (hES-MN) *in vitro* under standard Retinoic Acid (RA) mediated differentiation conditions. These hES-MN expressed marker combinations appropriate to each motor column, but were restricted to mostly rostral (cervical, $HOXA5^{+}$) phenotypes. We then based a strategy to generate more caudal hES-MNs on *in vivo* mechanisms. We show that the hES-MNs generated upon treatment with FGF showed significantly more caudal hox profiles (fewer cervical, more thoracic and lumbar, $HOXC8^{+}$ and $HOXD9^{+}$) while preserving and expanding motor column diversity to include subtypes which show differential sensitivities to ALS *in vivo*. The construction of more precise *in vitro* models of disease should help elucidate mechanisms of disease resistance and vulnerability, and identify novel therapeutic targets.

(13) EFFECT OF E-4031, PINACIDIL AND ISOPROTERENOL ON ACTION POTENTIAL CHARACTERISTICS AND CONTRACTILITY IN hiPS-DERIVED CARDIOMYOCYTES

José M. Di Diego, Michael Xavier Doss, Robert Goodrow, Yuesheng Wu, Jackie Treat, Charles Antzelevitch
Cardiac Research Institute at Masonic Medical Research Laboratory, Utica, NY

Background: Human induced pluripotent stem cell (hiPS)-cardiomyocytes exhibit electrophysiological properties that could potentially be used as models for safety pharmacology. In this study we tested the effect of E-4031, pinacidil and isoproterenol.

Method: Embryoid bodies (EBs) were made from a human iPS cell line reprogrammed with Oct4, Nanog, Lin28 and Sox2 using a serum free differentiation protocol supplemented with growth factors for selective cardiac differentiation. Beating clusters (BC) were micro-dissected from EBs ranging between 19 and 70 days of maturity and plated on gelatin coated dishes. Using sharp microelectrodes we characterized stable action potential (AP) recordings from spontaneously beating clusters superfused with HEPES-Tyrode's solution (KCl=4 mM; 37°C) before and after exposure to either 5 µM E-4031 (n=6), 10 µM pinacidil (n=6) or 1 µM isoproterenol (n=7). Contractility of beating clusters was assessed using a video edge detection system.

Results: Spontaneous rate and AP characteristics varied widely among preparations: 1) Cycle Length (CL): 327 to 2600 msec; 2) APD_{90}: 102 to 506 msec; 3) AP amplitude: 58 to 121 mV; 4) V_{max}: 5 to 86 V/sec. E-4031 (5 µM; n=6) significantly increased $cAPD_{90}$-B (Bazett's correction) from 351.7 ± 29.4 to 504.3 ± 121.7 ms ($p < 0.05$) and generated multiple phase 3-EADs in 1/6 preparations (56-70 days-old BCs). Addition of ranolazine (15 µM) reduced $cAPD_{90}$-B towards control levels and abolished EADs. Pinacidil (10 µM) and isoproterenol (1 µM) decreased APD_{90} from 275.5 ± 34.8 to 246.0 ± 33.0 ($p < 0.05$) and from 244.7 ± 34.9 to 188.7 ± 22.0 msec ($p < 0.05$), respectively, in 19-49 days-old BCs. Motion detection signals revealed that the EADs was accompanied by aftercontractions (E-4031) and that isoproterenol has a positive inotropic effect.

Conclusion: AP and contractility studies on beating cardiomyocyte clusters derived from hiPSC-EBs show that the effects of E-4031, ranolazine, pinacidil and isoproterenol are consistent with those reported in native cardiomyocytes.

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(14) EPIGENETIC REGULATION OF STEM CELL SELF-RENEWAL AND DIFFERENTIATION

Raffaella Di Micco[1], Guangtao Zhang[2], Michael Ohlmeyer[2], Weijia Zhang[3], Martin Walsh[2], Ming-Ming Zhou[2], Eva Hernando[1]
[1]Department of Pathology, New York University School of Medicine, New York, NY; [2]Department of Structural and Chemical Biology; [3]Department of Medicine, Mount Sinai School of Medicine, New York, NY

The full potential of hESCs in research and clinical applications requires a detailed understanding of the genetic and epigenetic mechanisms that govern the unique properties of hESCs. Studies show that the pluripotent state of stem cell is maintained by high-level of expression of stem cell-specific transcription factors including Oct-3/4, Sox2, Klf4 and c-Myc (OSKM). hESC possess an unusual "open" chromatin structure that allows readily access of the human genome by transcription factors and transcription machinery thus resulting in elevated plasticity. Active chromatin domains are marked by histones H3 and H4 lysines acetylation and di,tri-methylated H3K4, and transcription factors and chromatin remodeling complexes are key determinants of hESCs identity. We sought to investigate the role of bromodomain (BrDs)-containing proteins in governing hESCs self-renewal and pluripotency. BRD-containing proteins regulate chromatin dynamics through modulating acetylation-mediated protein-protein interactions in control of gene transcription. We discovered a small-molecule chemical compound that inhibits acetyl-lysine binding activities of BrDs in gene transcription, and is capable of effectively reducing the expression of OSKM genes and the number of undifferentiated colonies. Instead the expression of neuroectodermal lineage, neural crest and melanoblast markers increased following compound treatment, without any overt effect on cell viability. RNA sequencing (Illumina) of compound-treated ESCs revealed differentially expressed genes of the PDGF, TGFβ and WNT signaling pathways. Further, we have observed that this BrD inhibitor (BrDi) affects RNA polymerase recruitment to promoters of stem cell genes, with concomitant reduction of active histone marks and displacement of BrD-containing proteins. Using this novel chemical probe, we are dissecting the bromodomain regulated-mechanisms that control differentiation and stem cell self-renewal.

(15) INDUCTIVE ANGIOCRINE SIGNALS FROM SINUSOIDAL ENDOTHELIAL CELLS ARE REQUIRED FOR LIVER REGENERATION

Bi-Sen Ding[1], Daniel J. Nolan[1], Jason M. Butler[1], Daylon James[1], Alexander O. Babazadeh[1], Zev Rosenwaks[2], Vivek Mittal[3], Hideki Kobayashi[1], Koji Shido[1], David Lyden[4], Thomas N. Sato[6], Sina Y. Rabbany[1,5], Shahin Rafii[1,*]

[1]Ansary Stem Cell Institute, and Department of Genetic Medicine, Howard Hughes Medical Institute, [2]Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, [3]Department of Surgery, [4]Department of Pediatrics, Weill Cornell Medical College, New York, NY; [5]Bioengineering Program, Hofstra University, Hempstead, NY; [6]Graduate School of Biological Sciences, Nara Institute of Science and Technology, Ikoma, Nara, Japan

Background: Endothelial cells (ECs) induce organogenesis during embryogenesis, even before the development of circulation. These findings suggest that ECs establish an instructive vascular niche, which stimulate organ regeneration through elaboration of EC-derived paracrine (angiocrine) trophogens. However, the precise mechanism by which liver-specific subsets of ECs promote organ regeneration in the adults is unknown.

Results: Here, we demonstrate that liver sinusoidal endothelial cells (LSECs) constitute a unique population of phenotypically and functionally designated $VEGFR3^{+}CD34^{-}VEGFR2^{+}$VE-$cadherin^{+}FactorVIII^{+}CD45^{-}$ ECs, which through the release of angiocrine hepatogenic trophogens initiate and sustain liver regeneration induced by 70% partial hepatectomy (PH). After PH, residual hepatic vasculature remains intact without experiencing hypoxia or structural damage, allowing for studying physiological liver regeneration. Employing this model, we show that inducible genetic ablation of VEGF-A receptor-2 (VEGFR2) in the LSECs abolishes the initial burst of hepatocyte proliferation (days 1-3 after PH) and subsequent hepato-vascular mass reconstitution (days 4-8 after PH), by inhibiting the upregulation of EC-specific transcription factor *Id1*. Consequently, *Id1*-deficient mice manifest defects throughout liver regeneration, due to diminished expression of LSEC-derived hepatogenic angiocrine factors, including hepatocyte growth factor (HGF) and Wnt2. In *in vitro* co-cultures, VEGFR2-Id1 activation in LSECs stimulates hepatocyte proliferation. Notably, intrasplenic transplantation of $Id1^{+/+}$ or $Id1^{-/-}$ LSECs transduced with Wnt2 and HGF ($Id1^{-/-}$ $Wnt2^{+}HGF^{+}$ LSECs) reconstructs an instructive vascular niche in the liver sinusoids of $Id1^{-/-}$ mice, initiating and restoring hepato-vascular reconstitution.

Conclusions: Therefore, in the early phase of liver regeneration, VEGFR2-Id1-mediated inductive angiogenesis in LSECs through release of Wnt2 and HGF instigates hepatic proliferation. Subsequently, VEGFR2-Id1 dependent proliferative angiogenesis regenerates liver mass.

Future directions and impact: Therapeutic co-transplantation of inductive angiogenic $Id1^{+}Wnt2^{+}HGF^{+}$ LSECs with hepatocytes or hepatic progenitor cells offers an efficacious strategy to attain durable liver regeneration.

(16) AN INTEGRATED OCT4 NETWORK FOR PLURIPOTENCY OF EMBRYONIC STEM CELLS

Junjun Ding[1], Francesco Faiola[1], Huilei Xu[2], Avi Ma'ayan[2], Jianlong Wang[1,*]

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

Oct4 is a well-known transcription factor that plays fundamental roles in stem cell pluripotency and somatic cell reprogramming. Extensive studies have documented downstream target genes of Oct4, however, only limited information is available on the Oct4 protein complexes and their intrinsic protein-protein interactions that form the transcriptional machinery regulating expression of the Oct4 target genes. Here, we have employed an improved affinity purification approach combined with mass spectrometry to purify the Oct4 protein complexes in mouse embryonic stem cells (ESCs), which has led us to the discovery of novel Oct4 partners and signaling pathways important for stem cell pluripotency. We have identified 198 Oct4-interacting proteins constituting numerous

transcription factors and multiple chromatin modifying complexes with documented as well as newly proved functional significance in stem cell maintenance. Using bioinformatics tools we have also identified several novel kinase signaling pathways that are important for regulation of ESC self-renewal, proliferation and survival. Finally, we have constructed an integrated Oct4 network combining the protein-protein and protein-DNA interaction datasets of major pluripotency factors, which provides a great resource for further understanding the mechanisms controlling ESC self-renewal and pluripotency and for exploring alternative factor-based reprogramming strategies.

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(17) MODULATING INTRACELLULAR SIGNALING TO IMPROVE iPS REPROGRAMMING

Claudia A. Doege, Skylar Travis, William B. Vanti, Asa Abeliovich
Department of Pathology, Columbia University, New York, NY

Introduction: Induced pluripotent stem (iPS) cells can be generated from a variety of somatic cell types. Remarkably, only 4 transcription factors (Oct4, Sox2, Klf4, Myc) are sufficient to reprogram the epigenome. Since iPS cells hold great promise for regenerative medicine, understanding the mechanisms of reprogramming and overcoming major limitations, such as low efficiency of human iPS cell generation, are essential for future clinical applications. Therefore, our research is focused on improvement of iPS cell generation.

Progress Toward Specific Aims: Intracellular signaling cascades appear to be an exciting target to improve iPS cell generation and manipulation of signaling pathways may be achieved via genetic or chemical approaches. Here, we screened a chemical library of 100 compounds and found 5 drugs with pro-reprogramming properties targeting a variety of signaling pathways. Interestingly, hit compound U73122 might work via phospholipase C (PLC) gamma inhibition and thus, modulate chromatin remodeling. We are studying the effects of pro-reprogramming drugs *in vivo* applications, as teratoma formation and germline transmission.

Future Directions: We are going to study the effects of those iPS cells in an murine Parkinsonism model (transplantation experiment) and examine the influence of U73122 on tumor development in adult chimaeric mice.

Impact: Since efficient and safe reprogramming are critical for future clinical applications of iPS cells, chemical approaches to improve reprogramming might offer certain advantages. Small chemical compounds have rather shortlived effects and presumably less side effects as compared to genetic approaches. Thus, chemical alterations of intracellular signaling seems to be a very promising tool to increase efficiency and safety of iPS cell generation.

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(18) NEURAL PROGENITOR CELL RESPONSE TO HEMODYNAMICALLY GENERATED ENDOTHELIAL DERIVED EXTRACELLULAR MATRIX

Karen S. Ellison[1], Christopher M. Zwolinski[1], Natacha DePaola[2], Deanna M. Thompson[1]
[1]Department of Biomedical Engineering and the Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY; [2]Department of Biomedical Engineering and the Pritzker Institute of Biomedical Science and Engineering, Illinois Institute of Technology, Chicago, IL

This study demonstrates that adult neural progenitor cells exhibit a differential response to endothelial cell-derived extracellular matrix produced under hemodynamic flow compared to statically-produced extracellular matrix. *In vivo*, neural stem cells reside in close proximity to blood vessels, suggesting that endothelial-produced factors may influence neural stem cell fate. *In vitro*, Shen *et al*. (2004) demonstrated that soluble factors released by endothelial cells stimulate self-renewal and inhibit differentiation of embryonic neural stem cells. It is well known that the

endothelial phenotype is dependent on the local hemodynamic environment and can alter both extracellular matrix and soluble factors. However, neural stem cell response to endothelial derived products produced under hemodynamic stimulation has not been explored. In this work, we examined neural progenitor response to endothelial-produced extracellular matrix. Briefly, confluent mouse brain microvascular endothelial cells (mbEnd.3) were either (1) exposed to shear stress (10 dynes/cm^2- dynamic) or (2) traditionally cultured (static). The endothelial cells were chemically-lysed to isolate the extracellular matrix (ECM) and generate ~20-30 mm thick substrates. The ECM substrates were qualitatively visualized by immunostaining and increases in laminin and fibronectin were observed with only modest enhancement of collagen IV. Adult primary murine neural stem cells were isolated from the sub-ventricular zone, expanded to generate neurospheres, dissociated and seeded onto the ECM substrates. Neural progenitor cells attached, spread, and infiltrated the dynamically-produced ECM, while the statically-produced ECM generated only small neurospheres at the ECM surface. Current work is underway to characterize any changes in cell proliferation or differentiation following exposure to the EC-ECM as well as a more quantitative analysis of the ECM. Future work will be focused on soluble factors generated dynamically and the neural progenitor cell response. This work will generate a better understanding of the neural stem cell response to physiologic endothelial-derived cues.

(19) ERYTHROBLASTS WITH EXTENSIVE *EX VIVO* SELF-RENEWAL CAPACITY EMERGE FROM THE YOLK SAC AND EMBRYONIC STEM CELLS

Samantha E. England, Jenna Frame, Kathleen E. McGrath, James Palis
University of Rochester Medical Center, Dept. of Pediatrics and Center for Pediatric Biomedical Research, Rochester, NY

Adult-derived erythroid “progenitors” are capable of limited (<10^6-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 (>10^{30}-fold) proliferation *in vitro*. These cultures are composed of cells that divide daily and resemble proerythroblasts by morphology and ckit/ter119 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 an 8-16-fold increase in cell numbers, consistent with the proerythroblast identity of ESREs. Cell maturation is accompanied by the loss of c-kit and the upregulation of ter119 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. ESRE can be readily generated from murine ES cells differentiated as embryoid bodies. 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.

(20) EVALUATING THE INTERACTIONS BETWEEN POLARIZED MACROPHAGES AND HUMAN MESENCHYMAL STEM CELLS IN AN *IN VITRO* PLATFORM

Donald O. Freytes, Jung W. Kang, Ivan Marcos, Gordana Vunjak-Novakovic
Department of Biomedical Engineering, Columbia University, New York, NY

Introduction: The outcomes of cardiac therapies, by injection of cells or implantation of engineered constructs, will inevitably depend on inflammatory signals at the site of injury. Repair of damaged tissue is mediated by the recruitment of monocytes and their subsequent differentiation into macrophages, which in turn remove the cellular debris and secrete factors that impact cellular recruitment, angiogenesis, and ECM deposition. Recent findings have shown the dynamic

incidence of polarized macrophages with pro- (M1) and anti-inflammatory (M2) properties following myocardial infarction (MI). Repair cells such as mesenchymal stem cells (hMSCs) interact with macrophages during the host tissue response ultimately impacting tissue repair.

Methods: Human THP-1 monocytes were differentiated using phorbol 12-myristate 13-acetate (PMA) and polarized into M1 and M2 macrophages by the addition of LPS/IFN-γ and IL-4/IL-13 respectively. To analyze the reciprocal action between macrophages and hMSC, THP-1 derived macrophages were co-cultured with hMSCs in RPMI medium supplemented with heat-inactivated serum using a transwell system for 2 days. hMCS were also cultured with M1 (TNF-α, IL-1β, IL-6, and IFN-γ) and M2 (IL-10, TGF-β1, TGF-β3, and VEGF) associated cytokines. Following the culture period, cytokine expression and cell numbers were quantified.

Results: There was an increase in the number of hMSCs during co-culture with M2 macrophages when compared to hMSC co-cultured with M1 macrophages, suggesting supportive effects of M2s on hMSC growth. Culture of M2 associated cytokines confirmed this protective effect. Cytokine secretion by macrophages was also increased after co-culture with hMSCs when compared to culture in polarization medium.

Conclusion: Taken together, our data suggest that M2 macrophages may aid in the survival of hMSCs, while in turn the hMSCs help maintain cytokine expression of polarized macrophages *in vitro*. There seems to be a dynamic and reciprocal effect between macrophages and hMSCs that may ultimately dictate the type of reparative response elicited by cell therapies utilizing hMSCs.

Future Direction: Future studies will be completed in two directions: (1) confirming these findings using primary human macrophages, and (2) evaluating the effects of macrophages on cardiomyocytes derived from human embryonic stem cells.

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(21) HEAVY METALS ALTER H3K27 HISTONE MODIFICATION PATHWAYS IN MOUSE EMBRYONIC STEM CELLS

Sanket R. Gadhia and Frank A. Barile
Dept. of Pharmaceutical Sciences, St. John's University, College of Pharmacy, Queens, NY

The fetal basis of adult diseases (FeBAD) states that fetal exposure to carcinogens initiates processes that result in abnormal cell growth in adult life. Environmental exposure to metals fulfills this characterization by stimulating mutations or epigenetic changes in fetal life, resulting in DNA damage or histone modifications later in development, respectively. These perturbations alter gene expression, change transcription rates or interfere with DNA repair mechanisms. We tested the hypothesis that metals alter epigenetic pathways, by measuring cytotoxicity and histone lysine mono-methylation in mouse embryonic stem (mES) cells. Cell viability, total histone protein, and H3K27 mono-methylation (H3K27me1) were quantified in differentiated cultures of mES cells after exposure to arsenic, copper, cadmium, nickel, mercury lead and lithium, for 1-hr and 24-hr, followed by a recovery period. The data demonstrates that maximum cell death occurred during the first few hours of exposure at about 24-hr IC50 concentrations. Overall, 24-hr exposure of cells to As, Cd, Hg, and Ni decreased cell proliferation to a greater extent than total histone protein. While Ni and Cd exposures for 24-hr significantly increased total histone protein production per cell, As and Hg exposures decreased it, suggesting latter specifically target histone protein production. In addition, low dose acute exposure to As, Cd, Hg and Ni decreased mono-methylation of lysine (K27) residue on histone H3 when compared to control cells. Thus, since these metals decrease H3K27me1, then they are capable of suppressing normal transcriptional activation. This supports the contention that trace quantities of metals are capable of suppressing pathways that maintain transcriptional activation, thus altering chromatin structure. The pathway contributes to understanding the basis of cancer initiation, promotion and progression in differentiated cells.

(22) POLYCOMB GROUP REGULATION OF SELF-RENEWAL OF ISOLATED MURINE NEURAL STEM CELLS

Mythily Ganapathi[1], Nathan Boles[2], Sally Temple[2], Randall H. Morse[1]
[1]Laboratory of Molecular Genetics, Wadsworth Center, New York State Department of Health, Albany, NY; [2]Neural Stem Cell Institute, Rensselaer, NY

Polycomb group (PcG) genes have emerged as important regulators of stem cell maintenance and differentiation. PcG genes exert their regulatory effects through two distinct complexes, termed PRC1 and PRC2. Previous work from our lab showed a role for Bmi1, a PRC1 member, in self renewal and proliferation of embryonic and adult mouse neural stem cells (NSCs). When Bmi1 was inhibited in NSCs derived from the subventricular zone (SVZ) of mice, self-renewal was dramatically curtailed. Conversely, when Bmi1 was over expressed in these NSCs in culture, it drastically enhanced the capacity of the NSCs to proliferate and self renew. We are currently using molecular techniques to dissect the genes and pathways involved in Bmi1 regulation of NSC self-renewal and differentiation capacity. To determine whether Bmi1 is functioning through the PRC1/PRC2 pathway, we are assessing the effect of knocking down EZH2, a PRC2 member, in isolated NSCs in which Bmi1 is expressed at endogenous levels or is over-expressed. We are also conducting co-IP experiments to determine which potential PRC1 components partner with Bmi1 in NSCs, as the PRC1 complex exists in more than one form in different cell types. In addition, we are conducting microarray studies to compare gene expression in NSCs expressing endogenous or elevated levels of Bmi1. The data obtained from these experiments will shed light on the polycomb target genes which are crucial for neural stem cell maintenance.

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(23) DEVELOPMENT OF HUMAN PROSTATE BENIGN AND CANCER STEM CELL SPECIFIC GENE EXPRESSION PROFILE

Kalyan J. Gangavarapu[1], Wiam Bshara[2], Latif A. Kazim[3], Barbara A. Foster[1], Wendy J. Huss[1,4]
Departments of [1]Pharmacology and Therapeutics, [2]Pathology, [3]Cell Stress Biology, [4]Urologic Oncology, Roswell Park Cancer Institute, Buffalo, NY

Benign and cancer stem cells share many properties, thus unique identifiers remain elusive. Quantifying prostate cancer stem cells in prostate cancer may provide prognostic insight, but only if cancer stem cells can be distinguished from benign stem cells. In the present study, two stem cell enrichment assays were tested to determine the level of enrichment for cells with stem cell properties in each population; side population assay based on ABCG2 transporter activity and ALDEFLUOR® assay based upon aldehyde dehydrogenase enzyme activity. The aim of the study is to test stem cell properties using tissue recombination technique and determine unique stem cell gene signatures within single cells. Several cell lines were analyzed for side and ALDEFLUOR® ($ALDH^{br}$) population. While CWR-R1 prostate cancer cells have an average side population of 3.3% and 3% $ALDH^{br}$ cells; DU145 prostate cancer cells have an average side population of 1.6% and 1.5% $ALDH^{br}$ cells; and RWPE-1 non-tumorigenic prostate cells have an average side population of 0.3% and 3% $ALDH^{br}$ cells. The average side population is 0.4% (non-tumor) and 0.2% (tumor) in freshly digested human prostate specimens and the specimens have an average of 2% (non-tumor) and 2% (tumor) of $ALDH^{br}$ cells. Tissue recombination with urogenital mesenchyme showed that side population cells from human prostate clinical specimen are more enriched with cells demonstrating stem cell properties, human prostatic ductal growth up to 3 generations, compared to the $ALDH^{br}$ cells that showed minimal growth after first generation. To identify the gene expression profile of the cells isolated based upon the side population and ALDEFLUOR® assays, 14 different genes were selected that are associated with the stem cell biology and prostate differentiation. Based upon the initial results from multiplex PCR, the genes selected for identifying the stem cell profile are grouped into 4 different groups with 1 control gene in each group. The multiplex PCR reaction with single cells is performed with the AmpliSpeed slide cycler (Advalytix) and analyzed by automated fluorescent capillary electrophoresis to determine the presence or absence of a gene. Differential correlation analysis is performed for all comparisons. The benign and cancer stem cell

profile is developed by comparing expression of each gene in the multiplex from single side population and ALDH^{br} cells from benign and cancer specimens and cell lines. For each gene, normalized log signal will be modeled as a function of benign/cancer effects, accounting for patient level variation by mixed modeling. Thus, these studies seek to identify a benign and cancer stem cell gene expression profile in order to distinguish cancer stem cells from benign stem cells to enhance prognostic value of quantitating cancer stem cells in prostate specimens containing areas of pathologic prostate cancer.

(24) ANALYSIS OF GENE-SPECIFIC DNA METHYLATION PATTERNS IN CELLS DERIVED FROM EQUINE ADULT AND FETAL TISSUES

Catherine H. Hackett[1*], Line Greve[2*], Lisa A. Fortier[1]
[1]Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY; [2]Faculty of Life Sciences, University of Copenhagen, 1950 Frederiksberg C, Denmark

Cellular pluripotency is associated with expression of the homeobox transcription factor genes NANOG, SOX2 and POU5F1 (the gene that codes for Oct3/4 protein). Some reports suggest that mesenchymal progenitor cells (MPC) may express increased quantities of these genes; creating the possibility MPCs are more “pluripotent” than other adult cell types. The objective of this study was to determine if equine bone marrow-derived MPCs had gene expression or DNA methylation patterns that differed from either early fetal-derived or terminally differentiated adult cells. Specifically, this study compared DNA methylation of the NANOG and SOX2 promoter regions and concurrent quantitative gene expression of NANOG, SOX2 and POU5F1 in fetal fibroblasts, fetal brain cells, adult chondrocytes, and MPCs. In addition, MPCs were assayed following adipogenic and osteogenic induction to evaluate if differentiation conditions affected either gene expression or DNA methylation patterns. Our hypothesis was that MPCs derived from equine bone marrow would have little to no expression of NANOG, SOX2 and POU5F1 and would be in an epigenetic state similar to terminally differentiated chondrocytes. Results indicate that NANOG and POU5F1 were not detectable in appreciable quantities in any tissue. MPCs and adult chondrocytes expressed equivalent, low quantities of SOX2. In contrast, significantly increased quantities of SOX2 were noted in both fetal-derived cell types compared to adult cells. Further, SOX2 expression inversely correlated with the DNA methylation pattern in the promoter region, such that as gene expression increased, DNA methylation decreased. Adipogenic and osteogenic culture conditions did not result in decreased gene expression or increased DNA methylation in the promoter regions, suggesting differentiation conditions did not induce epigenetic changes in MPCs. Results do not support previous reports that NANOG, SOX2 and POU5F1 are poised for increased activity in MPCs compared to other adult cells or that differentiation conditions had an effect on pluripotency gene expression

**These authors contributed equally to this work.*

(25) THE ROLE OF ENDOGENOUS HIPPOCAMPAL STEM CELLS IN TREATING NON-HUMAN PRIMATE MODELS OF DEPRESSION AND ANXIETY

Jeremy D. Hill[1], Jeremy D. Coplan[2], Anna V. Rozenboym[1-3], Jean Ee Tang[1], Steven Lin[1], Tarique D. Perera[1]
[1]Departments of Psychiatry, College of Physicians and Surgeons, Columbia University Medical Center and New York State Psychiatric Institute, New York, NY; [2]Department of Psychiatry, State University of New York at Brooklyn, Downstate Medical Center, New York, NY; [3]Department of Biological Sciences, Kingsborough Community College, CUNY, Brooklyn, NY

Background: Millions of people worldwide suffer from major depression and have inadequate responses to antidepressant medications. Understanding the neurobiology of this illness will significantly improve the efficacy and tolerability of treatment options. There is increasing evidence that the rates of neuron formation from stem cells in the hippocampus contributes to the pathogenesis of depression while the stimulation of neurogenesis plays a role in generating antidepressant effects. Currently, available treatments fail to address the issue of neuron loss and

consequently, provide symptomatic relief or slow disease progression without curing the underlying pathology. Curing these disorders maybe possible if lost neurons can be replaced by inducing stem cells to differentiate and become neurons or glia. The adult brain has a reservoir of endogenous stem cells that are ubiquitously present and therefore can potentially be induced to replace lost tissue anywhere in the brain. In our previous studies we have shown that irradiating (XRT) the hippocampus, and thus ablating all new neurons blocks the effects of antidepressants. Our current study is looking to see if XRT is itself a cause of depression or if there needs to be another stressor to induce depression symptoms.

Materials/Methodology: Three groups of socially-housed, adult female bonnets are being exposed to 27-weeks of control conditions or repeated separation stress and treated concurrently with saline placebo (Control-Placebo, N=0; Stress-Placebo, N=2) or fluoxetine (Control-Drug, N=8; Stress-Drug, N=4). Within these groups we have given XRT treatment to certain animals (XRT Stress-Drug, N=2; XRT Control-Drug, N=4). Dependent measures are weekly behavioral observations.

Results: Baseline behavioral observations show no significant difference in depression-like behaviors (anhedonia and subordinance) between the VFD (variable foraging demand) and LFD (low foraging demand) reared animals. There has also been no significant difference between groups for the Dominant behavior subset. XRT based differences in behavior will be available at the time of the Annual Meeting in May.

Conclusion: Our data provides evidence that all animals at baseline are similar in terms of depression-like behaviors and dominance behaviors. This non-significant finding will lead to the ability to detect subtle changes in behavior once the experimental procedures have taken place and we have successfully induced depression in the stress groups. We will also be able to detect if there is any change in behavior in the non-stress groups after treatment with fluoxetine.

(26) IMPLANTATION OF HEDGEHOG-ACTIVATED MSCs REGENERATES PERIOSTEAL LIKE BONE FORMATION IN A MURINE BONE ALLOGRAFT REPAIR MODEL

Chunlan Huang, Ming Xue, Mu Xiao, Fanjie Zeng, Xinping Zhang
Center for Musculoskeletal Research, University of Rochester, School of Medicine and Dentistry, Rochester, NY

Periosteum plays an essential role in skeletal repair and reconstruction. To the end of understanding the molecular pathways underlying periosteum-mediated repair and reconstruction, we isolated a population of mesenchymal stem cells (MSCs) from the periosteum at the cortical bone healing site. These cells express typical MSC markers and are capable of differentiating into osteoblasts, adipocytes, and chondrocytes. Characterization of these cells demonstrated that activation of the hedgehog (Hh) pathway effectively promoted osteogenic differentiation of periosteum MSC *in vitro.* When implanted subcutaneously in athymic nude mice, these hedgehog-overexpressing cells induced robust bone formation *in vivo*. To further determine the effects of Hh-activated periosteum MSCs in repair and reconstruction, we implanted the periosteum MSCs overexpressing an N-terminal sonic Hedgehog peptide around a devitalized bone allograft to repair a 4 mm segmental femoral bone defect in mice. Our results demonstrated that implantation of the Hh-activated MSCs induced robust bone formation around bone allografts. MicroCT imaging analyses showed a 3-fold induction of new bone formation around bone allografts as compared to the controls. A contiguous periosteal collar bone was regenerated around bone allografts engrafted with Hh-activated periosteum MSCs. In comparison, the control allografts demonstrated a complete absence of effective periosteal bone formation around allografts. These results strongly suggest that the engraftment of Hh activated periosteum MSCs is effective in enhancing skeletal repair and reconstruction. In an effort to identify Hh-responsive MSCs from other sources for repair and reconstruction, we examined the effects of Hh activation in cells isolated from murine neonatal dermis. We found that overexpression of Hh ligand or Gli1, a key activator of the Hh pathway in dermal cells induced ALP staining and osteoblastic differentiation, albeit not as potent as in periosteal MSCs. Further experiments are underway to determine the potential utility of dermis MSCs for repair and reconstruction.

(27) IMMUNOGENICITY OF MESENCHYMAL STEM CELLS IN THE CUTANEOUS ENVIRONMENT

Li Ting Huang[1] and Soosan Ghazizadeh[1,2]
[1]Department of Oral Biology & Pathology, [2]Department of Dermatology, Stony Brook University, Stony Brook, NY

Stem cell transplantation offers an opportunity to restore tissue function. However, unless transplanted stem cells are derived from the patient's own cells, the transplanted cells will be targeted for rejection by the immune system. Mesenchymal stem cells (MSCs) are attractive candidates for allogeneic cell-based therapies due to their multipotency and reportedly low immunogenicity. To explore the potential use of these cells in allogeneic settings, we have developed a stable skin graft platform in immunocompetent mice for the analysis of immune responses to allogeneic MSC transplantation in a stringent cutaneous environment. Using green fluorescent protein (GFP)-labeled MSCs and a skin reconstitution assay, we demonstrated that isogenic bone marrow-derived MSCs can replace dermal fibroblast during skin regeneration, in contrast to unpurified bone marrow cells. Inclusion of dermal fibroblasts with MSCs at a 1:1 ratio however, increased the efficiency and the size of the regenerated skin graft. MSC derived (GFP^+) cells contributed only to the dermal compartment of skin and persisted for at least 10 weeks after grafting, indicating their long term engraftment in skin. In contrast to isogenic MSCs, transplantation of allogeneic MSCs in this model resulted in an acute rejection of MSCs, as indicated by complete loss of GFP^+ cells within two weeks after grafting, similar to that observed for allo-fibroblasts. Therefore, despite the immunomodulatory properties of MSCs in vitro, our data showed immunogenicity of non-self MSCs *in vivo*. We are currently exploring whether MSCs modified with lentiviral vectors to express immunosuppressive factors, such as PD-L1 or CTLA4Ig, can protect allogeneic MSCs from immune rejection. Such knowledge will contribute to the eventual goal of generating universal stem cells for cutaneous and other cell-based therapies.

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(28) GENOME-WIDE PROFILING REVEALS KEY MOLECULAR CHANGES IN ERYTHROID PROGENITORS IN LATE GASTRULATION STAGE MOUSE EMBRYOS

Joan Isern[1], Zhiyong He[1], Stuart T. Fraser[1,2], Vincent Schulz[3], David Tuck[4], Patrick G. Gallagher[3], Margaret H. Baron[1,2]
[1]Medicine/Hematology-Oncology, [2]Black Family Stem Cell Institute, Mount Sinai School of Medicine, New York, NY; [3]Pediatrics and [4]Pathology,Yale University School of Medicine, New Haven, CT

Primitive erythroblasts (EryP) are the first cell type specified from mesodermal progenitors, toward the end of gastrulation in the mouse embryo. EryP progenitors arise in the yolk sac at embryonic day (E) ~E7.5-8.5 then begin to differentiate to erythroblasts that enter the circulation and continue to mature in a stepwise, synchronous fashion until their enucleation several days later. We have purified these first hematopoietic-committed progenitors from staged embryos based on the expression of a nuclear GFP transgene that is expressed specifically within the EryP lineage as early as E7.5. Genome-wide expression profiling allowed us to define the transcriptome from each stage of development and revealed highly dynamic changes during the progression from progenitor to maturing erythroblast. We have focused on the emergence of EryP progenitors in the yolk sac and on the transition to circulation stage. TRANSFAC analysis of promoters of differentially expressed genes allowed us to identify candidate transcriptional regulators, some of which have not previously been implicated in erythroid development (e.g. Nkx3.1, known previously as a regulator of prostate stem cells). We designed experiments to test predictions from our microarray analysis and found that EryP progenitor numbers are regulated by TGFβ1 and hypoxia. In most mammalian cells, the response to hypoxia is mediated by the transcription factor HIF-1. *Hif-1* is apparently not expressed in EryP. However, *Hif3a/Ipas,* a gene that encodes a dominant negative regulator of HIFs and that is thought to function as a feedback regulator in response to hypoxia, is expressed in EryP as early as E7.5 and is upregulated as the cells mature. The response to hypoxia by EryP may involve a pathway that is distinct from that of most other cells. EryP progenitors express genes

associated with aerobic glucose metabolism (the Warburg effect), a phenotype characteristic of cancer and other rapidly proliferating cells. Whether this glycolytic profile reflects the energy needs of these cells or a more unique feature of primitive erythropoiesis is under investigation. We have also generated mouse ES cell lines in which GFP is expressed exclusively within EryP; this system will facilitate evaluation of the functions of other genes identified by our microarrays. This study is the first lineage specific transcription profiling of a differentiating cell type in the early mouse embryo and will provide a strong basis for future work on normal erythropoiesis throughout ontogeny. It may also help guide efforts to direct the differentiation of stem/progenitor and cells of other lineages to an erythroid cell fate.

(29) ROLE OF TGF-β SIGNALING PATHWAYS IN DIRECTING MATURATION OF HEPATIC STEM/PROGENITOR CELLS AT EARLY DEVELOPMENTAL STAGE WITH CONJOINT EPITHELIAL AND MESENCHYMAL PHENOTYPE

Mukesh Kumar, Sriram Bandi, Bhaskar Das, Sanjeev Gupta
Albert Einstein College of Medicine of Yeshiva University, Bronx, NY

During fetal development, we found foregut endoderm-derived hepatic and pancreatic cells were characterized by conjoint epithelial/mesenchymal phenotype, which was reproduced in hESC-derived immature liver cells. As gain of maturity in stem cell-derived hepatic cells is of much interest for applications, we determined the role of outside-in signaling from TGF-β, which exerts pleiotropic effects extending to downregulation of epithelial properties. Restriction of HBV replication to hepatic conditions and inhibition of HBV replication by TGF-β offered convenient assays besides gene expression analyses to determine cell maturity. We blocked TGF-β signaling by a series of 18 synthetic compounds, which were studied for inhibition of TGF-β-induced phosphorylation of Ser/Thr on TGF-β receptor, along with a blocker of TGF-β receptor kinase activity demonstrated by decreased phosphorylation of intracellular Smad4. None of the compounds induced greater epithelial properties, e.g., E-cadherin, or lesser mesenchymal properties, e.g., vimentin, in hTERT-FH-B fetal human liver stem/progenitor cells. This was in agreement with unchanged expression of Oct-4, Nanog, albumin, or HNF4• . Also, levels of quantitative hepatic functions, i.e., ureagenesis, albumin secretion, or phenobarbital-induced p450 expression, were unaltered. Similarly, exposure of FH-B cells to TGF-β modulators followed by transduction with recombinant adenoviral HBV-GFP vector confirmed excellent transgene expression but no HBV replication, indicating maintenance of nonpermissiveness for HBV.

Conclusions: Inhibition of TGF-β-mediated intracellular signaling, including through inhibition of TGF-β receptor activity or of the intracellular TGF-β kinase activity, did not induce maturation in candidate hepatic stem/progenitor cells. The onset of permissiveness for HBV replication will provide convenient assays to identify whether hepatic maturation is being achieved.

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(30) A SURVIVAL ASSAY FOR HUMAN EMBRYONIC STEM CELL-DERIVED MOTOR NEURONS: A PLATFORM TO IDENTIFY MOTOR NEURON SURVIVAL FACTORS

Nuno Jorge Lamas[1-5*], Bethany Kerner[2-5*], Hynek Wichterle[2-5], Christopher E. Henderson [2-5]
[1]Life and Health Science Research Institute, School of Health Sciences, University of Minho, 4710-057 Braga, Portugal; [2]Jenifer Estess Laboratory for Stem Cell Research, Project ALS, [3]Center for Motor Neuron Biology and Disease, [4]Departments of Pathology, Neurology, and Neurobiology and Behavior, [5]Columbia Stem Cell Initiative, Columbia University Medical Center, New York, NY

Understanding the mechanisms that keep human motor neurons alive may be of fundamental value for therapeutics aiming to slow down or halt motor neuron degeneration in Amyotrophic Lateral Sclerosis (ALS) or Spinal Muscular Atrophy (SMA). Here, using Human Embryonic Stem Cell-derived Motor Neurons (hESC-MNs), we developed two robust and reproducible *in vitro* survival assays - based on the neurotrophic factor deprivation model - to screen libraries of factors for their potential to promote the survival of human motor neurons. One strategy employs use of anti-mitotics to prevent motor neuron proliferation and the other strategy combines anti-mitotic treatment with FACS purification of fluorescent human motor neurons. We expect that candidate factors

identified using these assays will provide new information about the requirements and mechanisms of survival and death of human motor neurons, contributing to the development of long-desired efficacious drug options to treat human motor neuron disease.

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**Equal contribution*

(31) OVEREXPRESSION OF TGFβ ACTIVATED KINASE DIRECTS MYOCARDIAL CELLS TO A SINOATRIAL NODE FATE

Stephanie Legros[1], Michael Xavier Doss[2], Kemar Brown[1] and Ann Foley[1]
[1]Weill Medical College of Cornell University, New York, NY; [2]Current address: Masonic Medical Research Laboratories, Utica, NY

Cellular transplantation is emerging as a therapeutic strategy to repair damage to the heart resulting from myocardial infarction. However, many obstacles remain before such cell based therapies become feasible, not the least of which is out current inability to direct the differentiation of specific myocardial sub-types. This is significant because grafting donor cells with inappropriate electrophysiological characteristics into the heart would most certainly result in the development of lethal cardiac arrhythmias. We hypothesized that TGFβ-activated kinase (TAK1) is important for cardiac differentiation and patterning because it links two pivotal pathways that are essential for the earliest stages of myocardial specification. One of these is mediated by agonists of TGFβ signaling, the other by antagonists of the canonical Wntβ-Catenin signaling pathway.

To test this, we made a lentivirus to overexpress TAK1 in mouse ES cells. These were differentiated as embryoid bodies (EBs) and assessed for continuous TAK1 mRNA overexpression by QRT-PCR. Effects on patterning were assessed by QRT-PCR, physiological criteria and immunocytochemistry. We found that TAK1 overexpression resulted in the formation of sinoatrial node-like cardiomyocytes at the expense of working myocardium. These cells had average rates of beating approximately twice as fast as cardiomyocytes formed from untransduced ES cells. In addition, these cells expressed markers characteristic of sinoatrial node differentiation. Specifically, they show increased expression of Tbx5, Tbx3, Shox2 and HCN4 with concomitant decreases in expression of most cardiac contractile proteins. Together these findings suggest that TAK1 overexpression directs myocardial cells toward a sinoatrial node fate.

(32) WNT 2 INHIBITS BACTERIAL-INDUCED INFLAMMATION IN INTESTINAL EPITHELIAL CELLS

Xingyin Liu[1], Rong Lu[1], Shaoping Wu[1], Yong-guo Zhang[1], Yinglin Xia[2]. Jun Sun[1]
[1]Department of Medicine, [2]Department of Biostatistics and Computational Biology University of Rochester, Rochester, NY

The Wnt signaling plays an essential role in intestinal proliferation and stem cell renewal, with investigations focused on the development and immune response. Bacterial infection is a common public health problem and could be chronic and increases the risk of cancer. However, we lack the studies on how bacteria regulate Wnt proteins and how Wnts modulate the host responses to pathogenic bacteria in intestinal mucosa.

This study investigated the effects of *Salmonella* infection on Wnt 2, one of the Wnt family members, in intestinal epithelia cells. Using *in vitro* and *in vivo* models, we found that Wnt 2 mRNA and protein expression were elevated after *Salmonella* infection. Pathogenic bacteria regulate Wnt 2 location in the intestine *in vivo*. Furthermore, we found that over-expression of Wnt 2 was a strategy for host defense. Wnt 2 was involved in the host protection by inhibiting cell apoptosis and inflammatory responses to *Salmonella* typhimurium infection. Whereas inflammatory cytokine IL-8 mRNA in the Wnt 2 transfected cells was undetectable, it was easily detectable in the cells with low level of Wnt 2

protein blocked by Wnt 2 siRNA. AvrA is a known bacterial effector protein that inhibits the inflammation in the bacterial-induced intestinal inflammation by stabilizing β-catenin, the down-stream target of Wnt signaling. We further hypothesized that AvrA is involved in the Wnt 2 signaling during bacterial colonization. We examined the effects of AvrA on induction of Wnt 2. We found that the stabilization of Wnt 2 was regulated through ubiquitination. Moreover, bacterial protein AvrA from *Salmonella* and *E. coli* stabilized Wnt 2 protein expression *in vitro* and *in vivo*. Hence, our study on the novel role of Wnt in *Salmonella*-infected model provides fundamental molecular insights into host-bacterial interaction in intestine. It emphasizes the importance of Wnt and stem cell niches in inflammation infection.

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(33) HEMATOPOIESIS OF THE GUT IMMUNE SYSTEM AND GUT IMMUNITY IN IMMUNODEFICIENT MICE

María C. López and David A. Lawrence
Wadsworth Center, New York State Department of Health, Albany, NY

The gut-associated immune system protects the host from a myriad of commensal bacteria while defending against orally invasive pathogens and mounting tolerance towards food antigens to control food allergies. Severe combined immunodeficient babies or immunosuppressed patients, either children or adults, who undergo bone marrow transplantation and suffer graft *vs*. host disease, have increased morbidity due to the lack of a functional gastrointestinal immune system. Investigation of human stem cell regeneration of the gut-associated immune system in an experimental model is highly warranted. No studies have mechanistically examined the development and functional activity of a gastrointestinal human immune system after stem cell engraftment. Mice lacking genes critical for the development of lymphocytes have been given human stem cells at birth, which differentiate into all of the known human lymphocyte subpopulations. Using human cord blood stem cells, we have produced a human immune system in NOD/SCID/IL2Rγ chain KO mice. We have been able to demonstrate the presence of B, T, NK and NKT cells in peripheral blood 12 weeks after mice were reconstituted with human stem cells. B cells presented an interesting phenotype since the majority of them expressed CD5, a marker of B1 cells. Furthermore, a significant percentage of B cells in peripheral blood were expressing CD23. Although, mesenteric lymph nodes and Peyer's patches were in early stages of development in mice 12 weeks after reconstitution, secretory IgA was not found in mouse feces. These results suggest that reconstitution of the gut immune system in immunosuppressed mice is much more delayed than reconstitution of peripheral blood as it has been suggested from few human studies. Further time points will be analyzed.

(34) TRANSGENE EXCISION HAS NO IMPACT ON *IN VIVO* INTEGRATION OF HUMAN iPS DERIVED NEURAL PRECURSORS

Tamara Major[1], Jayanthi Menon[1], Gordon Auyeung[1], Frank Soldner[2], Dirk Hockemeyer[2], Rudolf Jaenisch[2,3], Viviane Tabar[1*]
[1]Center for Stem Cell Biology and Department of Neurosurgery, Memorial Sloan Kettering Cancer Center, New York, NY; [2]The Whitehead Institute; and [3]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA

Recent advances have generated significant enthusiasm for the prospects of iPS cell-based therapy. However, there are concerns about the suitability of iPS cells for *in vivo* applications in view of the use of viruses encoding the reprogramming factors and the concern over residual transgene expression, which may alter iPSC differentiation potential or result in neoplastic transformation. Recently developed lentiviral vectors (Soldner *et al*., 2009) allow the excision of viral reprogramming factors and the derivation of transgene-free hiPSC lines. However, it is unclear if reprogramming strategy has an impact on the behavior of hiPS progeny. Here, we analyze the impact of

reprogramming strategy on the neural differentiation potential of viral factor-free, c-myc-free and conventionally reprogrammed four-factor human iPSC lines. We demonstrate similar and efficient differentiation into neurons, astrocytes and oligodendrocytes among all hiPS and ES (H9) line controls. In addition, we transplant iPSC-derived neural precursors into the subventricular zone (SVZ) of adult rats and analyze survival, integration and phenotypes after 3 months *in vivo*. Our data demonstrate survival, acquisition of phenotypes typical of subventricular zone progeny, including Type A neuroblasts (EGFR+/CD24+), Type B (EGFR+/GFAP+) and Type C (EGFR+/Dlx2+) cells. A proportion of grafted cells migrated anteriorly along the rostral migratory stream towards the olfactory bulb where a small percentage of cells acquired an interneuron phenotypic identity. There was no formation of teratoma or excessive graft growth as shown by a Ki-67 <1%. Thus, reprogramming strategies, such as the number of factors, their copy number or their excision had no impact on the iPS cells' ability to respond to *in vivo* cues and to acquire regional phenotypes within the adult brain. Neither the use of c-myc in reprogramming nor its excision impacted the proliferation rate or graft size of human iPS progeny *in vivo*.

(35) 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. Elevated levels of canonical Wnt signaling contribute to tumorigenesis in experimental systems and have been observed in many human cancers including breast cancer. Oncogenic effects of Wnt signaling in mammary tissue may result primarily from changes in the behavior of mammary stem cells and/or cancer stem cells. We are interested in how Wnt signaling alters stem cell behavior and increases the propensity for tumorigenesis. To do this we are investigating the effects of Wnt signaling on mammary stem cell abundance, self-renewal, and differentiation.

We are using a number of approaches to examine the role of Wnt signaling, both *in vivo* and *ex vivo*. Our primary assay is 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.

Using this system, we have compared the stem cell content of cultures derived from wild type mice with those from transgenic mice expressing Wnt1 from a mouse mammary tumor virus (MMTV) promoter. MMTV-Wnt1 mice exhibited significantly greater number of mammosphere forming units (MFUs) in culture compared to wild type. This increase in MFUs can be inhibited by addition of exogenous Dkk1, a known inhibitor of canonical Wnt signaling. As well as elevated MFU numbers caused by continuous Wnt expression, we observed an increase in MFU number after addition of soluble Wnt3a to wild type cultures. This increase could also be inhibited by treatment of the cells with Dkk1. These results indicate that a Wnt-mediated increase in MFU numbers can be achieved with a short-term signal, but that Wnt signaling is continuously required to maintain the elevated number of MFUs.

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

(36) DYSTROGLYCAN REGULATES THE ORGANIZATION AND GLIOGENIC CAPACITY OF THE NEURAL STEM CELL NICHE

Freyja K. McClenahan[1,2] and Holly Colognato[1]
[1]Department of Pharmacology, [2]Program in Neurosciences, SUNY Stony Brook, Stony Brook, NY

Extracellular matrix (ECM) proteins have been implicated in maintaining the architecture and cellular composition of the adult subventricular zone (SVZ), a neural stem cell niche of the vertebrate forebrain. Specifically, •6β1 integrin binding to laminin is required for activated adult NSCs to

associate with ECM structures and proliferate appropriately. Here we report that an organized laminin-rich SVZ ECM is present at birth and that the laminin receptor dystroglycan (DAG) is required for early postnatal SVZ architecture, progenitor proliferation and regulation of the wave of gliogenesis that occurs within the first two postnatal weeks. Utilizing the Cre/loxP approach to generate mice in which dystroglycan is deleted at the neural stem cell stage (DAG-CNS-KO), we found that dystroglycan-deficient radial glia were disorganized and failed to form apical endfoot associations with ventricle-associated laminins, while DAG-KO Sox2+ progenitors inappropriately retained their neonatal proliferative capacity longer than WT progenitors. DAG-KO Sox2+ nuclei were also found in an aberrant distribution, proliferating closer to the ventricular surface than WT cells. The developing white matter in early postnatal DAG-CNS-KO corpus callosa contained more oligodendrocyte precursor cells (OPCs) and fewer mature oligodendrocytes than did that in WT littermates, with a higher proportion of DAG-KO OPCs displaying an immature phenotype i.e. co-expressing the NSC markers Nestin and Sox2. These delays in oligodendrocyte differentiation in turn led to delayed myelination of the DAG-CNS-KO corpus callosum. Neurosphere differentiation assays furthermore revealed that NSC/progenitors isolated from DAG-CNS-KO mice produced more OPCs but fewer post-mitotic and myelinating oligodendrocytes. Together these findings suggest that dystroglycan-laminin interactions in the perinatal 'gliogenic' SVZ are important for stem and/or progenitor cell organization and gliogenic capacity, as well as in the timely maturation of oligodendrocyte lineage cells.

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(37) DEVELOPMENT OF EARLY AND DELAYED AFTERDEPOLARIZATION ACTIVITY IN HUMAN iPS CARDIOMYOCYTES

Emiliano Medei, Michael Xavier Doss, Serge Sicouri, José M. Di Diego, Robert Goodrow, Yuesheng Wu, Jackie Treat, Charles Antzelevitch
Masonic Medical Research Laboratory, Utica, NY

Background: Human induced pluripotent stem cell (hiPS)-derived cardiomyocytes exhibit diverse electrophysiological and pharmacological properties. One of the goals of hiPS cardiomyocyte development is to have available a human model suitable for safety pharmacology. The present study tests the hypothesis that agents known to induce early (EAD) and delayed (DAD) afterdepolarizations do so in hiPS cardiomyocytes.

Method: Embryoid bodies (EBs) were made from a human iPS cell line reprogrammed with Oct4, Nanog, Lin28 and Sox2 using a serum-free differentiation protocol supplemented with growth factors for selective cardiac differentiation. Beating clusters were micro-dissected from EBs 56-80 days of maturity and plated on gelatin-coated dishes. Standard microelectrode techniques were used to record action potentials (AP) from spontaneously and electrically stimulated EB clusters superfused with HEPES-Tyrode's solution (KCl=4 mM; 37°C) before and after exposure to either 5 μM E-4031 (n=6) or 2.5-5.0 μM ouabain (n=6). For the purpose of comparing data from EBs displaying widely disparate beating frequencies, we employed a Bazett's correction in an attempt to normalize the action potential duration measured at 90% reprolarization ($cAPD_{90}$-B).

Results: E-4031 (5 μM; n=6) significantly increased $cAPD_{90}$-B from 351.7 ± 29.4 to 504.3 ± 121.7 ms ($p<0.05$) and generated multiple phase 3-EADs in 1/6 preparations (56-70 days-old BCs). Addition of ranolazine (15 μM) reduced $cAPD_{90}$-B from 504.3 ± 121.7 to 444.8 ± 44.1 ms and abolished EADs. The effects of ouabain were examined using a pulse train of 10 beats at cycle lengths (CLs) of 300-1000 ms followed by a pause. Ouabain (2.5-5 μM) reduced phase 4 depolarization and induced DADs in 3/6 and DADs-induced triggered activity in 1/6 preparations. DAD amplitude increased progressively as CL was reduced consistent with the characteristics of DADs observed in native cardiomyocytes.

Conclusion: Our data indicate that cardiomyocyte clusters derived from hiPS generate action potential duration changes, EAD- and DAD-induced triggered activity consistent with toxicological effects observed in the clinic.

[*Supported by NYSTEM contract C026424*]

(38) IDENTIFICATION OF mRNAS AND THEIR TARGETS ON mRNAS AND LONG NON-CODING RNAS THAT REGULATE LIVER STEM CELL DIFFERENTIATION IN CIRRHOTIC HUMAN LIVER

David Moskowitz, Brian Kosmyna, Leslie Rogler, Brent Calder, Joe Matarlo, Remon Babewee, Charles E. Rogler
Departments of Genetics, Microbiology/Immunology and Medicine, Albert Einstein College of Medicine, Bronx, NY

Cirrhosis in human HBV carriers is a product of an ongoing disease process in which replication of hepatocytes is compromised and the liver calls upon its stem cell compartment to maintain hepatocyte mass. Using a novel triple staining protocol we have identified dumbbell-shaped lineages of differentiating stem cells (oval cells) in which bile duct cells are located at one end of the dumbel and hepatocytes on the other end of the structure. MicroRNAs 23b, 27b and 24 (miR-23b cluster) are detected in hepatocytes but not bile ductular cells and these miRNAs target Smads that are required for TGFβ signaling. *In vitro* treatment of bipotent liver stem cells with miR-23b cluster mimics blocks bile duct differentiation of the stem cells due to blockage of TGFb signaling which is needed for ductular differentiation.

Our aim was to identify targets of miRNAs that regulate liver stem cell differentiation. To do this we developed a novel Argonaute RNP immunoprecipitation/deep sequencing protocol that identifies targets of miRNAs. This protocol identified and validated a large set of specific miRNA target sites on mRNAs from cirrhotic human liver. A set of validated targets to miR-23b cluster miRNAs clustered in the focal adhesion pathway which is regulated during stem cell differentiation.

We also identified a major fraction of long non-coding RNAs, many of which are components of molecular machines involved in diverse cellular mechanisms. The highest targeted long non-coding RNA was H19 which has been implicated in oncogenic processes in the liver. This discovery provides a conceptual advance in our understanding of the scope of functions of Argonaute complexes (Ago) in mammalian cells.

These functions include enhancer methylation (by H19), nuclear speckle construction (NEAT1), telomerase-associated RNA-dependent RNA polymerase activity (RMRP), RNase P processing of pre-tRNAs (RPPH1), and alternative splicing (MALAT1). We have functionally validated large sets of microRNA targets and numerous specific target sites, and present new insights into the molecular characteristics of microRNA targeting. In addition, we offer a new biogenesis model for small interfering RNAs originating from long non-coding RNAs.

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

Matthew Murtha, Yatong Wang, Claudio Basilico, Lisa Dailey
Department of Microbiology, NYU School of Medicine, New York, NY

Mammalian ES cells represent a unique cell state characterized by the properties of self-renewal and pluripotency. The distinguishing properties of ES cells, and those of their differentiated derivatives, are largely governed by different sets of core Transcription Factors (TFs) that serve to establish cell-specific chromatin landscapes and hierarchies of gene expression. Thus a full understanding of these cell states, and the transitions between them, requires the identification of the target DNA elements and TFs comprising their distinct transcriptional circuitries. While the majority of analyses have focused on ChIP-based studies of Oct4, Sox2, and Nanog, additional factors contributing to pluripotency remain largely uncharacterized, and little is known regarding the TFs and target DNAs mediating the earliest events of lineage specification of ES cells.

To address these challenges, we have developed a new technology allowing the *functional* isolation of stage-specific promoter and enhancer elements from ES cells, and those that become active as ES cells transition towards specific differentiated cell lineages. Based on the classic observation that active promoter and enhancer elements lie within nucleosome-free regions (NFRs) within

cellular chromatin, we first devised a simple method for isolating NFR-DNAs from ES cells. Lentiviral libraries in which individual ES-derived NFRs drive expression of a GFP reporter gene were constructed. Following the transduction of these constructs into ES cells, NFR DNAs exhibiting differential activation of GFP in ES cells, differentiating ES cells or Neural Stem cells (NSCs) have been isolated, and are likely to represent stage-specific regulatory elements. A set of Sox2 target elements that exhibit activity in both ES cells and NSCs have also been functionally identified. Current efforts entail the High-throughput (Illumina) sequencing and subsequent bioinformatic characterization of each group of elements to facilitate the identification of the TFs that correlate with their stage-specific activities.

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(40) EFFICIENT TWO-FACTOR REPROGRAMMING OF EMBRYONIC FIBROBLASTS INTO PLURIPOTENCY

Alice Nemajerova, Oleksi Petrenko, Natasha Marchenko, Flaminia Talos, Angelina Vaseva, Ute M. Moll
Department of Pathology, Stony Brook University, Stony Brook, NY

Reprogramming human somatic cells into pluripotency represents a valuable resource for development of improved *in vitro* models of human diseases and for regenerative medicine. Currently most approaches to reprogram differentiated somatic cells into a pluripotent state utilize viral transduction of combinations of 4 transcription factors. These protocols are inefficient presumably because only a small proportion of cells carry the appropriate number and stoichiometry of proviral insertions to initiate the reprogramming process.

To gain a better understanding, we performed a systematic molecular and functional dissection of freshly isolated E12.5 mouse embryo fibroblasts (MEFs), the classic target cell for reprogramming. We found that MEFs independent of strain background are heterogeneous and can be FACS-sorted into distinct subpopulations based on differential expression of the cell surface markers Thy1 (differentiation marker) and Sca1 (stem/progenitor marker). Importantly, these subpopulations yield significantly different efficiencies in iPS generation with 4 reprogramming factors (OKSM), 3 factors (OKS) and 2 factors (KO or OS). Specifically, Thy1+ cells (which constitute the vast majority of the unsorted MEF population) represent a poor target for iPS production, whereas the preexisting DN (double negative) and Sca1+ single positive subpopulations represent the most reprogrammable cell types. The efficiency of reprogramming of DN and Sca1+ cells with 2 or 3 factors is comparable to or surpasses that achieved with the classic 4 factors. Our findings suggest that other mechanisms than the appropriate stoichiometry among exogenous transcription factors dictate the rate of reprogramming. Ongoing microarray analyses should yield functional insights into these still undefined determinants.

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(41) REPROGRAMMING USING HUMAN EGGS AND OOCYTES

Scott Noggle[1], Hector Martinez[1], Cathleen Crumm[2], Robert Prosser[2], Kiboong Oum[2], Sarah Druckenmiller[1], Matthew Freeby[3], Ellen Greenberg[3], Robin Goland[3], Mark V. Sauer[2], Rudolph L. Leibel[3,], Dieter Egli[1,3]
[1]The New York Stem Cell Foundation Laboratory, New York, NY; [2]Center for Women's Reproductive Care; [3]Naomi Berrie Diabetes Center, College of Physicians and Surgeons, Columbia University, New York, NY

The exchange of the oocyte's genome with the genome of a somatic cell, followed by the derivation of pluripotent stem cells, could enable the generation of specific cell types affected in degenerative human diseases. Such cells, carrying the patient's genome, might be useful for autologous

transplants and disease modeling. Indeed studies in mice have demonstrated the value of this approach for treating severe combined immune deficiency and Parkinson's disease, as well as for the in vitro study of ALS. The generation of stem cells by after reprogramming using oocytes is also a critical step towards determining whether stem cells generated by chemical induction (iPS cells) are equivalent to pluripotent stem cells derived from blastocysts.

However, logistical, legal and social considerations have slowed the progress of human reprogramming studies by limiting the availability of oocytes. Although a few experiments have been carried out, they have uniformly failed to produce patient derived pluripotent stem cell lines. It is unclear whether these failures reflect the preliminary nature of the studies or some fundamental block to reprogramming. Here we will share our experience in obtaining donated human oocytes and 1-cell zygotes, as well as their use in reprogramming studies.

(42) EINSTEIN COMPREHENSIVE HUMAN PLURIPOTENT STEM CELL CENTER

Ruchi Patel, Sumita Berry, Nathalie Lallier, Zipora Etzion, Julien Lajugie, Guillermo Simkin, Vladik Sandler, Uli Steidl, Eric E. Bouhassira
Albert Einstein College of Medicine, Bronx, NY

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. We will present data on automated production of transgene free iPS using plasmid transfection.

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. We will present GenPlay, a fast, easy to use and stable tool for rapid analysis and processing of genomic and epigenomic data that was developed by the stem cell genomic unit. It is written in Java and runs on all major operating systems. GenPlay recognizes a wide variety of common genomic data formats from micro-array or sequencing based platforms. GenPlay displays tracks adapted to summarize gene structure, gene expression, repeat families, CPG islands, SNPs etc. GenPlay can display custom tracks to visualize the results of RNA-Seq, ChIP-Seq, TimEX-Seq and SNP analysis. GenPlay can generate statistics (minimum, maximum, standard deviation, correlation between tracks, etc). GenPlay offers a library of operations to modify tracks and compare experiments. Some of the operations available are normalization operations, filters, indexation, signal saturation, smoothing (Gaussian, lowess, moving average), several peak finders, data summarization, track crossing and annotation etc. The software also offers graphical features such as scatter plots and bar charts to depict signal repartition.

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 immuno-deficient mice are kept in stock.

Number of users: In 2009 the Center served 35 users.

(43) GENERATION OF ES-DERIVED INTERNEURONS UTILIZING INDUCIBLE EXPRESSION OF NKX2.1

Timothy J. Petros[1], Carine Maurer[1], Stewart Anderson[1]
[1]*Department of Psychiatry, Weill Cornell Medical College, New York, NY*

Forebrain GABAergic interneurons are the primary source of inhibition in the cerebral cortex and play crucial roles in brain function. Interneurons comprise ~20% of cortical neurons and are divided into different subgroups based on their neurochemical markers, connectivity and physiological properties. Abnormal development and function of interneurons is implicated in the pathobiology of several neurological and psychiatric disorders, including schizophrenia, autism, and epilepsy. The lack of an efficient mechanism for the production, collection and selection of interneurons has hindered our ability to study the role of interneurons in disease etiologies and their potential as cell-based therapies. The current protocol to derive interneurons from embryonic stem cells (ESC) is handicapped by the small percentage of interneurons produced. In this project, we aim to enhance the production of interneurons by inducibly, reversibly, expressing the transcription factor Nkx2.1 to drive telencephalic ($Foxg1^+$) cells towards an interneuron fate ($Lhx6^+$). Using this cell line, we are working to establish a protocol that preferentially generates distinct interneuron subgroups by manipulating the timing of Nkx2.1 expression and the level of Sonic hedgehog (Shh) signaling. These experiments should greatly increase our ability to study interneurons and their potential roles in the pathobiology and treatment of disease.

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(44) EFFICIENT, ROBUST, EPIGENETICALLY YOUNG AMNIOTIC FLUID (AF) CELLS AS FOREFRONT OF REGENERATIVE MEDICINE AND POTENTIAL TRANSLATIONAL PATH FROM STEM CELL RESEARCH TO THERAPIES

Katalin Polgar
Departments of Medicine and Obstetrics Gynecology and Reproductive Science, Mount Sinai School of Medicine, New York, NY

Amniotic fluid (AF) obtained in the early second trimester of pregnancy (around 15 weeks) contains different cell types (Priest *et al*., 1978; Gosden, 1983; Polgar *et al*., 1989). AF contains approximately 1% precursor state multipotent cells and 99% terminally differentiated somatic cells mostly desquamated from the fetal skin. The 1% of the amniotic fluid cells categorized as amniotic fluid stem (AFS) cells (De Coppi *et al*., 2007; Fauza, 2004; Hipp and Atala, 2008; In't Anker *et al*., 2003; Li *et al*., 2009; Prusa *et al*., 2003; Siegel *et al*., 2008, Valli *et al*., 2009). AFS cells (De Coppi *et al*., 2007) and hAFDCs [human amniotic fluid-derived cells] (Li *et al*., 2009) represent a ''precursor state,'' not a pluripotent stem cell state (De Coppi *et al*., 2007; Li *et al*., 2009). The ''precursor state'' hAFDCs could be reprogrammed rapidly (6 days after infection) and efficiently (Li *et al*., 2009). By induction of pluripotency with the transcription factor quartet (OCT3/4, SOX2, KLF4 and c-MYC) (Takahashi *et* a.l, 2007) AF skin cells were reprogrammed twice as fast and two hundred percent more iPS colonies than adult and neonatal skin cells (Galende *et al*., 2010). AF-iPS cells were comparable to hES cells for morphological and growth characteristics, antigenic stem cell markers, stem cell gene expression, telomerase activity, *in vivo* and *in vitro* differentiation potential (Galende *et al*., 2010). AF-iPS cells may also have a more embryonic like epigenetic background, which may facilitate pluripotency. The ability to efficiently reprogram AF cells provides not only an abundant cell source for various basic studies and valuable insight into mechanisms of diseases but also a potential role in regenerative medicine. Amniotic fluid cells have therapeutic value in translational therapies that is being discussed in this presentation.

(45) PROSTAGLANDIN E2 PRODUCED IN RESPONSE TO BONE MARROW INJURY IMPROVES SURVIVAL OF PRIMITIVE HEMATOPOIETIC CELLS AND RECOVERY OF PERIPHERAL BLOOD COUNTS

Rebecca L. Porter, Regis J. O'Keefe, Laura M. Calvi
University of Rochester School of Medicine, Rochester, NY

Hematopoietic stem and progenitor cells (HSPCs) are responsible for the continual production of mature blood cells in the organism but are exquisitely sensitive to environmental and iatrogenic injury. Although Prostaglandin E_2 (PGE2) is known to have beneficial effects on hematopoietic cells, it is unclear whether it regulates HSPCs during injury. To investigate this we injured mice with increasing doses of total body radiation (TBI) and measured PGE2 levels in the bone marrow (BM) supernatant by ELISA at specific times post-TBI. PGE2 levels rapidly increased in a dose-dependent manner to sub-lethal TBI ($p=0.0030$). We next tested if augmentation of PGE2 signaling following 6.5Gy TBI could enhance the survival of HSPCs. Mice treated with 16,16-dimethyl-PGE2 (dmPGE2) immediately post-TBI and daily thereafter had significantly more LSK cells in their BM ($p<0.005$) and increased spleen colony-forming units ($p<0.05$) at 24hr post-TBI compared with vehicle-treated mice. Mice lacking the EP2 receptor did not display increased numbers of surviving LSK cells in response to dmPGE2 treatment post-TBI, suggesting that EP2 mediates the effects of dmPGE2 on HSPCs. At 72 hr post-TBI, BM cells from dmPGE2-treated mice had superior repopulating activity in transplantation assays compared with vehicle-treated BM cells, demonstrating that dmPGE2 treatment after injury enhances the survival of functional HSPCs. Further, surviving HSPCs post-TBI and dmPGE2 treatment differentiated normally, resulting in increased numbers of downstream progenitor cells in dmPGE2-treated mice 72 hr post-TBI ($p<0.05$). Finally, mice treated with dmPGE2 for 72 hr post-TBI displayed accelerated recovery of platelet levels and hematocrit in peripheral blood compared with vehicle-treated mice. In summary, PGE2 is a critical microenvironmental regulator of HSPC response to injury and amplification of this physiological signal with PGE2 agonists may represent a novel approach to accelerate recovery of peripheral blood counts in patients with hematopoietic system injury during a vulnerable time when few therapeutic options are currently available.

(46) VON WILLEBRAND FACTOR SECRETED BY ENDOTHELIAL CELLS REGULATES HUMAN MESENCHYMAL STEM CELL ADHESION TO ENDOTHELIAL CELLS

Irina A. Potapova[1], Ira S. Cohen[1], Sergey V. Doronin[1]
[1]*Department Physiology and Biophysics, Stony Brook University, Stony Brook, NY*

Human mesenchymal stem cells are promising candidates for stem cell assisted therapy. Results of animal models and initial clinical trials have shown that direct injection or systemic delivery of mesenchymal stem cells is beneficial for treatment of ischemic tissues such as infarcted myocardium. Effective application of mesenchymal stem cells in clinic, however, depends on understanding of molecular mechanisms of mesenchymal stem cell homing to injured tissues.

A number of studies suggest that monolayer cultured mesenchymal stem cells are homing impaired due to the loss of CXCR4 receptors and, subsequently, unable to activate integrins in response to SDF-1 released by injured tissue. We have shown that the loss CXCR4 is reversible. Culturing of mesenchymal stem cells in a 3D-environment restores the expression of CXCR4 and stimulates biosynthesis and secretion of angiogenic and anti-inflammatory factors.

We also showed that human mesenchymal stem cells sense a decrease of mitochondrial transmembrane potential in endothelial cells and selectively adhere to distressed/apoptotic endothelial cells. Molecular mechanism of mesenchymal stem cell adhesion to distressed/apoptotic endothelial cells includes the activation of p38 MAPK in endothelial cells mediated by von Willebrand factor secreted by endothelial cells in stress conditions.

Overall our findings demonstrate that the homing capacity of mesenchymal stem cells can be modulated *in vitro* without genetic manipulations and that von Willebrand factor is an auto/paracrine regulator of mesenchymal stem cell adhesion to endothelial cells under stress conditions.

(47) INVESTIGATING THE ROLE OF NRF2 IN MSC SURVIVAL AND FUNCTION

Tirumalai Rangasamy[1], Umayal Sivagnanalingam[1], Nandini Seshan[1], Masayuki Yamamoto[2], Steven Kleeberger[3], Darwin J. Prockop[4], Steve N Georas[1]
[1]Division of Pulmonary and Critical Care, Department of Medicine, University of Rochester, Medicine, Rochester, NY; [2]Department of Medical Biochemistry, Tohoku University School of Medicine, Sendai, Japan; [3]National Institute of Environmental Health Sciences, Research Triangle Park, NC; [4]Institute for Regenerative Medicine, Texas A & M Health Science Center, Temple, TX

Rationale: Nrf2 plays an important role in the proliferation and survival of multiple cell types and in protecting against various inflammatory lung diseases. However, very little is known about the role of Nrf2 in the differentiation and function of mesenchymal stem cells (MSC). The main objective of this study was to determine the role of Nrf2 in the differentiation and reparative function of MSC.

Methods and Results: We cultured mouse MSC or MSC overexpressing (lentiviral) MSC with cigarette smoke extract (CSE) alone or with sulforaphane. We have isolated MSCs from the bone marrows (BM) of Nrf2 WT and Nrf2 KO mice and characterized them using flow cytometer and differentiation assays. To investigate the therapeutic potential of MSCs, we instilled Nrf2 WT and Nrf2 KO MSCs into C57 mice 21 and 36 days after porcine pancreatic elastase treatment. CSE significantly induced apoptosis (by MTT assay) and markedly inhibited the proliferation (by BrDU kit), cell adhesion (by fibronectin kit) and colony forming units (CFU) of MSC. CSE (200-1000 µg/ml) significantly induced the secretion of IL-6 and VEGF by MSC. Treatment with sulforaphane or Nrf2 overexpression using lentiviral particles significantly increased the expression of antioxidant genes, inhibited the antiproliferative effect of CSE, and rescued MSC from CSE-induced apoptosis. Deletion of Nrf2 did not significantly affect the CFU, adipocyte and osteocyte differentiation capacity of BM-MSC. Administration of Nrf2 WT MSC significantly attenuated the inflammatory cell population, cytokines (IL-6, TNF•, and MIP2•), and MLI in the lungs of elastase treated mice when compared to its KO counterpart.

Conclusion: The results of the present study clearly revealed the critical role of Nrf2 in multiple physiologic functions of MSC, and in protecting them from harmful effects of CSE. Thus, enhancing Nrf2 expression in MSC in vivo represents a novel therapeutic strategy for emphysema.

(48) A TWO-COLOR MOUSE ES LINE FOR THE STUDY OF PROGENITORS AND PRECURSORS OF FOREBRAIN GABAERGIC INTERNEURONS

Efrain Ribeiro[1,2], Qing Xu[1,3], Asif Maroof[1,2], Lorenz Studer[2], Stewart Anderson[1]
[1]Weill Cornell Medical College, New York, NY; [2]Sloan Kettering Institute, New York, NY and Current address, [3]Mount Sinai School of Medicine, New York, NY

Stem cells have the potential to play a major role in shaping our conception of neurodegenerative diseases, not only in their etiology but also in how they can be treated. Recently, several studies have shown that progenitors of GABAergic interneurons, taken from the medial ganglionic eminence (MGE) of the developing forebrain, can be used therapeutically in rodent models of epilepsy and Parkinson's disease. However, to move this promising approach towards clinical trials, a better source of interneuron progenitors than embryonic tissue will need to be identified.

Mitotic progenitors of the MGE express the transcription factor Nkx2.1, whereas post-mitotic interneuron precursors express the Nkx2.1 target gene, Lhx6. Previously, we have modified a mouse embryonic stem cell line to express the green fluorescent protein (GFP) under control of Lhx6 promoter elements (mLhx6-GFP). GFP+ cells isolated from ventral forebrain differentiations of this line, and transplanted into neonatal neocortex, results in cells displaying characteristics of cortical interneurons in their migratory behavior, neurochemical labeling, and intrinsic physiological properties (Maroof *et al*., 2010).

A challenge with these studies has been relatively poor survival of postmitotic, GFP+ interneuron precursors through the FACS and transplantation procedure. To improve survival, as well as study other aspects of interneuron fate determination, we have generated a new mES line that expresses the fluorophor mCherry under control of Nkx2.1, and Lhx6-GFP (mNkx-Chry:Lhx-GFP). With FACS, the cells can be separated into three subpopulations, each of which represents a distinct stage in cortical interneuronogenesis. After 13 or 16 days of differentiation, we transplanted these three populations into neonatal mice that were sacrificed after four weeks to determine the fate potential of each cell type. While experiments are ongoing, this double-transgenic mNkx-Chry:Lhx-GFP ES line appears to be a valuable new tool in our efforts to use embryonic stem cells for clinically relevant studies of interneuron fate determination, and to bring interneuron cell-based therapy closer to a clinical reality.

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(49) miRS 23B, 27B AND 24-1 REGULATE CELL FATE DECISIONS IN THE LIVER

Leslie E. Rogler, Joseph Martolo, Brian Kosmyna, Charles E. Rogler
Departments of Medicine and Genetics Albert Einstein College of Medicine, Bronx, NY

The miR-23b, 27b and 24-1 cluster located on murine chromosome 13 regulates bipotent differentiation of liver stem/progenitor cells *in vitro* by down regulating TGF-β signaling in hepatoblasts (Rogler *et al*., Hepatology 50:575-584). Mutational analysis confirmed Smad3, 4, and 5 as miR23b cluster miRNA targets. We have used antagomiRs and LNA antagonists directed against miR-23b cluster miRNAs to explore their role in late fetal and early postnatal liver histogenesis and in liver fibrosis. Antagomir injection of fetal or neonatal mice led to an expansion of the CK19 positive cells within the liver and a repression of hepatocyte proliferation in both wild type and alb-TGFβ1 transgenic mice.

In the adult liver high levels of TGFβ induce hepatic stellate cell activation and fibrogenesis. Male TGFβ1 transgenic mice develop kidney and liver fibrosis (Sanderson et al., PNAS USA 92:2572-2576). Male TGFβ1 mice injected with either Antagomirs or LNA antagonists showed a striking reduction in liver fibrosis compared to age matched sham injected TGFβ1 transgenic males. To investigate the mechanism of action we isolated Vitamin A positive hepatic stellate cells. Upon *in vitro* activation these cells up regulated the expression of miR-27b compared to freshly isolated cells. During *in vitro* activation hepatic stellate cells gradually lose Vitamin A fluorescence. Transfection of mir23b cluster inhibitors reactivated Vitamin A fluorescence. These results suggest that miR-27b plays a necessary and sufficient role in genesis of fibrosis and may represent an important target for anti-fibrotic therapy. In summary, we have found that miR-23b cluster microRNAs have cell type specific effects. Their down regulation in developing cholangiocytes permits bile duct formation, up regulation in hepatocytes allows hepatocyte growth in the neonatal liver and up regulation in adult hepatic stellate cells leads to activation and fibrosis.

(50) ROLE OF SONIC HEDGEHOG IN REMYELINATION

Jayshree Samanta, Dave Marzan, Gurkirat Multani, Vitor Sousa, Gordon J. Fishell, James L. Salzer
Smilow Neuroscience Program, New York University Langone Medical Center, New York, NY

The failure of oligodendrocytes (OL) to remyelinate effectively in multiple sclerosis (MS) leads to progressive deterioration of neurological function due to conduction block and neuronal degeneration. A key therapeutic goal is to identify the mechanisms that limit remyelination in order to develop new strategies to enhance remyelination and repair in MS. Recent evidence suggests that progenitor cells in the subventricular zone (SVZ) give rise to OLs. Sonic Hedgehog (Shh) is a secreted morphogen required for the generation of OLs during development and for maintenance of stem cells in the adult SVZ. Binding of Shh to its receptor relieves the transmembrane protein smoothened (smo) from inhibition and results in transcription of Gli proteins, which are downstream

effectors of Shh signaling. To elucidate the role of Shh signaling in remyelination, we examined whether OLs are generated from Shh-responsive stem cells during remyelination. To this end, we used Gli1-CreER; Rgfp mice to genetically fate-map Shh-activated cells. In these mice, Tamoxifen treatment transiently activates CreER resulting in permanent expression of cytoplasmic GFP from the Rosa26 locus in Gli1-expressing cells and all their progeny. Thus, the fate of the progeny of cells activated by Shh at the time of tamoxifen treatment can be determined.

We induced demyelination in the corpus callosum (CC) via cuprizone diet or stereotaxic injection of 2% lysolecithin in these mice and analyzed remyelinating cells in the CC two weeks after returning them to regular diet. We observed GFP labeled cells in the CC of cuprizone-fed but not control mice. Further analysis by immunohistochemistry showed that the labeled cells differentiate into mature OLs and astrocytes. This suggests that Shh-responsive cells are recruited to the site of demyelination where they contribute to remyelination. To determine whether Shh signaling remains active in remyelinating cells, we analyzed Gli1-LacZ mice where LacZ is expressed in cells only during active Gli1 transcription. We did not observe LacZ-labeled cells in the CC even up to a month after demyelination. This suggests that the Gli1-responsive stem cells, which migrate to the site of demyelination, do not actively signal through Shh. To examine whether Shh is required for the recruitment of stem cells during remyelination, we conditionally removed smoothened (smo) from Nestin expressing stem cells by using Nestin-CreER;Smo-floxed mice. Analysis of these mice showed that inhibition of Shh signaling results in an increase in the number of remyelinating OLs in the CC. We also examined Gli1-null mice which lack the expression of Gli1 and observed an increase in remyelinating cells in the CC. Thus, these studies indicate that Shh responsive stem cells contribute to remyelination but active Shh signaling is not required for the process. Further, inhibition of Shh signaling results in enhanced remyelination suggesting this pathway may be a useful therapeutic target for remyelination.

(51) CHARACTERIZATION OF THE RETROVIRAL SILENCING MACHINERY IN EMBRYONIC STEM CELLS

Sharon Schlesinger and Stephen P Goff
Columbia University, Biochemistry and Molecular Biophysics, New York, NY

The pluripotency of Embryonic stem cells (ESC) mandates stringent mechanisms for maintaining genomic integrity, among them the ability to silence potentially genotoxic attacks by endogenous and exogenous retroelements. Although infection of ESC by Moloney murine leukemia virus (MMLV) results in the successful integration of the proviral DNA into the genome, transcription from the viral promoter in the long terminal repeat (LTR) is potently silenced. This restriction is partly due to a large nuclear complex that binds to a conserved DNA element called the primer binding site (PBS). It was recently shown that the PBS of MMLV- PBSpro -binds a DNA binding Zinc-Finger protein called ZNF809, which further recruits TRIM28, a known transcriptional silencer. TRIM28 recruits factors involved in transcriptional silencing and heterochromatin formation.

In this work we try to characterize different aspects of the PBS-directed silencing machinery by comparing the wt MMLV (PBSpro) to a mutated virus (PBSgln). Using these viruses we show a high PBS-mediated restriction rate in ESC. The silencing machinery is active immediately after viral DNA integration and is retained at the same efficiency for weeks. Moreover, even the small sub-population of cells that escape silencing in a defined window of time are again silenced after one day. Interestingly, in cells infected with PBSgln or in ZFP809 KD cells the viral expression in ES and EC cells is still much lower than that seen in somatic cells. This implies the existence of an alternative silencing mechanism of a different and unknown nature. In addition, as many of the proteins in the TRIM28 complex are known modifiers of the epigenetic state, we examine both the state of histones on the DNA, and the state of methylation of the DNA itself. We show an ESC-specific enrichment of the viral LTR and PBS sequences with repressed chromatin markers.

(52) GENETIC BACKGROUND HAS AN IMPACT ON INDUCED PLURIPOTENT STEM (iPS) CELL GENERATION

Lauren V. Schnabel, Christian M. Abratte, John C. Schimenti, Lisa A. Fortier
Induced Pluripotent Stem Cell Core Laboratory, Cornell University, Ithaca, NY

There is growing evidence that the genomic signature of an individual significantly affects not only their susceptibility to disease, but also their response to therapy. The purpose of this study was to determine if the genetic background of an individual influences the ability to generate induced pluripotent (iPS) cells. Our hypotheses were that the efficiency of generating iPS cells and the pluripotent stability of the iPS cells would be strongly influenced by genetic background. In order to test these hypotheses, mouse embryonic fibroblasts (MEFs) were isolated from six strains of mice (NON/LtJ; C57BL/6J; DBA/2J; BALB/cJ; 129S1/SvlmJ; CAST/EiJ) selected based on genetic diversity and on differences in their ability to produce embryonic stem cell lines. The MEFs were reprogrammed via doxycycline inducible lentiviral infection with Oct4, Sox2, Klf4, and c-Myc expression vectors and their potential differences in efficiency to generate iPS cells assessed on primary transformation plates by comparing the number of total colonies, the percentage of colonies positive for alkaline phosphatase (AP) staining, and the percentage of cells positive for SSEA-1 (determined by flow cytometric analysis) from each strain. NON/LtJ and CAST/EiJ strains were statistically more efficient than C57BL/6J and DBA/2J strains in all parameters measured. Colonies from primary transformation plates were mechanically passaged onto 96 well plates and are currently being expanded. Established iPS cell lines from all six mouse strains will be assessed for pluripotency via their ability to form embryoid bodies *in vitro*, their ability to form teratomas in SCID mice, and their ability to generate chimeras. The results of this study suggest that genetic background does have an impact on iPS cell generation and will likely lead to further investigations into the molecular mechanisms regulating iPS cells derivation and to development of new methods for the generation of therapeutically useful iPS cells from all individuals.

(53) NOVEL INTEGRATIVE NUCLEAR SIGNALING MODULE FOR NEURONAL DEVELOPMENT AND REGENERATIVE MEDICINE

Michal K. Stachowiak and Ewa K. Stachowiak
State University of New York, Western New York Stem Cell Culture and Analysis Center, Buffalo, NY

Ontogeny requires the coordinated regulation of multi-gene programs by a plethora of extracellular and intracellular signals. As a result, stem cells transition between states of self-renewal, proliferative expansion and differentiation. Disruption of this regulation may cause oncogenic transformation in which stem cells are “arrested” in the proliferative state. Systems biology postulates computational modules which integrate environmental (extra- and intra-cellular) information to control entry into the cell cycle and promote perpetual self-renewal by the stem cells. We have identified an analogous Feed-Forward-And Gate network module that effects postmitotic development and neuronal differentiation by the stem cells. In the center of this module resides a novel gene-activating mechanism "Integrative Nuclear Fibroblast Growth Factor Receptor-1 (FGFR1) Signaling" (INFS). I will discuss how stochastic molecular collisions among nuclear proteins can lead to coordination of gene activities that enable neuronal development by diverse stem cell populations (embryonic stem cells, adult brain-derived and umbilical cord blood-derived neural stem cells) by different neurogenic signals. A new technology has been developed allowing direct INFS control of neuronal differentiation without the need of the external neurogenic signals. A recombinant form of FGFR1 has been engineered to reside exclusively in the nucleus and switch on neuronal differentiation. Nanotechnology-based *in vivo* transfection of such recombinant genes effectively reconstitutes developmental-like neuronogenesis in the adult brain.

(54) EFFECT OF MECHANICAL LOADING AND NANOFIBER ALIGNMENT ON HUMAN MESENCHYMAL STEM CELL DIFFERENTIATION

Siddarth D. Subramony, Michael S. Tracey, Amanda Su, Nancy M. Lee, Helen H. Lu
Department of Biomedical Engineering, Columbia University, New York, NY

Functional regeneration of soft tissue grafts utilized in ligament and tendon repair remains a significant challenge for current orthopaedic repair and reconstruction. Nanofiber scaffolds based on poly(lactide-*co*-glycolide) (PLGA) have been investigated for tendon and ligament tissue engineering. Additionally, human mesenchymal stem cells (hMSC) have been evaluated as a viable cell source for musculoskeletal tissue engineering applications. The objective of this study is to optimize scaffold design and culturing conditions in order to direct the differentiation of MSC into ligament fibroblasts and enable subsequent ligament regeneration. It is hypothesized that nanofiber alignment and/or mechanical stimulation would induce the differentiation of MSC towards the ligament fibroblast linage, and support collagen deposition and formation of a ligament-like tissue. To test this hypothesis, hMSCs were cultured on aligned and unaligned scaffolds in a custom bioreactor and exposed to 90 minutes of 1% strain at a frequency of 1 Hz twice daily. Cellular response was evaluated after 1, 7, 14 and 28 days of culture. It was observed that cell morphology on the nanofiber scaffolds was a function of fiber alignment, and the optimized dynamic loading induced the differentiation of hMSC into ligament-like cells, in the absence of chemical stimulation. In addition, differential integrin expression and upregulation of ligament-related markers were observed on both unaligned and aligned scaffolds, as well as in response to mechanical loading. It is anticipated the optimized nanofiber-scaffold system would induce MSC-mediated regeneration of ligament-like tissue, and enable functional soft tissue engineering.

(55) THE SKI STEM CELL RESEARCH FACILITY

Mark J. Tomishima, Viviane Tabar, Lorenz Studer
The Center for Stem Cell Biology and the Center for Cell Engineering, Developmental Biology Program, Sloan-Kettering Institute, New York, NY

The SKI Stem Cell Research Facility provides a number of services to the New York State stem cell community. Our primary missions are to provide: 1) *training*; 2) *genetic modification* of human pluripotent stem cells (hPSCs); 3) the *production* of human induced pluripotent stem cells (hiPSCs); and 4) *directing differentiation* of pluripotent stem cells. Here, we will update the NYS stem cell community on our progress since last year's meeting. Specifically, we will provide an overview of training opportunities provided by the lab. Advances and remaining challenges regarding the use of hPSC transgenesis will be addressed. Our lab now routinely produces hiPSCs without any genomic modification; we will describe our new workflow for producing such hiPSCs. Finally, we will provide an update on construction of the high-throughput module and our initial assays that make use of high-throughput screening approaches.

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(56) AN ADAPTATION OF SPEMANN'S ORGANIZER PRINCIPLE TO ADULT GERMINAL NICHES: PHYSIOLOGICAL CELL STRESS INDUCES VARIABLE MORPHOGEN EXPRESSION AND AXONAL CONNECTIVITY BRIDGES PHYSICAL GAP BETWEEN SITE OF MORPHOGEN PRODUCTION AND ACTION

Miguel Verbitsky[1], Maria J. Perez[2], Luis E. Gonzalez-Reyes[3], Andreas H. Kottmann[1]
[1]Columbia University, Psychiatry, New York, NY; [2]New York University, Skirball Institute, New York, NY; [3]Case Western University, Biomedical Engineering, Cleveland, OH

The ultimate promise of stem cell research is to devise strategies to force adult germinal niches to produce cell types that have been lost due to aging or disease.

Using gene expression tracer - and conditional gene ablation - strategies we found that variable expression of the morphogen Sonic Hedgehog (Shh) by mesencephalic dopamine (DA) neurons and

by somatic spinal motor neurons (MNs) of the adult CNS is a signal for structural and functional tissue deterioration and a regulator of cell fate in the germinal niche of the forebrain (SVZ) and the skeletal muscle, respectively.

Mesencephalic DA neurons elaborate transient topographically organized collateral axonal projections to migrating A and C cells of the SVZ. Shh expression by DA neurons is up-regulated in graded manner correlated with the severity of transient dysfunction pharmacologically induced in cholinergic neurons that are synaptically connected with DA neurons. The conditional ablation of Shh from 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 precursor populations translate into altered olfactory bulb cyto-architecture, increased numbers of dopaminergic, periglomerular neurons and ER81+ granule cells, and olfactory dysfunction.

MNs project to skeletal muscle forming tight neuro-muscular junctions (NMJs). Muscle stem cells are found enriched in close proximity to NMJs. Physical or chemical muscle damage up-regulates Shh expression specifically in those MNs that project to the injured muscle. MN restricted ablation of Shh results in a switch of cell fate determination from the production of slow twitch to fast twitch muscle fibers causing altered fiber composition of muscle.

Our data suggests that the manipulation of those signals that signify tissue deterioration and alter Shh expression in restricted neuronal populations could potentially result in the endogenous production of particular cell types desired for tissue replenishment.

(57) THE MAINTENANCE AND SELF-RENEWAL OF LEUKEMIA STEM CELLS BY AML1-ETO IS DEPENDENT ON SITE-SPECIFIC LYSINE ACETYLATION

Lan Wang[1], Alexander Gural[1], Xiao-Jian Sun[2], Xinyang Zhao[1], Fabiana Perna[1], Gang Huang[1], Megan A. Hatlen[1], Ly Vu[1], Fan Liu[1], Haiming Xu[1], Takashi Asai[1], Hao Xu[1], Tony Deblasio[1], Silvia Menendez[1], Francesca Voza[1], Yanwen Jiang[3], Philip A. Cole[5], Jinsong Zhang[4], Ari Melnick[3], Robert G. Roeder[2], Stephen D. Nimer[1*`]

[1]Molecular Pharmacology and Chemistry Program, Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center, New York, NY; [2]Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, New York, NY; [3]Division of Hematology/Oncology, Department of Medicine, Weill Cornell Medical College, New York, NY; [4]Department of Cancer and Cell Biology, University of Cincinnati College of Medicine, Cincinnati, OH; [5]Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD

The maintenance and self-renewal of hematopoietic stem cells (HSCs) is abnormally promoted by oncogenic proteins, such as AML1-ETO, which can transform HSCs to pre-leukemia stem cells. AML1-ETO, generated by the balanced chromosomal 8; 21 translocation, is found in acute myelogenous leukemia (AML), and has aberrant transcriptional activating or repressing properties.

While therapeutic targeting of leukemia stem cells remains elusive, the post-translational modifications of proteins that control the maintenance and self-renewal of leukemia stem cells could present such a targetable event. The lysine acetyltransferase, p300, functions as a transcriptional co-activator, acetylating histones and non-histone proteins, which control the proliferation of HSCs under some conditions. In this study, we found that lysine acetylation of AML1-ETO by p300 is indispensable for its self-renewal promoting effects in human hematopoietic stem and progenitor cells isolated from umbilical cord blood. Acetylated AML1-ETO is found in primary t(8;21)+ AML patient samples, and in leukemia cells isolated from our mouse AML model. Deletion of the region responsible for its interaction with p300 (which encompasses the nervy homology region 1 (NHR1) domain) results in the loss of acetylation. Deletion of the same region from AML1-ETO9a, a leukemogenic, alternatively spliced form of AML1-ETO, also results in loss of its leukemia promoting activity in a mouse model of AML. The inhibition of p300 using the chemical/peptide inhibitors or shRNAs, not only abrogates the acetylation of AML1-ETO, but also impairs its ability to activate its

target genes. These inhibitors promote apoptosis of the AML1-ETO9a driven leukemia cells and prolong the survival of mice that are secondary recipients of such leukemias. Thus, we have identified a new mechanism promoting leukemia, and have identified lysine acetyltransferases as a potential therapeutic target in AML.

(58) TRANSPLANTATION OF REPROGRAMMED EMBRYONIC STEM CELLS IMPROVES VISUAL FUNCTION IN A MOUSE MODEL FOR RETINITIS PIGMENTOSA

Katherine Wert[1,2], Nan-Kai Wang[1], Chyuan-Sheng Lin[1], Stephen H. Tsang[1]
[1]Bernard & Shirlee Brown Glaucoma Laboratory, Department of Pathology and Cell Biology, [2]Institute of Human Nutrition, College of Physicians & Surgeons, Columbia University, New York, NY

Introduction: Specialized retinal cells called the retinal pigment epithelium (RPE) maintain vision. Death of these cells leads to vision loss, inability to perform daily tasks, and even depression in many individuals with retinitis pigmentosa (RP) and other macular degenerations. Nearly 20% of Americans 65-75 years of age are expected to experience RPE loss due to macular degenerations, and its incidence is expected to double by 2020. A major obstacle to restoring vision by cell therapy is the availability of cadaver RPE donors for transplantation. Our central hypothesis is that RPE cells derived from embryonic stem (ES) cells can be used to successfully replace diseased counterparts.

Methods: Mouse C57BL/6J-Tyr$^{c\text{-}2j}$/J (C2J) ES cells were labeled with a yellow fluorescent protein (YFP) and made into RPE cells of the eye in culture. After becoming RPE-like cells, the cells were transplanted into the eyes of the RP mouse model. Imaging of live mice after transplantation determined that the cells survived the transplantation process. Electroretinograms were performed on the mice to evaluate their visual function.

Results: The ES cells that have become RPE-like cells expressed the correct markers for being RPE cells. After transplantation, the stem cells were imaged in the eyes of the mice for as long as 7 months. The mice that underwent the transplantation procedure showed a significant gain in vision over the 7-month period, while those that were injected with saline and other control groups did not show any restoration of visual function.

Conclusions: For the first time, ES cells were successfully used to replace diseased retinal cells and restore nervous system function in a mouse model of retinitis pigmentosa – a strategy to treat millions of humans with age-related macular degeneration and other forms of retinal disease.

(59) FUNCTIONAL CHARACTERIZATION OF MOTOR NEURONS GENERATED FROM PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS

Damian J. Williams[3]*, Derek H. Oakley[1,2]*, Gist F. Croft[1,2], Mackenzie W. Amoroso[1,2], Christopher E. Henderson[1,2], Hynek Wichterle[1,2] and Amy B. MacDermott[3]
[1]Project A.L.S./Jenifer Estess Laboratory for Stem Cell Research, [2]Departments of Pathology, Neurology and Neuroscience, Center for Motor Neuron Biology and Disease (MNC), and Columbia Stem Cell Initiative (CSCI), [3]Departments of Physiology and Cellular Biophysics, and Neuroscience, Columbia University, New York, NY

Motor neurons generated from patient-derived induced pluripotent stem cells have the potential to be excellent cellular models for Amyotrophic Lateral Sclerosis (ALS), but a functional characterization of these cells is necessary to ensure they have a true motor neuron phenotype. Live cell techniques such as Ca^{2+} imaging and electrophysiology are particularly well-suited for this purpose as they provide a method to directly monitor neuronal function. Preliminary Ca^{2+} imaging data show that spontaneous Ca^{2+} transients occur in motor neurons generated from multiple iPS cell lines and suggest that these transients are dependent on voltage-gated Na^{+} channels. Ca^{2+} transients can be induced by kainate and high K^{+}, which indicates that functional AMPA/kainate receptors and voltage-gated Ca^{2+} channels are present in these cells. These results suggest that induced pluripotent stem cell-derived motor neurons (iPS-MNs) have functional characteristics that

are consistent with a motor neuron phenotype. A functional characterization of these cells will provide the basis for a comparison between iPS-MNs generated from ALS patients and healthy controls. This comparison has the potential to reveal previously unidentified mechanisms involved in ALS pathophysiology.

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**equal contribution*

(60) PANCREATOGENIC DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS IN DISH AND MICROCARRIER CULTURES

Jincheng Wu[1], Yongjia Fan[1], Lye T. Lock[1], Suzanne G. Laychock[2], Emmanuel (Manolis) S. Tzanakakis[1,3,4]
[1]Department of Chemical & Biological Engineering, [2]Department of Pharmacology and Toxicology, State University of New York, Buffalo, NY; [3]New York State Center of Excellence in Bioinformatics and Life Sciences, Buffalo, NY; [4]Western New York Stem Cell Culture and Analysis Center, State University of New York, Buffalo, NY

Human embryonic stem cells (hESCs) and induced pluripotent cells (collectively termed human pluripotent stem cells or hPSCs) are a promising source of therapeutics for various maladies, which were considered incurable before. One such disease is diabetes mellitus, which is linked to reduced numbers of insulin-producing pancreatic cells. Essential for the realization of hPSC-based diabetes therapies are efficient differentiation strategies and the engineering of scalable bioprocesses for the generation of large quantities of insulin-producing cells.

Stem cells in static cultures were guided through definitive endoderm (DE), primitive gut tube (PGT) and pancreatic foregut (PFG) toward islet cell progeny. A protocol was developed involving physiological factors in serum-free media. The combination of factors, their concentrations and length of incubation were established by applying design-of-experiment methods. When probed by quantitative PCR (qPCR), immunostaining and flow cytometry, cells expressed markers such as SOX17 and FOXA2 (DE), HNF1B and HNF4A (PGT), and PDX1 and HNF6 (PFG) sequentially. Further differentiation toward pancreatic islet cells led to 8% of the cells exhibiting insulin transcription and secretion in response to known secretagogues.

We previously reported the expansion of undifferentiated hPSCs in a stirred-suspension microcarrier bioreactor and their directed differentiation to DE cells. Human PSC-derived DE cells in microcarrier cultures were subsequently coaxed to PGT and PFG cells with the same factors used in dish differentiations. Cells expressed stage-specific markers and the relative efficiencies of differentiation to DE, PGT and PFG stages were above 70%. These cells also gave rise to populations expressing pancreatic endocrine cell genes. Almost 6.5% of the cells were insulin-transcribing.

Currently our efforts focus on improving the yield of islet (especially insulin-producing) cells from hPSCs in bioreactor cultures and eliminating animal-derived components from our system. Our findings warrant further development of scalable bioprocesses for producing therapeutically useful stem cell derivatives in clinically relevant quantities.

(61) IDENTIFICATION AND CHARACTERIZATION OF ENDOTHELIAL NICHE FACTORS THAT STIMULATE SELF-RENEWAL AND NEUROGENESIS OF NEURAL STEM CELLS

Tao Zhang, Jianzao Zhang, Beverly Young, Sheila Le, Sally Temple, Jun Yan
Neural Stem Cell Institute, Rensselaer, NY

Neural stem cells (NSCs) can be derived from various developmental stages and different regions of the central nervous system (CNS). NSCs are influenced by their micro-environment or ‘niche’, which includes the blood vasculature. Niche factors regulate NSC survival, proliferation, and fate

choice. In prior studies (Shen *et al*., 2004), we demonstrated that blood vessel endothelial cells secrete factors that stimulate the self-renewal and neurogenesis of mouse embryonic and adult forebrain NSCs. The goal of this project is to identify and characterize the endothelial cell secreted factors that stimulate NSC self-renewal and neurogenesis.

The main strategy used was to separate protein components in endothelial cell conditioned-medium (CM) with high-performance liquid chromatography (HPLC) columns, then bioassay each fraction using mouse embryonic NSC cultures to monitor the activities. For the most active fractions, protein components were further identified using mass spectrometry/protein ID analysis.

After sequential separation of endothelial conditioned-medium on two HPLC columns, an anionic exchanger (Uno-Q) and a cationic exchanger (Uno-S), we have identified several candidate proteins for the NSC-promoting activity. We have been concentrating on testing two of the most likely candidates coded EF1 and EF2. These factors stimulate division of NSCs, and tests to determine whether they promote neurogenesis are ongoing. *In situ* hybridization images indicate these are expressed in forebrain germinal zones. Recently, we have produced lentiviral vectors overexpressing the niche factors for future studies of their ability to enhance NSC self-renewal and neurogenesis *in vitro* and *in vivo*.

Our findings should further the understanding of interactions between NSCs and endothelial cells. The identified active factors may be used to improve NSC cultures and may also provide potential therapeutic applications.

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(62) REGULATION AND FUNCTION OF A NOVEL HUMAN EMBRYONIC STEM CELL PLURIPOTENCY FACTOR

Xin Zhang[1], Safak Yalcin[1], Dung-Fang Lee[1], Tsung-Yin J. Yeh[2], Seung-Min Lee[1], Su Jie[1], Sathish Kumar Mungamuri[1], Pauline Rimmelé[1], Marion Kennedy[3], Rani Sellers[4], Markus Landthaler[5], Thomas Tuschl[5], Nai-Wen Chi[2], Ihor Lemischka[1,6,7], Gordon Keller[3], Saghi Ghaffari[1,6-8]
[1]Department of Gene and Cell Medicine, Mount Sinai School of Medicine, New York, NY; [2]Department of Medicine, University of California, San Diego, La Jolla, CA; [3]McEwen Center for Regenerative Medicine, University Health Network, Toronto, Ontario, Canada; [4]Department of Pathology, Albert Einstein College of Medicine, New York, NY; [5]Howard Hughes Medical Institute, Laboratory for RNA Molecular Biology, The Rockefeller University, New York, NY; [6]Black Family Stem Cell Institute; [7]Department of Regenerative & Developmental Biology; [8]Department of Medicine Division of Hematology, Oncology, Mount Sinai School of Medicine, New York, NY

Understanding mechanisms of regulation of stem cell function has important implications for stem cell therapy and regenerative medicine. In the past few years the longevity transcription factors Forkhead Box O (FoxO) have emerged as critical regulators of tissue specific stem cells. The FoxO family comprises four members in mammals (FoxO1, FoxO3, FoxO4 and FoxO6) that are evolutionarily conserved regulators of stress response and function as tumor suppressors. Here we show that FoxO1 is the most highly expressed FoxO in undifferentiated self-renewing human ES (hES) cells. In addition, using loss and gain of function approaches in two hES cell lines we demonstrate that FoxO1 is a critical regulator of hES cell pluripotency. Consistent with this, knock down of FoxO1 leads to the erroneous induction of mesoderm and endoderm differentiation under undifferentiation self-renewal conditions of hES cells. We further show that FoxO1 regulation of pluripotency is conserved in mouse ES cells. We demonstrate that this function is not through regulation of apoptosis, cell cycle, or oxidative stress in hES cells but is mediated by FoxO1 (and not other FoxO) binding to and activation of OCT4 and SOX2 promoters. FoxO are phosphorylated by AKT serine threonine protein kinase, a critical regulator of pluripotency that mediates FoxO's nuclear exclusion and suppression in response to growth factors or cytokines. We demonstrate that in pluripotent hES cells, FoxO1 is nuclear and phosphoAKT is not the predominant signal controlling FoxO1 subcellular localization and transcriptional activity. Finally, our results suggest that distinct FoxO isoforms are subject to distinct regulation in hES cells. Altogether these findings support a

non-redundant function for FoxO1 in the regulation of hES cell pluripotency and have critical implications for stem cells, development and reprogramming. Further investigation into regulation of FoxO's function in stem cells will have important consequences for targeting these proteins in therapy.

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LIST OF PARTICIPANTS

Abeliovich, Asa
Columbia University
aa900@columbia.edu

Abratte, Christian
Cornell University
ca258@cornell.edu

Aldosary, Tagreed
National Guard Health Affairs
t_dosary@yahoo.com

Alexandrova, Evguenia
Stony Brook University Medical Center
kim.distefano@stonybrook.edu

Almonte, Ronnie
The New School for Liberal Arts
almor184@newschool.edu

Amoroso, Mackenzie
Columbia University-Project ALS
mw2578@columbia.edu

Anders, David
NYSTEM
anders@wadsworth.org

Anderson, Stewart
Weill Cornell Medical College
saa2007@med.cornell.edu

Antzelevitch, Charles
Masonic Research Medical Laboratory
ca@mmrl.edu

Arango, Victoria
Columbia University-NYSPI
va19@columbia.edu

Armantrout, Jann
Empire State Stem Cell Board
armantrout@dor.org

Ascienzo, Terry
Wadsworth Center
tka03@health.state.ny.us

Auvergne, Romane
University of Rochester
romane_auvergne@urmc.rochester.edu

Bach, Erika
New York University School of Medicine
erika.bach@nyu.edu

Bahou, Wadie
Stony Brook University Medical Center
wbahou@notes.cc.sunysb.edu

Barajas-Martinez, Hector
Masonic Medical Research Laboratory
barajash@mmrl.edu

Baron, Margaret
Mount Sinai School of Medicine
margaret.baron@mssm.edu

Battista, Daniela
Memorial Sloan-Kettering Cancer Center
battistd@mskcc.org

Benraiss, Abdellatif
University of Rochester
abdellatif_benraiss@urmc.rochester.edu

Bishop, David
Mount Sinai School of Medicine
david.bishop@mssm.edu

Bieker, James
Mount Sinai School of Medicine
james.bieker@mssm.edu

Boldrini, Maura
Columbia University-NYSPI
mb928@columbia.edu

Brautigam, Bonnie
Wadsworth Center
bjb08@health.state.ny.us

Brivanlou, Ali
The Rockefeller University
cooperb@rockefeller.edu

Brown, Anthony
Weill Cornell Medical College
amcbrown@med.cornell.edu

Burke, Kelly
New York State Psychiatric Institute
kb2475@columbia.edu

Bynum, David
State University of New York at Stony Brook
cesame@stonybrook.edu

Cai, Chenleng
Mount Sinai School of Medicine
chenleng.cai@mssm.edu

Calder, Elizabeth
Weill Cornell Graduate School of Medical Sciences
elc2015@med.cornell.edu

Campbell, Christine
State University of New York at Buffalo
cc59@buffalo.edu

Castren, Maija
University of Helsinki
maija.castren@helsinki.fi

Chamany, Katayoun
The New School for Liberal Arts
chamanyk@newschool.edu

Chang, Chan-Jung
Albert Einstein College of Medicine
chan-jung.chang@phd.einstein.yu.edu

Chang, Stephen
New York Stem Cell Foundation
schang@nyscf.org

Chatterjee, Sujash
New York University School of Medicine
sujash.chatterjee@nyumc.org

Chaurasia, Pratima
Mount Sinai School of Medicine
pratima.chaurasia@mssm.edu

Chen, Julia
Columbia University
jcc2219@columbia.edu

Chen, Shaw-ree
University of Rochester
shawree_chen@urmc.rochester.edu

Chou, Kathy
NYSTEM
yxc10@health.state.ny.us

Christiano, Angela
Columbia University Medical Center
amc65@columbia.edu

Clavero, Sonia
Mount Sinai School of Medicine
sonia.clavero@mssm.edu

Cohn, Janet
NYSTEM
jsc02@health.state.ny.us

Colognato, Holly
State University of New York at Stony Brook
colognato@pharm.stonybrook.edu

Croft, Gist
Columbia University-Project ALS
gist.croft@gmail.com

Curchoe, Carol
New York University School of Medicine
carol.curchoe@nyumc.org

D'Souza, Sunita
Mount Sinai School of Medicine
dsouzs01@mssm.edu

Dailey, Lisa
New York University School of Medicine
lisa.dailey@nyumc.org

Davis, Brian
GE Global Research
davis@research.ge.com

Dees, Richard
University of Rochester
richard.dees@rochester.edu

Desclaux, Mathieu
Columbia University-Project ALS
mjd2170@columbia.edu

Dewhurst, Stephen
University of Rochester Medical Center
stephen_dewhurst@me.com

Di Diego, Jose
Masonic Medical Research Laboratory
didiego@mmrl.edu

Di Micco, Raffaella
New York University School of Medicine
raffaella.dimicco@nyumc.org

Doege, Claudia
Columbia University
cad2114@columbia.edu

Doetsch, Fiona
Columbia University
fkd2101@columbia.edu

Doss, Michael Xavier
Masonic Medical Research Laboratory
xavier@mmrl.edu

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

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

Essayagh, Sanah
Mount Sinai School of Medicine
essayagh@gmail.com

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

Feaster, Moses M.
Rockefeller University
mfeaster@rockefeller.edu

Fischbach, Gerald
Simons Foundation
asharkey@simonsfoundation.org

Fortier, Lisa
Cornell University
laf4@cornell.edu

Freytes, Donald
Columbia University
df2328@columbia.edu

Fuchs, Elaine
The Rockefeller University
fuchs@rockefeller.edu

Gadhia, Sanket
St. John's University
sanket.gadhia08@stjohns.edu

Ganapathi, Mythily
Wadsworth Center
mythilyg@yahoo.com

Gardner, Connie
Wadsworth Center
csg01@health.state.ny.us

Gelman, Julia
New York University School of Medicine
Julia.gelman@med.nyu.edu

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

Ghazizadeh, Soosan
State University of New York at Stony Brook
sghazizadeh@notes.cc.sunysb.edu

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

Goldman, Steven
University of Rochester
steven_goldman@urmc.rochester.edu

Griffin, Nona
The New School for Liberal Arts
nrgriff@comcast.net

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

Gross, Steven
Weill Cornell Medical College
ssgross@med.cornell.edu

Gruber Mica, Yvonne
Memorial Sloan-Kettering Cancer Center
grubery@mskcc.org

Guijarro, Maria
New York University Medical Center
maria.guijarro@med.nyu.edu

He, Wu
New York Blood Center
whe@nybloodcenter.org

Heicklen, Alice
Columbia University
aah2289@columbia.edu

Henderson, Christopher
Columbia University Medical Center
ch2331@columbia.edu

Hernando, Eva
New York University School of Medicine
eva.hernando-monge@nyumc.org

Hill, Jeremy
Columbia University
hilljer@pi.cpmc.columbia.edu

Hoey, David
Columbia University
dh2460@columbia.edu

Hoffman, Ronald
Mount Sinai School of Medicine
Ronald.hoffman@mssm.edu

Huang, Li Ting
State University of New York at Stony Brook
liting.huang@sunysb.edu

Jordan, Craig
University of Rochester
craig_jordan@urmc.rochester.edu

Kalderon, Daniel
Columbia University
ddk1@columbia.edu

Kang, Min Jung
Memorial-Sloan Kettering Cancer Center
kangm@mskcc.org

Kaufman, Judy
Monroe Community College
jkaufman@monroecc.edu

Kerner, Bethany
Columbia University-Project ALS
blj2109@columbia.edu

Kieburtz, Karl
University of Rochester Medical Center
karl.kieburtz@ctcc.rochester.edu

Kohn, Matthew
NYSTEM
mjk09@health.state.ny.us

Kolbet, Jason
NeoStem
jkolbert@neostem.com

Kottmann, Andreas
Columbia University
ak139@columbia.edu

Kumar, Mukesh
Albert Einstein College of Medicine
mkumar@aecom.yu.edu

Lamas, Nuno
Columbia University-Project ALS
njl2117@columbia.edu

Lehmann, Ruth
New York University Medical Center
Ruth.Lehmann@med.nyu.edu

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

Li, Xiajun
Mount Sinai School of Medicine
xiajun.li@mssm.edu

Lin, Carol
Columbia University
CSL27@columbia.edu

Loghin, Evelina
GE Global Research
loghine@research.ge.com

Loomis, Mario
Member, Empire State Stem Cell Board
mario@drloomis.com

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

Lu, Helen
Columbia University
hl2052@columbia.edu

Major, Tamara
Memorial Sloan-Kettering Cancer Center
majort@mskcc.org

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

Markowitz, Dina
University of Rochester
dina_markowitz@urmc.rochester.edu

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

Marzan, Dave
New York University Sackler Institute
dave.marzan@gmail.com

Matta, Khushi
Roswell Park Cancer Institute
khushi.matta@roswellpark.org

McClenahan, Freyja
State University of New York at Stony Brook
fmcclena@ic.sunysb.edu

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

Medei, Emiliano
Masonic Medical Research Laboratory
medeie@mmrl.edu

Mickunas, Edmund
Advanced Cell Technology
emickunas@advancedcell.com

Monastersky, Glenn
Rensselaer Polytechnic Institute
monasg@rpi.edu

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

Natesan, Sridaran
Sanofi-aventis
sridaran.natesan@sanofi-aventis.com

Nemajerova, Alice
Stony Brook University Medical Center
kim.distefano@stonybrook.edu

Niebuhr, R. Gustav
Syracuse University
rgniebuh@syr.edu

Noble, Mark
University of Rochester
mark_noble@urmc.rochester.edu

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

Oakley, Derek
Columbia University-Project ALS
dho2101@columbia.edu

Osada, Masako
The City College of New York
mosada@ccny.cuny.edu

Palis, James
University of Rochester
james_palis@urmc.rochester.edu

Paluh, Janet
State University of New York at Albany
jpaluh@uamail.albany.edu

Paul, Jeremy
Skirball Institute, New York University School of Medicine
jeremy.paul@med.nyu.edu

Pedersen, Roger
University of Cambridge
roger@stemcells.acm.ac.uk

Peek, Ken
Wadsworth Center
kep03@health.state.ny.us

Petros, Timothy
Weill Cornell Medical College
tip2003@med.cornell.edu

Pettinger, Michael
The New School for Liberal Arts
pettingm@newschool.edu

Porter, Rebecca
University of Rochester
rebecca_porter@urmc.rochester.edu

Rafferty, Lani
Wadsworth Center
ldr01@health.state.ny.us

Rafii, Shahin
Weill Cornell Medical College
srafii@med.cornell.edu

Rangasamy,Tirumalai
University of Rochester
tirumalair@hotmail.com

Recio, Janine
New York University of School of Medicine
Janine.reci@gmial.com

Reilly, Joyce
Wadsworth Center
jej02@health.state.ny.us

Reizis, Boris
Columbia University
bvr2101@columbia.edu

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

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

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

Rooke, Heather
International Society for Stem Cell Research
hrooke@isscr.org

Roxland, Beth
Department of Health
ber01@health.state.ny.us

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

Rucker, Lakia
NYSTEM
lsr06@health.state.ny.us

Russell, John
Syracuse University
jrussell@syr.edu

Ryther, Mary
Wadsworth Center
mlr06@health.state.ny.us

Salzer, James
New York University School of Medicine
James.Salzer@NYUMC.org

Samanta, Jayshree
New York University
jayshree.samanta@nyumc.org

Sandler, Vladislav
Albert Einstein College of Medicine
Vladislav.sandler@gmail.com

Schimenti, John
Cornell University
jcs92@cornell.edu

Schnabel, Lauren
Cornell University
lvs3@cornell.edu

Schneider, Bruce
FDA CBER
Bruce.Schneider@fda.hhs.gov

Schwartz-Orbach, Lianna
The New School for Liberal Arts
schw1821@newschool.edu

Shang, Linshan
New York Stem Cell Foundation
lshang@nyscf.org

Sicouri, Serge
Masonic Medical Research Laboratory
sicouris@mmrl.edu

Sim, Fraser
State University of New York at Buffalo
fjsim@buffalo.edu

Simon, Marcia
State University of New York at Stony Brook
marcia.simon@stonybrook.edu

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

Spiegel, Allen M.
Albert Einstein College of Medicine
allen.spiegel@einstein.yu.edu

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

Southard, Laurel
Cornell University
les3@cornell.edu

Stachowiak, Ewa
State University of New York at Buffalo
eks1@buffalo.edu

Stachowiak, Michal
State University of New York at Buffalo
mks4@buffalo.edu

Stansfield, Hope
Rensselaer Polytechnic Institute
stansh@rpi.edu

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

Studer, Lorenz
Memorial Sloan-Kettering Cancer Center
studerl@mskcc.org

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

Subramony, Siddarth
Columbia University
sds2156@columbia.edu

Sun, Jun
University of Rochester
jun_sun@urmc.rochester.edu

Suzuki, Gen
State University of New York at Buffalo
gsuzuki@buffalo.edu

Tabar, Viviane
Memorial Sloan-Kettering Cancer Center
tabarv@mskcc.org

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

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

Terrenoire, Cecile
Columbia University
ct2068@columbia.edu

Thams, Sebastian
Columbia University-Project ALS
st2650@columbia.edu

Thompson, Deanna
Rensselaer Polytechnic Institute
thompd4@rpi.edu

Tomishima, Mark
Memorial Sloan-Kettering Cancer Center
tomishim@mskcc.org

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

Tumbar, Tudorita
Cornell University
tt252@cornell.edu

Tzanakakis, Emmanuel
State University of New York at Buffalo
emtzan@buffalo.edu

Upadhyay, Ambuj
Mount Sinai School of Medicine
ambuj.upadhyay@mssm.edu

Vacaru, Andrei
Mount Sinai School of Medicine
andrei.vacaru@mssm.edu

Van Buskirk, Robert
Binghamton University
rvanbus@binghamton.edu

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

Walsh, Martin
Mount Sinai School of Medicine
martin.walsh@mssm.edu

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

Wang, Lan
Memorial Sloan-Kettering Cancer Center
wangl5@mskcc.org

Wang, Su
University of Rochester Medical Center
su_wang@urmc.rochester.edu

Wang, Zhu
Columbia University
zw2103@columbia.edu

Wargaski, Julia
Parsons the New School for Design
wargaskj@newschool.edu

Wert, Katherine
Columbia University
kjw2126@columbia.edu

Wichterle, Hynek
Columbia University
hw350@columbia.edu

Williams, Damian
Columbia University
dw2471@columia.edu

Wolosin, J. Mario
Mount Sinai School of Medicine
jmario.wolosin@mssm.edu

Xenopoulos, Panagiotis
Memorial Sloan-Kettering Cancer Center
pxenopou@temple.edu

Yan, Jun
Neural Stem Cell Institute
junyan@nynsci.org

Yee, Frances
The Rockefeller University
fyee@rockefeller.edu

Yu, Manshan
New York University School of Medicine
manshan.yu@nyumc.org

Yuan, Jenny
Columbia University
xy2126@columbia.edu

Yun, Chi
New York University RNAi Core
chi.yun@nyumc.org

Zhang, Xinping
University of Rochester Medical Center
Xinping_Zhang@URMC.rochester.edu

Zhant, Hailan
Mount Sinai School of Medicine
hailan.zhant@mssm.edu

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

EMPIRE STATE STEM CELL BOARD

Nirav R. Shah, M.D., M.P.H., Chair†*
Commissioner, *New York State Department of Health*

David C. Hohn, M.D., Vice Chair†*

Jann K. Armantrout †

Fr. Thomas Vincent Berg, LC, Ph.D.†

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PROGRAM-AT-A-GLANCE

MAY 24 – Concourse Lobby and Breakout Rooms, Proshansky Auditorium

8:00 AM	Registration Opens and Continental Breakfast
8:30 AM	Education Initiatives Workshop
9:00 AM	Shared Facilities Workshop
10:30 AM	Break
11:00 AM	Plenary I – Stem Cells in Cancer and Other Diseases
12:30 PM	Lunch
1:30 PM	Opening Remarks
1:45 PM	Keynote: Stem cells of the skin: their biology and clinical promise
2:35 PM	Plenary II – Stem Cell Biology
3:15 PM	Break
3:45 PM	Plenary III – Tissue Engineering and Regenerative Medicine
5:15 PM	Reception/Poster Session

MAY 25 – Concourse Lobby, Proshansky Auditorium

8:00 AM	Registration Opens and Continental Breakfast
9:00 AM	Translation Workshop/Panel Discussion
10:30 AM	Break
12:00 PM	Lunch
1:00 PM	Plenary IV – Disease Models and Therapeutic Approaches
2:30 PM	Break
3:00 PM	Plenary V – Pluripotency, Reprogramming and Differentiation
4:30 PM	Closing Remarks