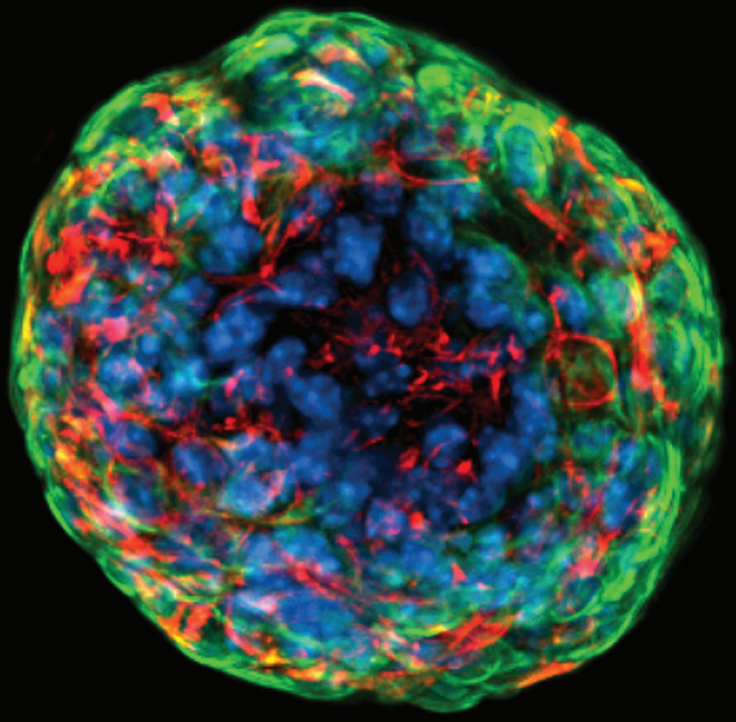
NYSTEM *2013*



Collaboration & Renewal

May 22 & 23, 2013
CUNY Graduate Center
365 Fifth Avenue, New York, NY

Wadsworth Center
NEW YORK STATE DEPARTMENT OF HEALTH

NYSTEM NEW YORK STATE STEM CELL SCIENCE

NYSTEM 2013: Collaboration & Renewal

PROGRAM COMMITTEE

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Special thanks to:

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Tracy Godfrey for program composition

Prostate Sphere cover photo courtesy of:
Maher Hanoun, Sandra Pinho and **Paul S. Frenette**,
Albert Einstein College of Medicine, Bronx, NY.

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GENERAL INFORMATION

Sessions

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

Meals

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

Posters

The poster sessions are in the breakout rooms across the Concourse Lobby from the auditorium. Posters should be put up by 1:00pm on May 22, prior to the start of the Opening Remarks, and should remain up through the second day.

Please refer to the Poster Abstracts section of the program book for poster assignments. If your poster is assigned an odd number, please be prepared to present it at Poster Session I, 4:00 - 6:00pm on May 22. If your poster is assigned an even number, please be prepared to present it at Poster Session II, 11:00am - Noon on May 23. Posters must be removed by 5:00pm Thursday.

For more information about NYSTEM, please visit: www.stemcell.ny.gov

PROGRAM SCHEDULE

WEDNESDAY, MAY 22, 2013

Noon	Registration and Poster Set-up
1:00 - 1:10	**OPENING REMARKS**
PLENARY I: Single Cell Analyses	
1:10 - 2:10	Chair**: Fiona Doetsch**, *Columbia University Medical Center*
1:10 - 1:30	**Jan Vijg**, *Albert Einstein College of Medicine* SINGLE CELL GENOMICS
1:30 - 1:50	**David Keefe**, *New York University School of Medicine* A NOVEL METHOD TO STUDY TELOMERE LENGTH IN INDIVIDUAL STEM CELLS
1:50 - 2:10	**El-ad David Amir**, *Columbia University* TRAJECTORY DETECTION ORDERS HALLMARKS OF EARLY HUMAN B CELL DEVELOPMENT
2:10 - 2:40	**BREAK and POSTERS**
PLENARY II: Stem Cells in Cancer	
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2:40 - 3:00	**Iannis Aifantis**, *New York University School of Medicine* DISTINCT THRESHOLDS OF MYC PROTEIN HALF-LIFE CONTROL SELF-RENEWAL OF NORMAL AND CANCER STEM CELLS
3:00 - 3:20	**Maria Themeli**, *Memorial Sloan-Kettering Cancer Center* HUMAN IPS CELL-DERIVED ANTIGEN-TARGETED T CELLS ERADICATE CD19-POSITIVE TUMOR CELLS
3:20 - 3:40	**N. Sumru Bayin**, *New York University School of Medicine* SELECTIVE TARGETING AND ABLATION OF GLIOBLASTOMA STEM CELLS
3:40 - 4:00	**Zhongwei Cao**, *Weill Cornell Medical College* TUMOR VASCULAR NICHE INDUCES AGGRESSIVE BEHAVIOUR AND CHEMORESISTANCE IN LYMPHOMA STEM CELLS
4:00 - 6:00	**POSTER SESSION I and RECEPTION**

THURSDAY, MAY 23, 2013

8:00am	Registration and Continental Breakfast

PLENARY III: Stem Cell Biology

9:00 - 11:00	Chair: **Gordana Vunjak-Novakovic**, *Columbia University*
9:00 - 9:20	**Janet Paluh**, *University at Albany* EVALUATION OF STEM CELL PLURIPOTENCY USING LTA-PDMS TRANSPARENT GRIDS FOR 3D TEMPLATING AND TIMELAPSE MONITORING
9:20 - 9:40	**George Eng**, *Columbia University* ELECTROMECHANICAL CONDITIONING OF HUMAN STEM CELL DERIVED CARDIOMYOCYTES
9:40 - 10:00	**Elena Ezhkova**, *Icahn School of Medicine at Mount Sinai* POLYCOMB SUBUNITS EZH1 AND EZH2 REGULATE THE MERKEL CELL DIFFERENTIATION PROGRAM IN SKIN STEM CELLS
10:00 - 10:20	**Raffaella Di Micco**, *New York University School of Medicine* BET PROTEINS REGULATE EMBRYONIC STEM CELL IDENTITY BY CONTROLLING TRANSCRIPTIONAL ELONGATION OF CORE PLURIPOTENCY GENES
10:20 - 10:40	**Xiajun Li**, *Icahn School of Medicine at Mount Sinai* GENOMIC IMPRINTING IS VARIABLY LOST DURING IPS CELL DERIVATION
10:40 - 11:00	**Hans-Willem Snoeck**, *Columbia University Medical Center* HIGHLY EFFICIENT GENERATION OF AIRWAY AND LUNG EPITHELIAL CELLS FROM HUMAN PLURIPOTENT STEM CELLS
11:00 - Noon	**POSTER SESSION II**
Noon - 1:00	**LUNCH and POSTER VIEWING**
1:00 - 1:10	**COMMISSIONER'S REMARKS, INTRODUCTION OF KEYNOTE**
1:10 - 2:00	**KEYNOTE**: TAKING STEM CELL AND GROWTH FACTOR THERAPY TO THE CLINIC FOR LOU GEHRIG'S DISEASE (ALS) **Clive Svendsen**, *Cedars-Sinai Medical Center*
2:00 - 3:15	**PANEL: NAVIGATING THE MAZE OF STEM CELL THERAPIES** Moderator: **Lorenz Studer**, *Memorial Sloan-Kettering Cancer Center* Panelists: **Steven Goldman**, *University of Rochester* **Clive Svendsen**, *Cedars-Sinai Medical Center* **Sally Temple**, *Neural Stem Cell Institute*

3:15 - 3:45	**BREAK and POSTER VIEWING**

PLENARY IV: NYSTEM Success Stories

3:45 - 4:45	Chair: **Ira Cohen**, *Stony Brook University*
3:45 - 4:05	**Jian Feng**, *University at Buffalo* CELL CYCLE AND P53 GATE THE DIRECT CONVERSION OF HUMAN FIBROBLASTS TO DOPAMINERGIC NEURONS
4:05 - 4:25	**Alexandre Gaspar-Maia**, *Icahn School of Medicine at Mount Sinai* THE HISTONE VARIANT MACROH2A ACTS AS AN EPIGENETIC BARRIER FOR REPROGRAMMING
4:25 - 4:45	**Ulrich Steidl**, *Albert Einstein College of Medicine* H2.0-LIKE HOMEOBOX (HLX) INDUCES UNLIMITED CLONOGENICITY AND A BLOCK IN DIFFERENTIATION IN HEMATOPOIETIC STEM CELLS AND COOPERATES WITH FLT3-ITD IN THE INDUCTION OF ACUTE MYELOID LEUKEMIA
4:45	**CLOSING REMARKS**
5:00	**ADJOURN**

Keynote Address by Clive Svendsen, Ph.D.

Taking Stem Cell and Growth Factor Therapy to the Clinic for Lou Gehrig's Disease (ALS)

Taking stem cells to the clinic for ALS (Lou Gehrig's disease) has been a long and windy road for my laboratory. We started in 2003 and were ready in 2005 - but a trisomy 7 in the lead cell line we wanted to use stopped the project. It took 3 years to sort this out and come up with a non trisomy line that could be expanded and engineered to release the powerful growth factor GDNF. We are now funded by the California Institute of Regenerative Medicine to perform cell manufacturing, pre clinical animal studies, regulatory filing of an IND and an 18 patient Phase I/II trial. I will discuss how we made it as an academic program and the advantages and disadvantages of partnering with industry along the way.

Director, Regenerative Medicine Institute, Cedars-Sinai Medical Center

Professor in Residence, UCLA

Dr. Svendsen did his predoctoral training at Harvard University and received his Ph.D. from the University of Cambridge in England where he established a stem cell research group before moving to the University of Wisconsin in 2000 to become Professor of Neurology and Anatomy, Director of an NIH funded Stem Cell Training Program and Co-Director of the University of Wisconsin Stem Cell and Regenerative Medicine Center. In 2010 he moved to Los Angeles. One focus of his current research is to derive stem cells from patients with specific disorders. These cells can then be "reprogrammed" to a primitive state and used as powerful models of human disease. Dr. Svendsen led the first groups to successfully model both Spinal Muscular Atrophy and more recently Huntington's Disease using this technology. The other side of his research involves cutting edge clinical trials. He was involved with one of the first growth factor treatments for Parkinson's Disease and is currently working closely with neurosurgeons, neurologists and other scientists to develop novel ways of using novel stem cell and growth factor combinations to treat patients with both Parkinson's and ALS.

PANEL: NAVIGATING THE MAZE OF STEM CELL THERAPIES

Lorenz Studer, M.D.

Memorial Sloan-Kettering Cancer Center, New York, NY

A native of Switzerland, Lorenz Studer graduated from medical school in 1991 and received his doctoral degree in neuroscience at the University of Bern in 1994. While there, he initiated studies with Christian Spenger, leading to the first clinical trial of fetal tissue transplantation for Parkinson's disease in Switzerland. Dr. Studer next pursued his research interests at the National Institutes of Health (NIH) in Bethesda, Maryland, where he worked in the laboratory of Ron McKay. At the NIH he pioneered the derivation of dopamine cells from dividing precursor cells. In 1998, he was first to demonstrate that the transplantation of dopamine cells generated in culture improves behavioral symptoms in Parkinsonian rats.

In 2000, he moved to New York City where he started his research program at the Memorial Sloan-Kettering Cancer Center (MSKCC). Early contributions of his lab include the *in vitro* derivation of midbrain dopamine neurons from ES cells, nuclear transfer ES cells and parthenogenetic stem cells. His laboratory was also first to demonstrate “therapeutic cloning” in a mouse model of a CNS disorder, and he has pioneered studies on the directed differentiation, high-throughput screening and genetic modification of human ES cells. His most recent work increasingly focuses on the translational application of human pluripotent stem cells in disease modeling, drug discovery and cell therapy. He currently leads a large multidisciplinary consortium to pursue the first clinical application of human ES cell derived dopamine neurons for the treatment of Parkinson's disease. He received numerous awards for his work including the Boyer Young Investigator award and, most recently, the Annemarie Opprecht Award.

Dr. Studer is the Director of the Sloan-Kettering Center for Stem Cell Biology. He is a Member of the Developmental Biology Program and the Department of Neurosurgery at MSKCC and a Professor in Neuroscience at Weill-Cornell.

Steven A. Goldman, M.D., Ph.D.

University of Rochester Medical Center, Rochester, NY

Steven Goldman is the Edward and Alma Vollertsen Rykenboer Professor of Neurology at the University of Rochester, and Co-Director of its Center for Translational Neuromedicine. He is emeritus Chairman of Rochester's Neurology department, where he is Chief of its Division of Cell and Gene Therapy, and holds an additional appointment as the Dean Zutes Chair in Biology of the Aging Brain. Dr. Goldman moved to Rochester in 2003 from the Weill Medical College of Cornell University, where he was the Nathan Cummings Professor of Neurology. 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. He interned in Medicine and did his residency in Neurology at both New York Hospital-Cornell and the Memorial Sloan-Kettering Cancer Center, before joining the Cornell faculty.

Dr. Goldman is interested in cell genesis and neural regeneration in the adult brain, with a focus on the use of neural stem and progenitor cells in treating demyelinating and neurodegenerative diseases. His lab focuses on the use of stem and progenitor cells for the treatment of neurodegenerative disorders such as Huntington's Disease as well as for the treatment of glial diseases such as the pediatric leukodystrophies and multiple sclerosis He also has a strong interest in the conversion of resident stem and progenitor cells into brain cancers. He has published over 200 papers in his field, over 100 as first or senior author. Dr. Goldman is a recipient of the Jacob Javits Neuroscience Investigator Award of the NIH, and has been elected to the Association of American Physicians and the American Society for Clinical Investigation, as well as the American Neurological Association. He remains active clinically, with subspecialty interests in stroke and myelin disease as well as in neuro-oncology, in which he is board-certified. Dr. Goldman serves on several NIH committees, and is a member of the FDA Cellular, Tissue and Gene Therapy Advisory Committee. His work is supported by NINDS and NIMH, NYSTEM, the Mathers and Adelson Foundations, the Department of Defense, CHDI, the National MS Society, and Biogen Idec.

Sally Temple, Ph.D.

Neural Stem Cell Institute, Rensselaer, NY

Sally Temple, Ph.D., is the Co-Founder and Scientific Director of the Neural Stem Cell Institute located in Rensselaer, NY. Dr. Temple's group is focused on studying neural stem cells, and using this knowledge to develop therapies for central nervous system (CNS) disorders.

A native of York, England, Dr. Temple received her undergraduate degree at Cambridge University, Cambridge UK, her PhD in optic nerve development from University College London, UK and completed her postdoctoral work on spinal cord development at Columbia University, NY, USA.

In 1989, Dr. Temple discovered that the embryonic mammalian brain contained a rare, multipotent stem cell that could be extracted and grown in tissue culture to produce both CNS neurons and glia. Her group has continued to make pioneering contributions to the field of neural stem cell research, identifying factors intrinsic to these cells as well as external signaling molecules from the niche that participate in their self-renewal and differentiation. Recently, she helped identify a novel, accessible adult human CNS stem cell in the retinal pigment epithelium, which offers new possibilities for developing therapeutics for retinal disease.

SINGLE CELL GENOMICS

Jan Vijg

Albert Einstein College of Medicine, New York, NY

A NOVEL METHOD TO STUDY TELOMERE LENGTH IN INDIVIDUAL STEM CELLS

David L Keefe[1], Fang Wang[1,2], Xinghua Pan[3*], Keri Kalmbach[1], Michelle Smith[1], Xiaoying Ye[2], Danielle Antunes[1,4], Yu Yin[2], Lin Liu[2*], Sherman M. Weissman[3]

[1]Department of Obstetrics and Gynecology, New York University Langone Medical Center, New York, NY; [2]Nankai University, College of Life Sciences, Tianjin, China; [3]Department of Genetics, Yale University School of Medicine, New Haven, CT

Telomere length measurements currently require a large population of cells or replicating cells, which masks telomere length heterogeneity in single cells or fluorescent in situ hybridization in metaphase arrested cells, which poses significant technical challenges. We established a simple and robust approach for Single Cell Telomere length measurement (SCT-pqPCR), and successfully optimized a multiplex pre-amplification specific for telomeres and reference genes from individual cells, such that the amplicon provides a consistent ratio (T/R) of telomeres (T) to the reference genes (R) by qPCR. The average T/R ratio of multiple single cells corresponded to that of a given cell population measured by regular qPCR, and correlated well with those of telomere restriction fragments (TRF) and Q-FISH measurements. Telomere length measured by SCT-pqPCR was also validated using two sister cells (totipotent stem cell) from 2-cell embryos. Telomere length heterogeneity was identified in individual cells of various human and mouse cell types by SCT-pqPCR. We found that human oocytes and polar bodies have nearly identical telomere lengths, and that the telomere lengths progressively increase from the zygote, 2-cell to 4-cell embryo. This method will facilitate understanding of telomere heterogeneity likely linked with tumorigenesis, aging and associated diseases.

TRAJECTORY DETECTION ORDERS HALLMARKS OF EARLY HUMAN B CELL DEVELOPMENT

El-ad David Amir

Columbia University, New York, NY

DISTINCT THRESHOLDS OF MYC PROTEIN HALF-LIFE CONTROL SELF-RENEWAL OF NORMAL AND CANCER STEM CELLS

Iannis Aifantis

New York University School of Medicine, New York, NY

While transcriptional regulation of stem cell pluripotency and differentiation has been extensively studied, only a small number of studies have addressed the roles for post-translational modifications in these processes. A key mechanism of post-translational modification is ubiquitination by the ubiquitin-proteasome system (UPS). We present here a combination of genetic and biochemical studies aiming at the identification of UPS members that can control both embryonic and adult stem cell self-renewal and differentiation. We particularly focus on the composition and function of the proteasome itself demonstrating qualitative and quantitative differences during stem cell differentiation. Moreover, we identify members of the UPS controlling the half-life of key embryonic and adult stem cell regulators (including the key transcription factor c-MYC) and study their function in physiology and disease. Finally, we propose that modulation of UPS activity can be pivotal for optimal cellular reprogramming and induced pluripotency.

HUMAN IPS CELL-DERIVED ANTIGEN-TARGETED T CELLS ERADICATE CD19-POSITIVE TUMOR CELLS

Maria Themeli, Christopher C. Kloss, Michel Sadelain

Center for Cell Engineering, Molecular Pharmacology and Chemistry Program, Memorial Sloan-Kettering Cancer Center, New York, NY

Pluripotent stem cells may be harnessed to generate tumor-targeted T lymphocytes for cancer immunotherapy. Merging induced Pluripotent Stem cell (iPS) and Chimeric Antigen Receptor (CAR) technology, we characterize here the phenotype and anti-tumor function of human iPS-derived T cells that are genetically targeted to a predetermined tumor-associated antigen.

To this end, we generated iPS cells from peripheral blood lymphocytes (T-iPS) and we genetically engineered them to express 1928z, a CAR currently used for adoptive immunotherapy to treat CD19 positive B-cell leukemias. Stably transduced 1928z-T-iPS lines were then directed to differentiate towards the T lymphoid lineage. After 20 days of differentiation around 80% of the cells were CD3+TCRαβ+, with some expressing CD8 and/or CD56. Most of the generated T lymphocytes had a CD45RA+CD62L- effector memory phenotype while 6% had a CD45RA+CD62L+ naïve phenotype. The generated T cells all expressed the 1928z CAR on the surface (1928z-TiPS-T). When cultured in the presence of the CD19 target-antigen, 1928z-TiPS-T cells rapidly responded by forming clusters, increased the expression of activation markers (CD69, CD25) and secreted cytokines (TNF-α, IFN-γ and IL-2). We were able to optimize conditions in order to achieve around 1000-fold expansion of functional 1928z-TiPS-T cells. The expanded cells still had an effector memory phenotype and up-regulated the expression of natural cytotoxicity receptors. Importantly, we found that 1928z-TiPS-T cells displayed highly specific lytic activity against CD19+ tumor cells lines *in vitro*.

In conclusion, we demonstrate that TiPS-derived CAR+ T cells display key canonical features of T lymphocyte function and can specifically eradicate target tumor cells. The genetic modification of human pluripotent stem cells with CARs is, thus an effective approach to rapidly and efficiently generate antigen-specific T cells with a clinically relevant function. Furthermore, the iPS cell technology is an excellent platform for safe genetic modifications to augment the potency of immune effectors.

SELECTIVE TARGETING AND ABLATION OF GLIOBLASTOMA STEM CELLS

N. Sumru Bayin[1], Taylor A. Wilson[1], August Dietrich[1], Tobias Abel[2], Moses V. Chao[3], Hae-Ri Song[1], David Zagzag[4], Christian J. Buchholz[2], Dimitris G. Placantonakis[1]

[1]Department of Neurosurgery, New York University School of Medicine, New York, NY; [2]Medical Biotechnology and Gene Therapy, Paul-Ehrlich-Institut, Langen, Germany; [3]Skirball Institute; [4]Department of Pathology, New York University School of Medicine, New York, NY

Glioblastoma multiforme (GBM) is the most common and lethal brain malignancy with 10,000 new cases in the US annually. Despite advances in surgery and chemoradiotherapy, the median survival is only 15 months. The poor prognosis highlights the need for novel therapeutic approaches. A small subpopulation of GBM cells behave as stem cells (GBM stem cells or GSCs) and are believed to be responsible for tumor growth, recurrence and resistance to chemoradiotherapy. Therefore, GSCs represent important therapeutic targets. The cell surface glycoprotein CD133 was previously shown to specifically mark tumor stem cells in many malignancies, including GBM. Here, we took advantage of the specific expression of CD133 in GSCs and tested the hypothesis that CD133-LV (Anliker *et al.,* 2010), a non-replicating lentivirus displaying a single-chain antibody against CD133 on its envelope, can selectively and efficiently transduce CD133+ GSCs. Using primary human GBM cultures, in which CD133+ cells represent 1.4±0.1% (n=4) of all cells, and a CD133-LV constitutively expressing fluorescent proteins, we found that CD133-LV transduced a subpopulation of GBM cells *in vitro* and *in vivo*, consistent with the small percentage of CD133+ cells in the cultures. In contrast, pantropic lentivirus produced 20-fold higher transduction efficiencies. To assess the specificity of transduction, we treated cultures with an antibody raised against CD133 and observed a more than 2-fold decrease in transduction efficiency. Furthermore, we showed that CD133-LV does not transduce normal mouse brain and human embryonic stem cell-derived neurons. By using a CD133-LV, which expresses *Pseudomonas* exotoxin, we selectively ablated CD133+ and impaired tumorsphere formation *in vitro*. We propose that CD133+ GSC-selective gene therapy may be a powerful new strategy in the treatment of GBM.

TUMOR VASCULAR NICHE INDUCES AGGRESSIVE BEHAVIOUR AND CHEMORESISTANCE IN LYMPHOMA STEM CELLS

Zhongwei Cao[1], Bi-Sen Ding[1], Peipei Guo[1], Sharrell B. Lee[1], Jason M. Butler[1], Koji Shido[1], Joseph M. Scandura[2], and Shahin Rafii[1]

Departments of [1]Genetic Medicine; [2]Medicine, Weill Cornell Medical College, New York, NY

Tumor endothelial cells (ECs) promote cancer progression in ways that extend beyond their role as passive conduits supporting tumor metabolism. ECs can deploy specialized instructive signals that are ordinarily used to direct tissue repair but that can be co-opted to support tumor progression. However, it is not known how the vascular niche function is subverted by malignant cells to orchestrate tumor propagation and confer resistance to chemotherapy (chemoresistance). Here, we show that lymphoma stem cells (LSCs) functionalize a tumorigenic vascular niche by triggering FGFR1-mediated signals in ECs and causing them to deploy Notch ligand, Jagged1 (Jag1), that in turn provides juxtacrine support for LSCs. This tumorigenic, feed-forward cycle results in uncontrolled expansion of aggressive mouse and human LSCs and provides a nidus to generate chemoresistant lymphomas. We combined clonal analysis with xenobiotic-free EC co-cultures to show that FGFR1-induced expression of Jag1 on ECs, specifically initiates and sustains the expansion of $Myc^{+}CD44^{+}IGF1R^{+}CSF1R^{+}$ LSCs. Notably, Jag1 expressed by tumor ECs conferred non-LSC lymphoma cell subpopulation with functional and phenotypic attributes of LSCs. Using an inducible knockout strategy to ablate Fgfr1 or Jag1 in the ECs of Eμ-Myc lymphoma-harboring mice ($Myc^{+}Fgfr1^{i\Delta EC/i\Delta EC}$/$Myc^{+}Jag1^{i\Delta EC/i\Delta EC}$), we demonstrate that tumorigenic vascular niche uses FGFR1-Jag1 paracrine loop to promote Notch-dependent expansion and hepatic invasion of $Myc^{+}CD44^{+}IGF1R^{+}CSF1R^{+}$ LSCs. Similarly, we found xenografted human lymphoma cells upregulated Jag1 in tumor ECs adjacent to Notch-activated lymphoma cells. This paracrine loop has potential clinical importance because $Jag1^{+}$ tumor ECs bestowed Notch-dependent protection against chemotherapy to perivascularly localized LSCs. EC-specific deletion of *Jag1* enhanced survival of $Myc^{+}Jag1^{i\Delta EC/i\Delta EC}$ mice and sensitized tumors to chemotherapy. Therefore, LSCs do not solely rely upon intrinsic genetic defects, but also require extrinsic signals from tumor-primed vascular niche. These results open the door to new strategies selectively targeting FGFR1 or Jag1 to treat chemoresistant lymphomas by breaking tumorigenic vascular niche.

EVALUATION OF STEM CELL PLURIPOTENCY USING LTA-PDMS TRANSPARENT GRIDS FOR 3D TEMPLATING AND TIMELAPSE MONITORING

Martin L. Tomov[1], Zachary T. Olmsted[1], Janet L. Paluh[1]

Nanobioscience, College of Nanoscale Science and Engineering (CNSE), University at Albany, SUNY, Albany, NY

The prevalent use of embryoid bodies (EBs) as an efficient precursor of lineage differentiation and as bioreagents in development of graft or organoid structures for tissue engineering necessitates a re-evaluation of methods used to prepare and evaluate EB 'performance'. EBs formed in free solution as non-templated 3D aggregates generate a mixed population with varied sizes and shapes including fused larger and misshapen EBs. This mixed character introduces variation into the experimental design that complicates interpretations of results. We are developing new techniques for statistical use of EBs and their response to differentiation cues in evaluation of pluripotency of human embryonic stem cell (hESC) lines. Uniform EBs of tunable size and shape are generated in a transparent template allowing time-lapse evaluation of EB formation and rate, then dispersed and patterned for high throughput analysis of differentiation. The first step uses lithography-templated arrays (LTA) spin coated with the desired hydrogel to template and track EB formation. Here we describe work with PDMS 200 and 500 micron templates (LTA-PDMS) seeded with mechanically passaged 2D hESC cell clusters or one day old 3D stem cell aggregates. Templated EBs are next dispersed and re-patterned using a multi-layered SU-8 based device to allow individual tracking of EB differentiation responses. The multi-lineage differentiation potential of our EBs cultivated in LTA-PDMS grids are then readily compared. Templated EBs formed in 200 micron wells out-perform larger EBs obtained from 500 micron wells or in solution without templating and demonstrate the importance of controlling EB size as a critical factor in differentiation. We discuss new information provided by our approach for EB templating, dispersion and differentiation monitoring. This strategy is invaluable for evaluation of hESC lines and use of such lines in tissue engineering, cancer stem cell analysis, and regenerative medicine applications.

[Supported by NYSTEM contract # C026186]

ELECTROMECHANICAL CONDITIONING OF HUMAN STEM CELL DERIVED CARDIOMYOCYTES

George Eng[1,2], Benjamin W. Lee[1,2], Mark Gagliardi[5], Kristy Brown[4], Gordon Keller[5], Gordana Vunjak-Novakovic[1,3]

[1]Department of Biomedical Engineering; [2]College of Physicians and Surgeons; [3]Department of Medicine; [4]Department of Pathology and Cell Biology, Columbia University, New York, NY; [5]McEwen Centre for Regenerative Medicine, University Health Network, Toronto, Ontario, Canada

Introduction: Differentiated human cardiomyocytes, from either embryonic or induced pluripotent stem cells have great potential to ameliorate heart disease through therapeutic engraftment into the diseased heart. One of the key concerns is the electromechanical mismatch between the host and therapeutic cells. Most of the native heart development occurs in the presence of electrical signals. We previously showed that electrical field stimulation improves conductive and contractile properties of neonatal rat cardiomyocytes, but the conditioning of human embryonic stem cell derived cardiomyocytes (hESC-CM) has not been explored. Our objective was to determine if electrical stimulation at suprathreshold amplitudes following molecular induction of cardiac differentiation modulates contractile function of hESC-CMs.

Methods: hESCs were differentiated into CMs in form of embryoid bodies (EB) using staged molecular induction for 20 days in culture, and then transferred into microbioreactors and stimulated for 7 days by square wave signals (5V/cm, 2 ms) at 0, 0.5, 1 and 2Hz. After the stimulation was discontinued, the contraction amplitude, autonomous frequency, strain distribution, ultrastructure, gene expression, distribution of cardiac-specific proteins, and the function of the KCNH2 channel were measured.

Results: Electrical stimulation modulated the autonomous beating frequency of the hESC-CMs over the physiologic range (0.5 – 2 Hz). Seven days of electrical stimulation synchronized the autonomous contractions at the stimulation frequency, improved contractile protein assembly and myofibrillar ultrastructure and modified global gene expression in a frequency-dependent manner. We identified the molecular basis for the observed changes: the voltage gated inward rectifier potassium channel, encoded by KCNH2, which was critical for electromechanical conditioning, as demonstrated by the lack of effects following channel inhibition and the recovery following washout.

Conclusions: Electrical conditioning of hESC-CM markedly improved the contractile protein assembly and enabled control of autonomous beating frequency, via KCNH2 channel.

Future Directions: Current efforts are focused on maturation of iPS-CMs by electromechanical conditioning.

[Supported in part by NYSTEM contract C026449]

POLYCOMB SUBUNITS EZH1 AND EZH2 REGULATE THE MERKEL CELL DIFFERENTIATION PROGRAM IN SKIN STEM CELLS

Elena Ezhkova, Evan Bardot, Julian Valdes, Carolina Perdigoto, Jisheng Zhang

Department of Developmental and Regenerative Biology, Icahn School of Medicine at Mount Sinai, New York, NY

Chromatin regulators have recently emerged as important regulators of stem cell control. *In vitro* studies of embryonic stem cells have identified the Polycomb chromatin repressor complex as the key regulator of differentiation, but it remains unclear as to whether Polycomb-mediated mechanisms control fates of multipotent stem cells *in vivo*.

Here we show that removal of Ezh1 and Ezh2, key Polycomb subunits, from mouse skin results in a marked change in fate determination in epidermal stem cells, leading to an increase in the number of lineage-committed Merkel cells, a specialized subtype of skin cells involved in mechanotransduction. By dissecting the genetic mechanism we showed that the Polycomb complex restricts differentiation of epidermal stem cells by directly repressing the transcription factor *Sox2*. Ablation of Sox2 results in a dramatic loss of Merkel cells indicating that Sox2 is a critical regulator of the Merkel cell differentiation program. We show that Sox2 directly activates transcription of Atoh1, the obligate regulator of Merkel cell differentiation. Concordantly, ablation of Sox2 attenuated the *Ezh1/2*-null phenotype, confirming the importance of Polycomb-mediated repression of the Sox2/Atoh1 network to maintain the epidermal stem cell state. Together these findings define a novel regulatory network by which the Polycomb complex maintains the stem cell state and governs differentiation *in vivo*.

BET PROTEINS REGULATE EMBRYONIC STEM CELL IDENTITY BY CONTROLLING TRANSCRIPTIONAL ELONGATION OF CORE PLURIPOTENCY GENES

Raffaella Di Micco[1,2], Barbara Fontanals Cirera[1,2], Aristotelis Tsirigos[3], Panagiotis Ntziachristos[2,4], Yoshiya Yonekubo[1,2], Guangtao Zhang[5], Elena Rusinova[5], Guillermo Gerona-Navarro[5], Marta Cañamero[6], Michael Ohlmeyer[5], Iannis Aifantis[2,4], Ming-Ming Zhou[5], Eva Hernando[1,2]

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Transcription factors and chromatin remodeling complexes are key determinants of Embryonic Stem Cell (ESC) identity. The bromodomain and extra-terminal domain (BET) family of proteins regulates chromatin dynamics through modulating acetylation-mediated interactions critical for gene transcription. Here, we investigated the functional role of BET proteins in governing ESC properties, self-renewal and pluripotency. Small-molecule chemical inhibition of the BET bromodomain acetylated lysine binding activity significantly reduced the number of undifferentiated ESC colonies, without affecting their viability. Moreover, BET inhibition compromised the ability of ESCs to self-renew or form embryoid bodies (EBs). Global gene expression profiling of compound-treated human ESCs revealed a marked suppression of pluripotency genes, including *OCT4* and *PRDM14.* Conversely, the expression of epithelial to mesenchymal transition (EMT) markers and neuroectodermal lineage genes was increased following compound treatment. Deep sequencing of chromatin immunoprecipitation (ChIP-seq) of compound-treated hESCs revealed preferential displacement of the BET proteins BRD3 and BRD4 from stem cell genes. ChIP-based assessment of RNA polymerase II (Pol II) density along gene sequences and of the transcriptional elongation marks H3 lysine 36 trimethylation (H3K36me3) and Pol II serine-2 phosphorylation (Pol II pS2) revealed a specific defect in transcriptional elongation of pluripotency genes, following BET displacement. Among BET proteins, BRD4 was preferentially displaced with Pol II from down-regulated stem cell genes. Consistently, silencing of *BRD4*, but not *BRD2* or *BRD3*, phenocopied the effects of BET chemical inhibition on ESCs. Further, teratomas derived from *Brd4*-depleted ESCs exhibited a marked defect in multidifferentiation potential. Markedly, BET inhibition or *Brd4* depletion impaired the somatic reprogramming of fibroblasts to induced pluripotent stem cells (iPSCs). Overall, our study identifies a key role of BET proteins, particularly BRD4, in regulating the transcriptional elongation of the pluripotency network. Our findings define a new level of regulation of ESC identity, which could be exploited to improve the efficiency of ESC differentiation and somatic reprogramming.

GENOMIC IMPRINTING IS VARIABLY LOST DURING IPS CELL DERIVATION

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Derivation of induced pluripotent stem (iPS) cells from somatic cells is mainly an epigenetic reprogramming process. The epigenetic marks associated with genomic imprinting are established during gametogenesis and are stably maintained in somatic cells after fertilization. There are some reports and often times contradictory results regarding the status of genomic imprinting in iPS cells. To gain further insight into this epigenetic reprogramming phenomenon, we derived twelve iPS clones from the mouse embryonic fibroblast (MEF) cells that carry the OSKM transgene for inducible expression of four reprogramming factors (*Oct4*, *Sox2*, *Klf4* and *c-Myc*). We found that the parental DNA methylation imprint was variably lost in these iPS clones at most imprinted regions examined. Because these MEF cells were generated from the male and female mice with different genetic background, we were able to assess the inheritance of original DNA methylation imprint at the *Snrpn* and *Zac1* imprinted regions. Based on bisulphite sequencing, DNA methylation imprint at both imprinted regions on the maternal chromosomes was variably lost in almost all iPS clones. Consistent with loss of genomic DNA methylation imprint at the *Snrpn* and *Peg3* imprinted regions, loss of mono-allelic expression of the corresponding *Snrpn* and *Zim1* imprinted genes was indeed confirmed in these iPS clones based on allele-specific RT-PCR analysis. Furthermore, we found this loss of parental genomic imprinting in iPS cells was likely caused by the reprogramming process during iPS cell derivation. Prolonged culture of iPS cells may contribute to the loss of genomic imprinting in some imprinted regions. These results might have some implications for future therapeutic applications of iPS cells. Since DNA methylation imprint can be completely erased in some iPS clones at multiple imprinted regions, iPS cell reprogramming may also be employed to dissect the underlying mechanisms of erasure and maintenance of genomic imprinting in mammals.

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HIGHLY EFFICIENT GENERATION OF AIRWAY AND LUNG EPITHELIAL CELLS FROM HUMAN PLURIPOTENT STEM CELLS

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The ability to generate lung and airway epithelial cells from human pluripotent stem cells (hPSCs) has applications in regenerative medicine for lung diseases, drug screening and disease modeling, and provides a model to study human lung development. Functional lung and airway epithelial cells have not been generated from hPSCs with high efficiency and purity, however. Here, we established, based on developmental paradigms, a highly efficient method for directed differentiation of hPSCs into lung and airway epithelial cells. Long-term differentiation yielded cultures where >90% of the cells were committed to a lung or airway epithelial fate and contained goblet, Clara, ciliated, type I and, after addition of maturation media containing dexamethasone, predominantly type II alveolar epithelial cells. Cell expansion was 35-fold, attesting to the high efficiency of this strategy. Inhibiting or removing agonists to signaling pathways critical for early lung development in the mouse, retinoic acid, Wnt and BMP, modeled defects observed in corresponding genetic mouse knockouts, thus validating this approach. Importantly, the type II alveolar epithelial cells generated were capable of surfactant protein-B uptake, providing evidence of specific function.

CELL CYCLE AND P53 GATE THE DIRECT CONVERSION OF HUMAN FIBROBLASTS TO DOPAMINERGIC NEURONS

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The direct conversion of fibroblasts to induced dopaminergic (iDA) neurons and other cell types has significant impact on many practical applications and fundamental understanding of cell biology. The low efficiency of these relatively fast conversions, which generally manifest themselves within days, suggests that conditions additional to the requisite transcription factors must be met to enable highly efficient cellular reprogramming. Here, we show that reduction in p53 expression significantly improved the efficiency of generating iDA neurons from human fibroblasts using Ascl1, Nurr1, Lmx1a and miR124. In addition, cell cycle arrest at the G1 phase by serum withdrawal greatly enhanced the conversion. Furthermore, a combination of small-molecule compounds and neurotrophic factors that facilitate the differentiation, survival and maturation of dopaminergic (DA) neurons significantly boosted the conversion efficiency so that 93% of the cells at day 9 were $Tuj1^+$ and 59% were TH^+. The iDA neurons expressed markers for midbrain DA neurons, had Ca^{2+}-dependent DA release and selective DA uptake, and exhibited spontaneous action potentials and spontaneous excitatory postsynaptic currents. The results suggest that cell cycle arrest at G1 readies mitotic cells such as fibroblasts for conversion to postmitotic cells such as neurons. Reduction in p53 level appears to remove a key barrier of cellular reprogramming, as is found in the derivation of iPS cells. The optimal combination of survival factors enables the manifestation of the efficient conversion process. These three parameters (cell cycle, p53 and environment) may be important to cellular reprogramming in general.

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THE HISTONE VARIANT MACROH2A ACTS AS AN EPIGENETIC BARRIER FOR REPROGRAMMING

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The chromatin template imposes an epigenetic barrier during the process of somatic cell reprogramming. Here we report that the histone variant macroH2A is not essential for the process of somatic cell differentiation but acts as a barrier to induced pluripotency. MacroH2A is a unique histone variant, consisting of an N-terminal region similar to canonical histone H2A and a non-histone macro domain at its carboxyl terminus, making it the largest known histone variant.

Here we show that during development and *in vitro* differentiation macroH2A1 and macroH2A2 isoforms become enriched in the chromatin fraction. Using fibroblasts isolated from macroH2A double knockout mice, we observed enhanced reprogramming efficiency compared to fibroblasts from wild type animals. We further show that macroH2A isoforms act cooperatively in this process, by reprogramming macroH2A1 and macroH2A2 single knockout cells, and through re-expression of such variants in the double knockout fibroblasts. Quantitative MassSpectrometry (qMS) analysis of histone post-translational modifications during reprogramming shows that the absence of macroH2A variant does affect the histone mark landscape. Genome wide ChIP-seq analysis in wild type fibroblasts reveals that macroH2A and H3K27me3 domains co-localize and occupy pluripotency genes. While the absence of macroH2A does not affect H3K27me3 localization in fibroblasts, macroH2A is highly enriched at a set of Utx target genes that need to be reactivated early during iPS reprogramming. Finally, macroH2A double knockout induced pluripotent cells are able to differentiate properly *in vitro* and *in vivo*, indicating macroH2A is not essential for differentiation. However, dKO differentiated cells are more prone to be rescued to a pluripotency state. Given these findings, we propose that macroH2A isoforms provide a redundant silencing layer or terminal differentiation 'lock' at critical pluripotency genes that presents as an epigenetic barrier when differentiated cells are challenged to reprogram.

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H2.0-LIKE HOMEOBOX (HLX) INDUCES UNLIMITED CLONOGENICITY AND A BLOCK IN DIFFERENTIATION IN HEMATOPOIETIC STEM CELLS AND COOPERATES WITH FLT3-ITD IN THE INDUCTION OF ACUTE MYELOID LEUKEMIA

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Less than one third of patients with acute myeloid leukemia (AML) achieve durable remission with current treatment regimens, and prognostication and risk stratification are challenging. We found that a novel non-clustered homeobox gene, H2.0-like homeobox (*HLX*), regulates early hematopoiesis and promotes AML in mice and humans. *HLX* is 2 to 16 fold overexpressed in more than 80% of patients with AML, in both blast cells and in highly purified stem cell compartments. Higher levels of *HLX* are associated with poor overall survival in AML patients (N=601, $p=2.3x10^{-6}$). ShRNA-mediated inhibition of HLX in both murine and human AML cells significantly inhibits leukemic growth and clonogenic capacity, overcomes the differentiation block, and prolongs survival in a xenotransplantation model. When we analyzed pre-leukemic hematopoietic stem and progenitor cells (HSPC) in a PU.1-knockdown AML mouse model, we found a 4-fold elevation of *Hlx*, suggesting that *Hlx* is involved in malignant transformation. Overexpression of HLX in HSPC in a competitive, congenic transplantation model led to near complete depletion of long-term HSC and 16-fold enrichment of myeloid progenitors. Overexpression of HLX in HSPC *in vitro* led to a myeloid differentiation block and to formation of aberrant progenitors with unlimited serial clonogenicity. Internal tandem duplications of *FLT3* (FLT3-ITD) are seen in approximately 30% of all AML patients. We found that co-expression of HLX and FLT3-ITD leads to greatly enhanced cytokine independent growth of murine HSPC *in vitro*. When we co-expressed HLX and FLT3-ITD in HSPC and transplanted them into congenic recipient animals, we found that by 12 weeks post-transplantation mice developed aggressive AML with large numbers of leukemic blasts. In summary, our studies have identified *HLX* as a novel key transcription factor involved in the regulation of hematopoietic stem cells and AML pathogenesis, and suggest *HLX* and downstream pathways as promising new therapeutic targets in AML.

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F1: AN UPSTATE NEW YORK SHARED FACILITY FOR STEM CELL RESEARCH

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The Rensselaer Center for Stem Cell Research (RCSCR) was established in 2012 with a NYSTEM award for the construction and operation of a shared-use stem cell research facility. Our mission is to accelerate high-quality, high-impact stem cell research through innovative and integrative projects and project support. This double-laboratory resource provides infrastructure and training in stem cell culture, derivation, imaging and phenotypic analysis for academic, not-for-profit and commercial research in the Capital Region and throughout New York State. The RCSCR is a dedicated cell culture facility, equipped with biosafety cabinets, tri-gas incubators for normal and low oxygen studies, centrifuges, water baths, cold and cryogenic storage, and a water purification system. The laboratory also includes several imaging systems, including multiple inverted and stereo microscopes, an Olympus IX51 inverted epifluorescence microscope and two platforms unique to the Capital Region: an Olympus VivaView time lapse microscope and a Thermo Scientific Cellomics ArrayScan high-content imaging system. In addition, comprehensive project development and execution are enhanced by collaboration with adjacent Rensselaer research cores and their staff members, including animal research, advanced microscopy, bioimaging, molecular biology, biochemistry and proteomics.

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F2: EXPANSION OF PLURIPOTENT STEM CELL RESEARCH AT MSSM

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The main objective of the pluripotent stem cell (PSC) shared resource facility (SRF) is to make the latest stem cell technology available to scientists. We currently use the Sendai virus and the mRNA/micro RNA methodology to reprogram blood and fibroblast cells respectively into induced PSCs (iPSCs). The next step involves the differentiation of these cells into the diseased tissue-specific cells in order to recapitulate aspects of the disease. To facilitate the latter, we are generating various reporter systems using the latest Transcription Activator-Like Effector Nuclease (TALEN) technology. These reporters will allow us to establish conditions necessary to improve the differentiation of specific lineages and allow siRNA, micro RNA, small molecule/drug screens in novel cell populations. For example, we engineered the human H9 ESC line to express M2rtTA constitutively from the human *ROSA26* locus on chromosome 3 using a TALEN pair to target the human *ROSA26* locus between exon 1 and exon 2 with a donor plasmid. Targeting efficiency using this specific TALEN pair was 86%. In addition, we are targeting the human *AAVS1* locus with a cassette that will enable Cre-recombination mediated cassette exchange to place any gene or combination of genes under the control of the Dox-inducible TRE promoter. This cell line will allow us to study the effect of inducible transgene expression and/or knockdown of various genes during human development and differentiation, thus providing a valuable resource for the stem cell community. We continue to quality control stem cell lines, reagents and supply these reagents to the stem cell community. Lastly we also conduct classes to teach iPSC generation and differentiation into the lineage of choice. Taken together, these services will have a three-fold benefit. Firstly, it will alleviate the quality control burden of individual scientists and allow them to concentrate on important scientific questions. Secondly, it will allow collaborative projects involving PSCs 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 it will provide the Core with a source of revenue to meet its expenditures.

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F3: NYSTEM HIGH-THROUGHPUT SCREENING AND CHEMISTRY SHARED FACILITY AT COLUMBIA UNIVERSITY

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The NYSTEM High-Throughput Screening and Chemistry Shared Facility is designed to expedite new discoveries in stem cell science and catalyze new translational strategies. The facilities are now fully operational and heavily used, providing stem cell researchers with state-of-the-art tools for mechanistic studies and target discovery, including high-throughput screening, target identification and medicinal chemistry. An experienced staff of 7 leads users through all stages in the process from assay design and development to data analysis and finally chemical optimization. The screening facility offers access to libraries of small molecules and tools for RNAi experiments. Recently the facility has entered into an agreement to access genome-wide lentiviral shRNA clones at greatly reduced rates, making these available to our NYSTEM users. The screening center has added new tools to help facilitate discovery: to aid with data analysis we have implemented Pipeline Pilot from Accelrys, and to expedite hit validation we add the HP digital dispenser for rapid, accurate dose-response curves. Since 2011 the OCCC has provided 10 research labs with organic synthesis support. Of these projects 8 required synthesis, 3 PK or metabolism studies, and 4 initial assay development. In the CPS Facility, we have established the capability of designing and synthesizing both chemical probes and therapeutic candidates, using computational and medicinal chemistry. We have been able to start 12 projects. Some of projects still ongoing and some were successfully finished. The facility will aid in the prosecution of new stem cell-related targets and provide new approaches to the treatment of disease. This year, a total of 15 new projects were undertaken in the screening facility and will acknowledge NYSTEM support. For more information on services available from the screening center, contact screening@columbia.edu. For information on the chemical probe synthesis facility, contact cps@biology.columbia.edu.

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1: THE PRESENCE OF PRIMARY CILIA IS REGULATED DURING EARLY MOUSE DEVELOPMENT AND IN STEM CELLS DERIVED FROM EMBRYOS

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Primary cilia are microtubule-based organelles that project from the cell surface and are required for cells to respond to specific intercellular signals. To determine whether primary cilia have a role in specification of different cell lineages in both stem cell differentiation and during embryonic development, we have generated a double transgenic mouse expressing Arl13b-mCherry to mark the cilium axoneme and Centrin-GFP to mark centrioles. Mouse embryonic stem cells (mESCs) are derived from the inner cell mass (ICM) of the preimplantation embryo. At this stage of development, no cells in the embryo have primary cilia. As the embryo implants, the ICM proliferates forming the epiblast, which is surrounded by extraembryonic endoderm. We first detect primary cilia at this stage of development, but they are present only in the epiblast and not in other lineages. This suggests that the presence of cilia is highly regulated, and that cilia may play a role in the ability of cells to respond to the signals that specify early cell lineages. To investigate this further we generated double transgenic mESCs expressing Arl13b-mCherry and Centrin-GFP and show ~15% of these cells are ciliated, which contrasts both with absence of cilia on ICM cells that give rise to mESCs and the postimplantation epiblast, where most cells are ciliated. We are comparing the levels of pluripotency marker expression in non-ciliated verses ciliated mESCs, and whether the level of ciliation changes as cells differentiate to tell us if primary cilia facilitate cell signaling required to maintain pluripotency or to promote differentiation. We are also testing whether primary cilia are present on stem cells derived from the epiblast, Epi stem cells and from the extraembryonic endoderm, XEN stem cells. These studies will help us to understand the role of primary cilia in the early embryo and on stem cells and how they might enable cells to respond to the signals necessary for specification of different cell lineages.

2: VIRALLY-MEDIATED EPENDYMAL OVEREXPRESSION OF BDNF AND NOGGIN INDUCES FUNCTIONAL MEDIUM SPINY NEUROGENESIS IN BOTH A MOUSE MODEL OF HUNTINGTON'S DISEASE AND IN ADULT NON-HUMAN PRIMATES

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Huntington's disease (HD) is characterized in part by the loss of neostriatal neurons. Intracerebroventricular (ICV) delivery of adenoviral BDNF and noggin triggers the addition of new neurons to the neostriatum, and slows disease progression in R6/2 HD mice (J. Clin. Invest. 117:2889-2902, 2007). However, prior studies have not demonstrated either the functional integration of these new neurons or their sustained therapeutic value. We used adeno-associated virus (AAV4), an ependymotrophic vector that allows persistent transgene expression, to deliver BDNF and noggin to the ventricular ependyma of adult R6/2 mice. We found that a single injection of AAV4-BDNF and AAV4-noggin triggered the sustained recruitment of new medium spiny neurons in both wild-type and R6/2 mice. Trigenic R6/2 x nestin-CreER^{T2} x EYFP mice treated with either intra-ventricular AAV4-BDNF/noggin, or with BDNF and noggin proteins, exhibited the robust recruitment of new striatal neurons, whose origin from nestin$^+$ subependymal progenitors was confirmed by fate-mapping. Pallidal backfills in both R6/2 and wild-type reporter mice, followed by patch clamp, confirmed the maturation and circuit integration of new striopallidal neurons. Importantly, the AAV4-BDNF/noggin-treated R6/2 mice exhibited delayed motor deterioration and substantially increased survival. In addition, normal squirrel monkeys given ICV injections of AAV4-BDNF/noggin followed by mitotic labeling with BrdU similarly manifested the robust addition of newly generated striatal neurons. These BrdU$^+$ neurons expressed doublecortin, βIII-tubulin, NeuN, and/or DARPP-32. Importantly, the recruitment of BrdU$^+$/βIII-tubulin$^+$ striatal neurons was limited to those monkeys treated with adeno-BDNF/noggin (70.9 ± 13.7 BrdU$^+$/βIII-tubulin$^+$ cells/mm^3; n=3 treated monkeys), no BrdU$^+$/βIII-tubulin$^+$ striatal neurons were observed in adeno-null-GFP-treated monkeys (n=3 controls). These findings suggest that AAV4-BDNF/noggin-induced neuronal addition may provide a promising means of disease modification in HD.

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3: ABERRANT NEURAL STEM CELL PROLIFERATION AND INCREASED ADULT NEUROGENESIS IN MICE LACKING CHROMATIN PROTEIN HMGB2

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Neural stem and progenitor cells (NSCs/NPCs) are groups of cells found in the mammalian central nervous system (CNS). Neurogenesis in the sub-ventricular zone (SVZ) lining the lateral ventricle in the adult brain is responsible for the generation of neurons bound for the olfactory bulb (OB) – and as such is one of two places in the adult that functions as a potent neurogenic niche. In a previous study utilizing shotgun proteomics analysis our group identified factors that influence the cell cycle dynamics of the post-natal SVZ. Among the putative regulators is HMGB2, a chromatin associated protein with roles in transcription and DNA repair that we found to be dynamically expressed in differentiating NSCs throughout embryonic development, suggesting that it may regulate NSC maintenance. HMGB2$^{-/-}$ mice exhibited SVZ hyperproliferation evident through increased numbers of SVZ NSCs, considerably more concentrated neuroblast migration especially in the proximal rostral migratory stream (RMS) and aberrant increases in newly born interneurons in the olfactory bulb (OB) granule cell layer. These changes were accompanied by increased p21^{Cip1} expression, which may be a part of a previously unknown NSC/NPC cell cycle regulatory axis. Moreover approximately 50% of the mice exhibited ventriculomegaly. Taken together, our findings suggest that HMGB2 functions as a semi-autonomous regulator of neurogenesis in young adult mice through modulation of NSC proliferation, and identifies a potential target via which CNS repair could be amplified subsequent to neuronal loss due to trauma or disease-based mechanisms.

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4: CCNA2 GENE TRANSFER INDUCES CARDIAC REGENERATION AND RESTORES CARDIAC FUNCTION IN A PORCINE MODEL OF MYOCARDIAL INFARCTION

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Our previous work has demonstrated that viral delivery of cDNA encoding *CCNA2* induces cardiac regeneration with significant restoration of cardiac function in mouse and rat models of myocardial infarction (MI). We undertook these studies to determine if *CCNA2* could induce cardiac regeneration in a large animal model of MI, and sought to understand the cellular mechanism of regeneration.

Twenty juvenile swine underwent balloon angioplasty occlusion of the proximal left anterior descending artery (LAD) for 60 minutes followed by placement of a platinum coil to create a chronic occlusion. One week post-MI, 10^{12} replication-deficient adenovirus particles containing *CCNA2* (n=7) or null content (n=6) were injected directly into the peri-infarct zone via left lateral thoracotomy of surviving animals. Animals were sacrificed six weeks later. Hemodynamic measurements and imaging (echocardiography, LV angiography, and MRI) were performed at baseline, prior to surgery and at the time of sacrifice. Ventricular sections at sacrifice were analyzed for markers of proliferation. Adenoviral *CCNA2* was also used to transfect adult mammalian cardiomyocytes *in vitro* with use of adenoviral alpha-sarcomeric actinin-mCherry to select for cardiomyocytes. Cell division was analyzed by time-lapse microscopy.

Myocardial CCNA2 expression was confirmed after gene transfer and was absent in controls. By LV angiography, the *CCNA2* treated animals had improved ejection fraction (EF) from the time of gene transfer to sacrifice compared to controls (27.0 ± 19.5% vs. 9.2 ± 12.7%; p = 0.05). 3D echocardiography also showed improved EF in the *CCNA2* group (27.4 ± 17.2% vs. 8.4 ± 14.3%; p = 0.032). MRI quantitated EF was improved in the *CCNA2* group compared to controls at the time of sacrifice and the relative change from surgery to sacrifice was statistically far superior in the *CCNA2* group (18.4 ± 9.9% vs -3.8 ± 16.0%; p = 0.035). Anti-phosphohistone H3 antibody staining revealed a significantly higher mitotic index in tissues from *CCNA2*-injected animals compared with controls. Time-lapse microscopy images of murine adult mammalian cardiomyocytes transfected with the same adenoviral *CCNA2* injected into porcine hearts demonstrate that 3.3% of adult cardiomyocytes undergo complete cell division compared to 0% of adult cardiomyocytes transfected with null adenovirus.

These data highlight a novel pathway to cardiac regeneration in a large animal model of MI that is not dependent on stem cell transplantation.

5: HISTONE DEACETYLASE INHIBITORS TARGET PLURIPOTENCY GENES IN CORD BLOOD STEM CELLS AND AFFECT THEIR BEHAVIOR

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Cord blood (CB) hematopoietic stem cells (HSC) that undergo rapid *ex vivo* cell division inevitably lose their marrow-repopulating potential. To overcome this decline in HSC function, dividing CB-HSC were treated for 7 days with histone deacetylase inhibitors (HDACIs) *ex vivo* using a serum-free culture system supplemented with cytokines. This strategy led to a greater fraction of the CD34+ cells expressing CD90 that were in mitosis (G2/M), resulting in a 1.1-2.5x10^5 fold expansion of the absolute numbers of CD34+CD90+ cells. HDACI treatment reduced the expression of HDAC1, HDAC3 and HDAC5 suggesting that these HDACs influence the expression of gene programs which define the fate of dividing stem cells. The HDACIs treated CD34+ cells were characterized by up regulation of several pluripotency genes including OCT4/SOX2/NANOG/ZIC3 but not hTERT, increased levels of aldehyde dehydrogenase activity (ALDH) and enhanced expression of CD117 and CD184. These *ex vivo* treated cells retained the ability to establish multi-lineage hematopoiesis following their transplantation into both primary and secondary immune-deficient recipient mice. Transplanted HDACI treated cells did not form teratomas or develop hematological malignancies in primary and/or secondary recipient mice.

The effect of up regulation of pluripotency genes on CD34+CD90+ cell behavior was explored by direct targeting of their RNA. With the inhibition (>80%) of SOX2/OCT4/NANOG expression in HDACI treated CB-CD34+ cells by SiRNA total cell numbers (1.9X10^8±0.2x10^8 to 2.0x10^7±0.5x10^7/CB collection; p=0.0009) as well as the percentage of CD34+CD90+ cells (52.1±11.3% vs 21.3.0±10.1%, p=0.04.) were reduced after a week of culture. A further reduction in total cell number (8.9x10^8±1.0x10^8 to 9.0x10^7±3.2x10^7/CB collection; p=0.01) and the number of CD34+CD90+ cells population (14.0±1.0% vs 2.5±1.3%; p=0.002) occurred in the second week of culture, indicating that pluripotency genes are necessary for retention of stem cell phenotype and proliferative capacity. These data indicate that epigenetically reprogrammed pluripotency genes play an important role in self-renewal, maintenance and expansion of CB-CD34+CD90+ cells.

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6: MICROENVIRONMENTAL CHANGES IN SUBVENTRICULAR ZONE PROMOTE REPAIR PROCESS OF SUBCORTICAL WHITE MATTER AFTER DEMYELINATION

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Stem cells in the adult brain reside in specialized niche, which regulate their properties. The unique features of this microenvironment also aid in maintaining the tissue homeostasis. In the normal brain, subventricular zone (SVZ) neural stem cells termed as type B cells (NSCs; Type B) maintain apical ventricular contact and their basal processes are wrapped around blood vessels. These cells are relatively quiescent and slowly divide to give rise to transit amplifying progenitors also known as type C cells (NPCs; Type C). In normal physiology, the niche and cytoarchitecture of SVZ maintain the population balance of NSCs and NPCs. We investigated how this population dynamics is altered in pathological conditions and observed that in acute subcortical white matter injury model, the numbers of SVZ type B NSCs (GFAP +CD15+) are reduced and type C cells (EGFR+) increased. This is consistent with our previous results, that EGFR+ cells play a functional role in myelin repair after demyelination. NSCs also displayed a decreased self renewal and this is associated with downregulation of notch signaling pathway that plays a crucial role in maintaining “stemness” and it’s restored back to normal levels after remyelination. To further analyze these molecular changes, we studied the interactions of NSC’s with the niche by staining SVZ whole mounts from injured animals for laminin and observed changes in vascular network and laminin distribution. Also during peak demyelination the number of type B cells contacting the blood vessels were reduced from the ependyma as compared to control conditions. These findings suggest that during demyelination, SVZ type B cells lose contact with blood vessels (BVs) and this leads to a loss of self renewal capacity, generating higher number of type C cells, which initiate the remyelination process. Currently we are investigating what signaling cues lead to vascular abnormalities and how it is connected to notch signaling in NSCs.

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7: THE ROLE OF PRIMARY CILIA IN BONE MARROW STROMAL CELLS IN MECHANICALLY INDUCED OSTEOGENESIS

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Osteoporosis is a disease of low bone mass and occurs when the activity of bone-forming osteoblasts is insufficient to counteract that of bone-resorbing osteoclasts. *In vitro* studies have shown that physical stimulation influences the differentiation of bone marrow stromal cells (BMSCs) into osteoblasts. One potential mechanism is the primary cilium, which is an antennae-like organelle extending into the extracellular space. Previous *in vitro* studies demonstrate that primary cilia act as mechanosensors and chemosensors, and are important for lineage commitment. The objective of this study was to determine the role of primary cilia in BMSCs in loading-induced bone formation *in vivo*. Wild type mice were irradiated and transplanted with BMSCs from either $Kif3A^{fl/fl}$ (control) or $Cre\text{-}ER^{T2}$; $Kif3A^{fl/fl}$ (experimental) donors. Mice were injected with Tamoxifen to induce Cre recombination in animals receiving $Cre\text{-}ER^{T2}$; $Kif3A^{fl/fl}$ cells, generating chimeric experimental animals with impaired primary cilia formation only in BMSCs. Right ulnae were axially loaded at 3N for 120 cycles for 3 days, and left non-loaded ulnae served as internal controls. Calcein and alizarin dyes were injected to mark new bone formation. After sacrifice, ulnae were prepared for histological analysis and dynamic histomorphometry. Donor-derived BMSCs were detected within bone, and their presence indicates that transplanted BMSCs contributed to bone formation. Mechanical loading resulted in increases in the percent of mineralizing surface (MS/BS), mineral apposition rate (MAR) and bone formation rate (BFR/BS) in loaded ulnae compared to non-loaded ulnae for both groups. However, chimeric experimental animals exhibited a significantly reduced osteogenic response to loading. Relative (r) measurements were obtained by subtracting values of non-loaded from loaded ulnae. Reductions of 46% ($p<0.05$) in rMS/BS and 53% in rBFR/BS ($p<0.001$) were observed in experimental animals compared to controls. These results demonstrate that primary cilia in BMSCs are important for loading-induced bone formation.

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8: INVESTIGATION INTO THE ROLES OF THE TRANSCRIPTIONAL CO-REGULATORS HCF-1 AND HCF-2 IN MOUSE EMBRYONIC STEM CELLS

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HCF-1 is a highly conserved transcriptional co-regulator that participates in diverse biological processes. HCF-1 is known to partner with multiple different transcription factors and recruit additional chromatin modifiers to assemble appropriate transcriptional complexes for gene regulation. Among the HCF-1 partners, the transcription factors Ronin, FOXO, and E2F1 have been implicated to be critical for embryonic stem cell pluripotency and/or proliferation, but the direct role of HCF-1 in stem cell function has not been thoroughly investigated. To investigate the roles of HCF-1 in mouse embryonic stem cells (mESCs), we developed a tetracycline inducible HCF-1 knockdown (KD) mESC line. We found that HCF-1 KD reduces plating efficiency and mESC proliferation. However, mESCs retain alkaline phosphatase activity after HCF-1 KD. A preliminary experiment suggests that HCF-1 does not impact the expression of pluripotency factors under normal culture conditions. However, after LIF removal, pluripotency factor expression profiles differed between HCF-1 KD and control mESCs. We are further investigating the impact of HCF-1 KD on differentiation. We will examine pluripotency factor expression and alkaline phosphatase activity under different differentiating culture conditions. Also, we plan to mine the existing ChIP-seq data as a first step in identifying likely HCF-1 partners and HCF-1 regulated mESC functions. In addition to HCF-1, we are investigating HCF-2 because it is closely related to HCF-1, but little is known of its function. To facilitate this investigation, we generated HCF-2 KD mESC lines and we are generating a HCF-2 conditional knockout mESC line. A preliminary experiment suggests that HCF-2 KD also impacts pluripotency gene expression compared to control after induction of differentiation, but interestingly, in a manner different from that of HCF-1 KD. The role of HCF-2 in differentiating mESCs will also be further investigated.

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9: AN EXPANDED NANOG INTERACTOME FOR STEM CELL PLURIPOTENCY AND SOMATIC CELL REPROGRAMMING

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Nanog are well-known transcription factors that play fundamental roles in stem cell self-renewal, pluripotency and somatic cell reprogramming. However, limited information is available on Nanog associated protein complexes and their intrinsic protein-protein interactions that dictate the critical regulatory activities of Nanog. Here we employed an improved affinity purification approach combined with mass spectrometry to purify Nanog associated protein complexes in mouse embryonic stem cells (mESCs), and discovered 119 Nanog interacting proteins and many of them are novel Nanog partners important for self-renewal and pluripotency of mESCs. Notably, we found that Nanog are associated with multiple chromatin modifying complexes, such as NuRD complex, Polycomb complex, Lsd1 complex, MLL1 complex, Sin3 complex and Ten eleven translocation (Tet) family methylcytosine hydroxylase Tet1 and Tet2, with documented as well as newly proved functional significance in stem cell maintenance and somatic cell reprogramming. We will present an expanded Nanog interactome together with the physical and functional validation of the interactome. Our study establishes a solid biochemical basis for genetic and epigenetic regulation of stem cell pluripotency and provides a framework for further dissecting the molecular mechanism underlying Nanog's function in pluripotency and reprogramming.

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10: MECHANISMS OF SOMATIC CELL REPROGRAMMING

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Background: Somatic cell reprogramming has great promise for regenerative medicine. However, understanding the basic mechanisms of reprogramming is crucial for improvement of the iPSC technology toward clinical applications. Since only little is known about the epigenetic events in the early phase of iPSC generation, we aim to identify epigenetic regulators which mediate the early epigenetic events at endogenous pluripotency loci.

Methods: To define the early epigenetic changes during reprogramming we employed time course analyses of gene expression, transcription factor and histone binding via chromatin immunoprecipitation, as well as DNA methylation measurements. For the latter we used *Hpa*II digestion and *Msp*I digestion of glucosylated DNA to distinct between the DNA methylation species 5-methylcytosine and 5-hydroxymethylcytosine. Proteomic analysis and a functional screen identified candidate epigenetic regulators. Subsequent studies examined if and how these epigenetic regulators modulate the early epigenetic events in iPSC reprogramming.

Findings: We identified two epigenetic regulators, Parp1 and Tet2, which work in concert with the reprogramming factors to mediate the early epigenetic events in iPSC reprogramming. They are recruited to the endogenous pluripotency loci (*Nanog*, *Esrrb*) in the early phase of iPSC reprogramming, preceding transcription of these loci. While Parp1 functions in the regulation of 5-methylcytosine, Tet2 is essential for the generation of 5-hydroxymethylcytosine. Parp1 and Tet2 are needed to establish the histone modifications that typify an activated chromatin state, and Parp1 promotes accessibility to the Oct4 reprogramming factor.

Impact: These data support necessary but distinct roles for Parp1 and Tet2 in the regulation of epigenetic marks and local chromatin structure at pluripotency loci during an early stage of iPSC reprogramming that precedes transcription at these loci. The data further suggest that 5-hydroxymethylcytosine does not simply represent an intermediate in the 5-methylcytosine demethylation process, but functions as an epigenetic mark, possibly recruiting *trans*-acting factors that promote chromatin remodeling.

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11: INDIRECT REGULATION OF NEURAL PROGENITOR CELLS BY FLOW STIMULATED ENDOTHELIAL CELLS

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Neurogenesis is most abundant in highly vascularized regions of the brain. Endothelial cells (ECs) within the microvasculature are known to promote neural stem cell (NSC) and neural progenitor cell (NPC) proliferation and inhibit differentiation directly via cell-cell contact and indirectly through extracellular matrix (ECM) and soluble factors. To date, NPC-EC *in vitro* models use statically-cultured ECs, however, dynamically cultured ECs exhibit profound changes in soluble factor and ECM secretion. Utilizing an *in vitro* model to collect soluble and insoluble factors produced by dynamically stimulate (10 dynes/cm^2) ECs, we show significant increases in bFGF and EGF in both isolated fractions and significant increases in laminin, fibronectin, and collagen IV in the ECM.

Adult NPCs from the subventricular zone (SVZ) were evaluated in response to dynamically or statically generated EC-factors: conditioned medium (CM) and ECM. Increases in cell number and spreading were seen by NPCs cultured on dynamic ECM compared to static ECM, presumably due to increased matrix proteins and sequestered growth factors. NPC response to the CM was evaluated as adherent cultures and as neurospheres. Viable, adherent NPCs were more abundant when cultured in EC-CM than non-CM, with static EC-CM exhibiting a greater increase in cell number over flow CM. Neurosphere forming capacity was evaluated by pre-conditioning SVZ cells for 10 days in EC-CM or non-CM then dissociated cells were seeded in a neural cell forming colony assay. Pre-conditioned cells from EC-CM formed larger and more numerous colonies with the flow-CM group generating the largest and most numerous colonies. Colonies greater than 1 mm are being evaluated to distinguish NSC from NPC derived colonies.

Clear differences exist in NPC response to EC factors in a flow-dependent manner, suggesting traditional static models are unrepresentative of the native microenvironment. Inclusion of flow-mediated changes may further enhance our understanding of the neurovascular niche.

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12: NUCLEAR GENOME TRANSFER IN HUMAN OOCYTES ELIMINATES MITOCHONDRIAL DNA VARIANTS

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Mitochondrial DNA mutations transmitted maternally within the oocyte cytoplasm often cause life-threatening disorders. Here we explore the use of nuclear genome transfer between unfertilized oocytes of two donors in order to prevent the transmission of mitochondrial mutations. Nuclear genome transfer did not reduce developmental efficiency to the blastocyst stage and genome integrity was maintained provided that spontaneous oocyte activation was avoided through the transfer of incompletely assembled spindle-chromosome complexes. Mitochondrial DNA transferred with the nuclear genome was initially detected at levels below 1%, decreasing in blastocysts and stem cell lines to undetectable levels, and remained undetectable after passaging for over one year, clonal expansion, differentiation into neurons, cardiomyocytes or β-cells, and after cellular reprogramming. Stem cells and differentiated cells had mitochondrial respiratory chain enzyme activities and oxygen consumption rates indistinguishable from controls. These results demonstrate the potential of nuclear genome transfer to prevent the transmission of mitochondrial disorders in humans.

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13: NAC1 IS DISPENSABLE FOR STEM CELL MAINTENANCE BUT IS REQUIRED FOR SOMATIC CELL REPROGRAMMING

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Nac1 (Nucleus Accumbens-1) belongs to the Bric-a-brac Tramtrac Broad complex/Pox virus and Zinc finger (BTB/POZ) family of transcription factors. It is ubiquitously expressed and originally identified in the nucleus accumbens of rat brain as a cocaine-inducible gene. Nac1 is also enriched in mouse embryonic stem cells (mESCs), interacts directly with Nanog, and shares many target genes with several stem cells pluripotency factors. Here we show that Nac1 null mESCs can be maintained indefinitely and are pluripotent, as demonstrated by *in vitro* and *in vivo* assays. However, loss of Nac1 skews differentiation of mESCs toward the trophectoderm lineage. In addition, somatic cell reprogramming using Nac1 deficient mouse embryonic fibroblasts demonstrates that Nac1 is required for efficient generation of high quality iPSC colonies. The molecular mechanism underlying the distinct function of Nac1 in stem cell maintenance and somatic cell reprogramming is currently unknown and will be discussed in our study.

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14: SUSTAINED LEVELS OF FGF2 MAINTAIN UNDIFFERENTIATED STEM CELL CULTURES WITH BIWEEKLY FEEDING

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An essential aspect of stem cell culture is the successful maintenance of the undifferentiated state. Many types of stem cells are FGF2 dependent, and pluripotent stem cells are maintained by replacing FGF2-containing media daily, while tissue-specific stem cells are typically fed every 3rd day. Frequent feeding, however, results in significant variation in growth factor levels due to FGF2 instability, which limits effective maintenance due to spontaneous differentiation. We report that stabilization of FGF2 levels using controlled release PLGA microspheres improves expression of stem cell markers, increases stem cell numbers and decreases spontaneous differentiation. The controlled release FGF2 additive reduces the frequency of media changes needed to maintain stem cell cultures, so that human embryonic stem cells and induced pluripotent stem cells can be maintained successfully with biweekly feedings.

15: ZFP281 RECRUITS THE NURD REPRESSOR COMPLEX TO MEDIATE NANOG AUTOREPRESSION AND INHIBITS SOMATIC CELL REPROGRAMMING

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The homeodomain transcription factor Nanog plays an important role in embryonic stem cell (ESC) self renewal and is essential for acquiring ground state pluripotency during reprogramming. Enforced expression of Nanog liberates ESCs from requisite LIF dependence and accelerates somatic cell reprogramming. Understanding how *Nanog* is transcriptionally regulated is important to further dissect mechanisms of ESC pluripotency and somatic cell reprogramming. We previously showed that a close partner of Nanog, Zfp281, functions as a transcriptional repressor to restrict *Nanog* expression in mouse ESCs. Here we report that Nanog is subjected to a negative auto-regulatory mechanism, i.e. autorepression, in ESCs, and that such autorepression requires the coordinated action of Zfp281. Mechanistically, Zfp281 maintains the integrity of the NuRD repressor complex and recruits it onto the *Nanog* enhancer/promoter region to mediate *Nanog* autorepression. Functionally, Zfp281-mediated Nanog autorepression presents a roadblock for direct molecular reprogramming of somatic cells or fusion-based reprogramming of somatic and ES cell heterokaryons. Our results identify both a novel transcriptional regulatory mode of *Nanog* gene expression and a transcription repressor as a novel barrier to efficient reprogramming, and shed new light into the mechanistic understanding of Nanog function in pluripotency and reprogramming.

[Supported by NYSTEM contract # C026420]

16: EQUINE BONE MARROW-DERIVED MESENCHYMAL STEM CELLS (MSCs) ARE HETEROGENEOUS IN MHC CLASS II EXPRESSION AND CAPABLE OF INCITING AN IMMUNE RESPONSE *IN VITRO*

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Bone marrow-derived MSCs are routinely used in human and veterinary medicine to enhance tissue regeneration. Allogeneic MSCs have been investigated as an available source of stem cells for time-of-diagnosis treatment. Although late passage MSCs have been well characterized, it is more common to use early passage MSCs in a clinical setting to maintain stemness. The immunophenotype of early passage MSCs has not been previously described. The purposes of this study were to immunophenotype P2-P8 MSCs and to compare the immunogenicity of MSCs with differing MHC II phenotypes through mixed leukocyte reactions (MLRs). Our hypotheses were that MSCs would be heterogeneous in MHC II expression and that MHC II negative MSCs would have low immunogenicity *in vitro* while those that were positive would be immunogenic. MSCs and peripheral blood leukocytes (PBLs) were obtained from horses of known MHC haplotype. Immunophenotyping of MHC II was performed using flow cytometry. Modified one-way MLRs were performed using MHC-matched and mismatched responder PBLs and stimulator PBLs and MSCs. Following 5 days of culture, PBLs were stained with CD3/APC antibodies. Proliferation of gated CFSE-labeled CD3+ responder T cells was evaluated via CFSE attenuation using flow cytometry and reported as number of cells in the proliferating T cell gate. MSCs (n=10 horses, 13 bone marrow aspirates) varied widely in MHC II expression from P2-P8. MSCs from 11 of 13 aspirates were positive for MHC II expression at P2 and 6 of those 11 remained positive through P8. MSCs from only one horse were MHC II negative from P2-P8 which was repeatable on a second aspirate. MLR results revealed that MHC II positive MSCs caused significantly increased responder T cell proliferation compared to MHC II negative and MHC-matched MSCs. These results suggest that MSCs must be immunophenotyped and confirmed as MHC II negative prior to allogeneic application.

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17: INTERACTIONS BETWEEN CARDIAC REPAIR CELLS AND POLARIZED MACROPHAGES

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Introduction: The outcome of engineered cardiac therapies depends in part on the inflammatory environment at the site of injury. Following myocardial infarction, there is a dynamic infiltration of pro-inflammatory (M1) and pro-healing (M2) macrophages that play a significant role during the healing process. Repair cells delivered into the injured myocardium are inevitably subjected to this inflammatory environment, which could impact the repair cells' function and survival. The present study investigated how this environment may affect stem cells and stem cell-derived cardiomyocytes.

Methods: Mesenchymal stem cells (MSCs) and pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) were cultured in the presence of M1 and M2 macrophages. Monocytes were differentiated and polarized into M1 and M2 macrophages by the addition of LPS/IFN-γ/M-CSF and IL-4/IL-13/M-CSF respectively. The effect of macrophage polarization on the growth and function of MSCs or hPSC-CMs was determined by measuring cell growth, differentiation potential, and maturation.

Results: The inflammatory cell type (M1 vs M2) present during co-culture affected the growth and gene expression of mesenchymal stem cells. The cardiac cell population seems to also be affected by the type of inflammatory cells present during co-culture.

Conclusions: The type of inflammatory cells present at the site of implantation of engineered cardiac patches may affect the behavior of the repair cells.

Future Directions: Current efforts are focused on the development of *in vitro* culture systems in which the health of the cardiac patch can be monitored *in vitro* while exposed to inflammatory cells.

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18: HOMOLOGOUS RECOMBINATION DNA REPAIR GENES PLAY A CRITICAL ROLE IN REPROGRAMMING TO A PLURIPOTENT STATE

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Induced pluripotent stem (iPS) cells hold great promise for personalized regenerative medicine. However, recent studies show iPS cell lines carry genetic abnormalities, suggesting reprogramming may be mutagenic. Here we show that ectopic expression of the reprogramming factors increases the levels of phosphorylated histone H2AX, one of the earliest cellular responses to DNA double strand breaks (DSBs). Further mechanistic studies uncover a direct role of the homologous recombination (HR) pathway, a pathway essential for error-free repair of DNA DSBs, in reprogramming. This role is independent of the use of integrative or non-integrative methods to introduce reprogramming factors, despite the latter being considered a safer approach that circumvents genetic modifications. Finally, deletion of the tumor suppressor p53 rescues the reprogramming phenotype in HR-deficient cells primarily through restoration of reprogramming-dependent defects in cell proliferation and apoptosis. These novel mechanistic insights have important implications for the design of safer approaches to create IPS cells.

19: CONTROLLING THE BREAST STEM CELL STATE AND LINEAGE SPECIFICATION BY TRANSCRIPTION FACTORS SLUG AND SOX9

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Regulatory networks orchestrated by key transcription factors (TFs) play a central role in the determination of stem cell states and subsequent differentiation into various lineages. We have previously identified two TFs, Slug and Sox9, that act cooperatively to determine the mammary stem cell (MaSC) state. Inhibition of either Slug or Sox9 blocks MaSC activity in primary mammary epithelial cells. Conversely, transient coexpression of exogenous Slug and Sox9 suffices to convert differentiated luminal cells into MaSCs with long-term mammary gland-reconstituting ability. The transient expression of Slug and Sox9 induces MaSCs by activating autoregulatory gene expression programs, which sustains the stem cell state. Using conditional knock-out and transgenic reporter animals, we found that, in addition to their role in bipotent stem cells, Slug and Sox9 control the specification of bipotent mammary stem cells into unipotent luminal or basal progenitor cells. Outside the stem cell compartment, Sox9 is specifically expressed in luminal progenitor cells and Slug is expressed in the basal lineage. Consistent with this expression pattern, deficiency of Sox9 inhibits the formation of luminal progenitor cells, whereas Slug is involved in the maintenance of the basal lineage. This demonstrates Slug and Sox9 each controls the specification of a major lineage in the mammary gland.

The key regulator of mammary stem/progenitor cells may contribute to breast cancer pathogenesis. Indeed, we have shown that coexpression of Slug and Sox9 promotes the tumorigenic and metastasis-seeding abilities of human breast cancer cells and is associated with poor patient survival, providing direct evidence that human breast cancer stem cells are controlled by key regulators similar to those operating in normal murine MaSCs.

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20: IDENTIFICATION OF THE OPTIMAL BIOLOGIC TO ENHANCE ENDOGENOUS STEM CELL RECRUITMENT

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Bone marrow derived mesenchymal stem cells (MSC's) demonstrate promise for musculoskeletal regenerative therapy. Unfortunately, this therapy is not widely available because obtaining autologous stem cells is costly, time consuming, and not presently approved by regulatory agencies. An alternate approach to the direct delivery of stem cells is to exploit the concept of in situ tissue engineering whereby methods are used to recruit the body's endogenous reservoir of local stem cells. Biologics such as bone marrow aspirate concentrate and platelet rich plasma (PRP) have successfully enhanced recovery from musculoskeletal injuries. These biologics contain platelet-derived growth factor (PDGF) and transforming growth factor- β (TGF-β) which could act as chemotactics for stem cells. The purpose of this study is to identify the optimal biologic for recruitment of stem cells. Our hypothesis is that PRP will result in the greatest migration of MSC's because of the milieu and concentration of growth factors contained in PRP. A microfluidics device was used to compare the ability of biologics to recruit stem cells. Cells were placed in the center well with six channels leading to different biologics. Preliminary results indicate that all biologics resulted in migration of stem cells. Live-cell imaging over the course of two days is also being performed to track the migratory patterns of the cells.

[Supported by NYSTEM contract # CO28091]

21: LEUKEMOGENIC TRANSFORMATION OF HEMATOPOIETIC CELLS BY OSTEOBLAST PRECURSORS

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Osteoblasts, the bone forming cells, have been implicated in self-renewal and expansion of hematopoietic stem cells (HSCs) and the fate of malignant stem cells. However, the molecular basis of these functions remains poorly understood. Here we show that constitutive activation the Wnt/β-catenin signaling pathway in osteoblast precursors in mice (*βcat(ex3)$_{osb}$*) disrupts hematopoiesis by shifting the differentiation potential of HSC progenitors to the myeloid lineage. As a result, there is accumulation of granulocyte/monocyte progenitors and development of acute myeloid leukemia (AML). This AML phenotype is associated with clonal evolution at the cytogenetic level since clonal abnormalities could be detected in leukemic blasts from *βcat(ex3)$_{osb}$* mice. Progression towards cells autonomous AML was shown following bone marrow transplantation from *βcat(ex3)$_{osb}$* to wild-type lethally irradiated mice. Consistent with these data, malignancy-inducing osteoblasts, detected by nuclear accumulation of β-catenin in bone marrow biopsies, were identified in 38% of patients with myelodysplasia (MDS) or AML. Specifically, β-catenin localized to the nucleus of osteoblasts in 15 of 44 patients with MDS (34%), 9 of 26 patients with AML (34.61%), and 12 of 26 patients (46.1%) with AML that arose from prior MDS. In contrast, β-catenin localized to the cell membrane of osteoblasts of all healthy controls examined. Activation of β-catenin signaling was detected specifically in osteoblasts from the patients with nuclear accumulation of β-catenin in osteoblasts. Moreover, β-catenin-activated osteoblasts increased proliferation of human HSCs in co-cultures, increased the percentage of early myeloid cells (CD34+/CD33+) but decreased the percentage of late differentiated myeloid cells (CD34+/CD11b+/CD14+) suggesting maturation arrest of the myeloid lineage and accumulation of immature myeloid cells. These findings demonstrate that genetic alterations in osteoblast precursors can induce AML in mice and are associated with AML development in humans. They also provide a molecular basis for the leukemogenic transformation.

22: SMALL MOLECULE INHIBITION OF ANDROGEN/β-CATENIN ACTIVITY IN PROSTATE CANCER

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The androgen receptor (AR) is the major therapeutic target in aggressive prostate cancer. However, targeting AR alone can result in disease recurrence since it has been suggested that AR pathway can be activated by alternative signaling pathways. Therefore, simultaneous targeting of multiple pathways could in principle be an effective new approach to treating prostate cancer. Here we provide proof-of-concept that a small molecule inhibitor of nuclear β-catenin activity (called iCRT3) can inhibit both AR and Wnt/β-catenin signaling pathways that are often misregulated in prostate cancer. The idea underlying the approach takes advantage of the fact that AR interacts with, and is transcriptionally regulated by β-catenin, the main effector of Wnt pathway. Treatment with iCRT3 ablated prostate cancer cell growth by disruption of both β-catenin/TCF and β-catenin/AR protein interaction, reflecting the fact that TCF and AR have overlapping binding sites on β-catenin. The iCRT3 treatment also resulted in decreased occupancy of β-catenin on the AR promoter and diminished AR and AR/β-catenin target gene expression. Surprisingly, iCRT3 treatment resulted in decreased AR binding to target genes, providing new insight into the unrecognized function of β-catenin in prostate cancer.

Growing evidence shows that Wnt/β-catenin signaling is highly active in “cancer stem cells (CSCs)”, a small population of cancer cells defined by their ability to self-renew as well as differentiate to a daughter cell type. In prostate cancer, CSCs has been suggested to be resistant to conventional AR antagonist treatment resulting in disease recurrence. Thus, it is important to develop therapeutic approaches to selectively target the self-renewing cell population. Using the sphere formation assay, which has been widely used to study CSCs biology, we saw that iCRT3 treatment as well as β-catenin knockdown blocked renewal of sphere forming cells. Importantly, iCRT3 inhibited tumor growth in an *in vivo* xenograft model, indicating its promising therapeutic potential.

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23: HUMAN CORD BLOOD DERIVED STEM CELLS PROMOTE WOUND HEALING AND HAVE THERAPEUTIC POTENTIAL FOR PATIENTS WITH RECESSIVE DYSTROPHIC EPIDERMOLYSIS BULLOSA

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There has been a growing interest in utilizing bone marrow (BM) or cord blood (CB) for the treatment of degenerative diseases. Preclinical and clinical studies also indicated that stem cells in the BM or CB contribute to wound healing and may rescue a group of basement membrane defects, recessive dystrophic epidermolysis bullosa (RDEB) that is caused by mutations in *COL7A1* gene. Recently, CB- derived multipotent stem cells, USSCs, have been applied in several animal models of degenerative diseases with beneficial outcomes. Here, we demonstrated that CB-USSCs share several embryonic stem cell properties and could be induced to express hallmark genes of keratinocyte differentiation. In the wounding model, a single USSC intradermal injection promoted epithelialization and facilitated formation and remodeling of epidermis, accompanied by a significantly accelerated rate of wound healing on days 6-10 post wounding ($F_{(1,168)}$=50.8 $p<0.01$). Moreover, USSCs constitutively express Col7A1 and human specific Col7A1 was detected at the epidermal-dermal junction of the healed skin treated with USSCs. *In vivo* BLI revealed specific migration of USSCs from a distant intradermal injection site toward the wound, as well as following systemic injection. Temporal quantification on the total bioluminescence indicated an overall 59.9% signal loss over 3 days followed by a 95.06% loss at 1 week. The bioluminescence in the area of wound was then maintained at ~0.5-1% level till the end of the experiment (3 month). *In vitro* chemotaxis analysis suggested that the specific migration of USSCs to the wound may be mediated via the interaction between CXCR4 and SDF1. The therapeutic effects of USSCs on RDEB are also under investigation using Col7a1-/- mouse model. These results suggest significant beneficial effects of CB-USSCs on wound healing and raised the possibility of USSC's therapeutic benefit in the treatment of patients with RDEB.

24: MULTIPOTENCY OF RETINAL PROGENITORS REQUIRES THE JOINT FUNCTIONS OF THE HOMEOBOX GENES *Six3* AND *Six6* IN MICE

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Six3 and *Six6* are homeodomain-containing transcription factors with identical DNA-binding domains. They are largely co-expressed in retinal progenitors in mice with Six3 expression prior to *Six6*. In our previous studies, conditional inactivation of *Six3* at E8.5 mouse embryos demonstrated that the specification of retinal progenitor cells requires Six3-mediated suppression of Wnt8b at the anterior neural plate. On the other hand, inactivation of *Six6* in mice does not affect retinal cell fate determination, but causes defects in retinal proliferation.

In this study, individual inactivation of *Six6* or *Six3* in E10.5 mouse retinal cells did not cause any major defect in retinal progenitors and terminal cell differentiation. In contrast, simultaneous inactivation of both *Six3* and *Six6* lead to severe defects in the maintenance of retinal progenitors and multi-lineage retinal cell differentiation. In E14.5 *Six3;Six6* compound null retinas, we found that: 1) the expression of Sox2 (an essential regulator of the competency of retinal progenitors) and its downstream target Notch1 were severely reduced or ablated; 2) the expression of Math5, Brn3b and Islet 1 (the ganglion cell differentiation markers) was absent or severely down-regulated; 3) the expression of Otx2 and Crx (the photoreceptor cell differentiation markers) was absent or severely reduced; 4) the expression of NeuroD and Fox4N (the amacrine cell differentiation markers) was largely unaffected; 5) the expression of Otx1 (a specific marker for the ciliary margin) and Axin2 (an endogenous readout of Wnt/β-catenin signaling that promotes ciliary margin cell fate) was significantly elevated; 6) the expression Pax6 (an essential factor for the multipotency of retinal progenitors) was increased; 7) the expression of Rax (an essential transcription factor that is required for optic vesicle formation and Otx2 expression) was largely unaffected.

We conclude that the multipotency of retinal progenitors require the joint functions of the homeobox genes *Six3* and *Six6* in mice.

25: CONVERGENCE OF COMMON EPIGENETIC SIGNATURES BETWEEN PLURIPOTENT AND UNIPOTENT STEM CELLS

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As the precursor of germ cells, mammalian spermatogonial stem cells (SSCs) undergo unipotent differentiation while still possessing ultimate totipotent development potential to propagate across generations. Many groups reported that *in vitro* cultured SSCs are unusually capable of spontaneous conversion to multipotent adult spermatogonial-derived stem cells (MASCs), which share most defining features with embryonic stem cells (ESCs) including the capability of inducing teratomas and contributing to chimeras. Despite these impressive characteristics, the basic biology of SSCs is poorly understood. To explore the mechanisms that endow SSCs with the ability to establish and control multipotency *in vitro* and totipotency *in vivo*, we profiled global mRNA and chromatin changes in SSCs, MASCs, and haploid spermatids. We found that SSCs preserve over 60% of H3K4me3 and H3K27me3 promoter 'bivalent' histone modifications (K4+K27) as in ESCs. Many of these transcription 'poised' genes have essential functions in pluripotency maintenance and various developmental processes not restricted to germline lingeage. Additionally, we found that polycomb-group (PcG)-mediated H3K27me3 transcription repression features prominently in unipotent to multipotent progression. To achieve reprogramming, germline specific genes acquire promoter H3K27me3 marks and pluripotency genes lose them, turning from transcription 'poised' state (K4+K27) to activation. Interestingly, genes that maintain similar expression between SSCs and MASCs possess more ESC-like epigenomes in MASCs, indicating that the change in chromatin provides the environment for gene activation or silencing during reprogramming. Our study in round spermatids also showed that the SSC's epigenome, especially the 'bivalent' histone modifications at developmental promoters, is largely preserved in haploid germ cells and consistent with their orthologs in mature human sperms. Collectively, our findings explain how transcription associated histone modifications contribute to the establishment of multipotency when SSCs reprogram to MASCs. The comprehensive map of epigenome in both SSCs and spermatids also provides great prospect to the understanding of unique developmental features of the germline.

26: CHROMIUM-INDUCED DNA DAMAGE RESPONSES IN HUMAN IPS CELLS

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Inducible pluripotent stem (iPS) cells are derived from somatic cells through reprogramming. Recent studies have shown that iPS cells offer a great alternative for basic embryonic stem cell research, as well as for drug development. Chromium (VI) compounds are well established environmental carcinogens that produce genotoxic effects, leading to human cancers. Although extensive studies have been carried on toxic and carcinogenic effects of Cr(VI) compounds on somatic cells it remains entirely unknown as to the effect of Cr(VI) on pluripotent stem cells. In the present study, we examined the effect of Cr(VI) on some key cellular components during DNA damage responses in human iPS cells and compared the effect with that of selected cell lines. We found that Cr(VI) treatment (10 μM Na_2CrO_4) led to a slight increase in the total p53 protein level in human iPS cells, Tera-1 human teratoma cells, and BEAS-2B lung epithelial cells. While p53 phosphorylation at Ser15 exhibited a clear time-dependent increase in Tera-1 and BEAS-2B cells it was undetectable in human iPS cells. Phosphorylation of ATM (Ser1981) and H2AX (γ-H2AX) were either undetectable or significantly compromised in human iPS cells after Cr(VI) treatment. In contrast, ATM phosphorylation and γ-H2AX signals were easily detected in Tera-1 cells in response to Cr(VI) treatment. Interestingly, p53 phosphorylation at Ser20 was high in BEAS-2B cells but not detectable in human iPS cells after Cr(VI) treatment. These findings strongly suggest that iPS cells are likely more resistant to Cr(VI)-induced DNA damage compared with the somatic cells.

27: REGULATION OF THE HIPPO PATHWAY IN THE MESENCHYMAL LINEAGE BY THE TRANSCRIPTION FACTOR SOX2

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Adipocytes and osteoblasts arise from a common progenitor cell derived from mesenchymal stem cells (MSCs). Several lines of evidence suggest a reciprocal relationship between the adipocytic and osteoblastic lineage. While the two master regulators of osteogenesis and adipogenesis Runx2 and PPARγ, respectively have been identified, very few factors governing the early fate decisions of MSCs upstream of these factors are known. In this study, we have studied the interaction between Sox2 (the stem cell transcription factor), and YAP (the downstream effector of the Hippo pathway) in cells of the mesenchymal lineage. YAP is a transcriptional cofactor and its transcriptional activity is usually restrained by the kinase cascade of the Hippo pathway. Our studies indicate that Sox2 and YAP regulate self-renewal of MSCs and early fate decisions in osteo-adipo lineage. To our knowledge, this is the first study that establishes a direct role for the stemness transcription factor, Sox2, in regulating the Hippo pathway. Additionally, our work identifies a novel fate determining mechanism by Sox2 and YAP in the osteo-adipo lineage. YAP and TAZ have always been assumed to function similarly and TAZ has been reported to induce osteogenesis. Our studies suggest that YAP functions differently from TAZ in the osteo-adipo lineage.

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28: DYSTROGLYCAN IS AN ORGANIZER OF STEM CELL NICHE STRUCTURE AND FUNCTION IN THE DEVELOPING POSTNATAL VZ/SVZ

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The subventricular zone (SVZ) of the vertebrate forebrain undergoes extensive cellular reorganization during early postnatal development to transform into an adult neural stem cell niche. Intriguingly, this reorganization takes place as SVZ neural stem cells undergo their major wave of gliogenesis. Here we report that the adhesion receptor dystroglycan regulates the organization of extracellular matrix (ECM) in the emerging SVZ stem cell niche such that dystroglycan loss-of-function results in the failure of appropriate niche cell organization, dysregulated progenitor proliferation and increased gliogenesis. During the first postnatal week, radial glia transform into ependymal cells and adult neural stem cells, which together organize into pinwheel structures at the ventricular surface. In mice with neural stem cell-specific deletion of dystroglycan (DG-cKO), radial glia lost their apical associations with ventricular surface-associated laminin. Radial glial cell cycle exit and ependymal cell differentiation were delayed, with a subsequent delay in pinwheel formation. Ependymal cell differentiation can be recapitulated *in vitro* and blocking dystroglycan-ligand interactions during this process similarly delayed ependymal cell maturation and impaired pinwheel-like clustering. Dystroglycan loss-of-function also impacts the gliogenic potential of SVZ progenitor cells; a single intra-ventricular injection of dystroglycan blocking antibody in perinatal rats produced an acute increase in immature oligodendrocyte progenitors (OPCs) within the SVZ. This preceded an increase in progenitor proliferation, suggesting that an induction of gliogenesis occurred independent of progenitor amplification. Increased gliogenesis was also observed throughout the first postnatal week in the DG-cKO SVZ. However, the differentiation of dystroglycan-null OPCs was delayed, with the early postnatal corpus callosum containing more OPCs and a higher proportion of OPCs with an immature phenotype, resulting in delayed myelination. Together these findings suggest that dystroglycan-ECM interactions are important for the organization of a functional stem cell niche with normal gliogenic capacity, as well as for the timely maturation of oligodendrocyte lineage cells.

29: SUPPRESSION OF ADIPOGENESIS BY GOLD NANOPARTICLES *IN VITRO*.

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Nanoparticles are ubiquitous components of numerous technologies, which range from solar power panels to drug delivery and medical imaging vehicles. The additional potential hazards of materials due to length scale are just being recognized, but despite the growing body of research on this topic, relatively little has focused on the influence of nanoparticles on stem cell differentiation. Gold nanoparticles (AuNPs) are currently used in numerous medical applications since they are considered relatively inert. Herein, we describe their *in vitro* impact on human adipose derived stromal cells (ADSCs) using 13 nm and 45 nm citrate coated AuNPs. In their non-differentiated state, ADSCs were penetrated by the AuNPs and stored in vacuoles. Similar to dermal fibroblasts, the presence of the AuNPs in ADSCs increased population doubling times, and decreased both cell motility and cell-mediated collagen contraction. The degree to which the cells were impacted was a function of particle concentration, where the smaller particles required a sevenfold higher concentration to have the same effect as the larger ones.

In media promoting the differentiation of ADSC to adipocytes the total uptake of AuNPs was reduced. However, both 13 nm and 45 nm AuNPs inhibited adipogenesis as measured by secretion of adiponectin, a regulator of energy metabolism, and the accumulation of Oil red O staining lipid droplets. These effects were preceded by and correlated with early transient increases in the secretion of the adipogenesis inhibitor DLK1 and with relative reductions in fibronectin. Although removal of exogenous AuNPs reduces cellular NP levels resulting in the restoration of normal ADSC function, even transient changes in adiponectin levels may adversely impact energy metabolism, leading to systemic changes. Hence, careful choice of size, concentration and clinical application duration of AuNPs is warranted.

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30: CHARACTERIZATION OF TCF-DEPENDENT AND INDEPENDENT FUNCTIONS OF β-CATENIN IN COLORECTAL CANCER

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Misregulation of the Wnt/β-catenin signaling pathway is required for colorectal cancer (CRC), as mutations in the pathway are found in a vast majority of CRC patients. These mutations lead to an accumulation of β-catenin, which can translocate to the nucleus, where it interacts with transcription factors including the TCF/LEF family. Targets of β-catenin/TCF transcription include CyclinD1 and c-Myc, which function in cell growth and proliferation. The Wnt pathway has an important function in stem cells, as it has been implicated in both embryonic stem cells (ESCs) and cancer stem cells (CSCs). While much of the research on the Wnt pathway is focused on TCF-mediated transcription, β-catenin has been shown to interact with a number of other factors, including E-cadherin, which is critical for cell-cell adhesion, and pluripotency factors such as Oct4 and Klf4.

While β-catenin has been shown to be required in ESCs, evidence has shown that its interaction with TCF/LEF transcription factors is not. This lead us to investigate whether there is a similar requirement for β-catenin in CSCs which are responsible for tumor initiation. To investigate this, we used an *in vitro* sphere formation assay as a model for tumor initiation. We utilized a combination of TCF-transcriptional reporters, small molecule inhibitors, and inducible shRNA lines to assess the requirement of β-catenin/TCF transcription for maintenance of colorectal CSCs. We found that blocking the interaction between β-catenin and TCFs with small molecules developed by our lab reduces proliferation but does not prevent sphere formation or maintenance. In addition, we found a correlation between the expression of a TCF-transcriptional reporter and the ability of a single cell to form a sphere. Moving forward, we are translating our *in vitro* findings into an *in vivo* mouse model to determine the requirement for TCF transcription in tumor initiation.

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31: COMPLETE TCRα GENE LOCUS CONTROL REGION ACTIVITY IN T CELLS DERIVED *IN VITRO* FROM EMBRYONIC STEM CELLS

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Locus Control Regions (LCR) are cis-acting gene regulatory elements with the unique, integration site-independent ability to transfer the characteristics of their locus-of-origin's gene expression pattern to a linked transgene in mice. LCR activities have been discovered in numerous T cell lineage expressed gene loci. These elements can be adapted to the design of stem cell gene therapy vectors that direct robust therapeutic gene expression to the T cell progeny of engineered stem cells. Currently, transgenic mice provide the only experimental approach that wholly supports all the critical aspects of LCR activity. Herein we report manifestation of all key features of mouse T cell receptor (TCR)-α gene LCR function in T cells derived *in vitro* from mouse embryonic stem cells (ESC). High level, copy number-related TCRα LCR-linked reporter gene expression levels are cell type-restricted in this system and upregulated during the expected stage transition of T cell development. We further report that *de novo* introduction of TCRα LCR linked transgenes into existing T cell lines yields incomplete LCR activity. Together, these data indicate that establishing full TCRα LCR activity requires critical molecular events occurring prior to final T-lineage determination. This study additionally validates a novel, tractable and more rapid approach for the study of LCR activity in T cells, and its translation to therapeutic genetic engineering.

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32: MULTIMODAL SUPPORT CELL THERAPY FOR CNS INJURY AND DISEASE

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One of the most challenging aspects of treating injuries of the central nervous system (CNS) is the multitude of problems that need to be addressed to restore normal CNS function. These include cell death, glial scaring, limited axon regeneration, disruption of synaptic complexes, neuroinflammation and disruption of neurovascular supply.

As many of the processes are controlled during normal development by astrocytes, we have hypothesized that transplantation of astrocytes into the degenerating CNS will promote recovery by limiting detrimental processes, and by enhancing tissue reorganization. Until recently attempts to use astrocytes to ameliorate CNS injury have been disappointing or require genetic modification in order to produce any benefit. Our research on CNS development and on treatment of spinal cord injury has identified a novel population of astrocytes that is exceptionally effective at CNS repair. These astrocytes are generated by directed differentiation of purified, fetal glial precursors using chemically defined growth conditions and the cytokine bone morphogenetic protein-4 (BMP4). When transplanted into the injured rat spinal cord, these BMP-induced astrocytes promote survival of injured neurons, reduce the expression of axon-growth inhibitory molecules, and promote extensive regeneration of large numbers of endogenous axons and remarkable behavioral recovery. By comparing the properties of these astrocytes with other fetal- or postnatal-derived astrocytes, we find increased expression of numerous neurotrophic factors, extracellular matrix proteins and biochemical properties that make BMP-induced astrocytes uniquely suited to promote neuronal survival, support synapse formation and tissue revascularization. These properties also make these astrocytes highly suitable for the treatment of neurodegenerative diseases, in which tissue homeostasis has been disrupted. Because of the wide-ranging activity of this unique cell population, we refer to these astrocytes as MPACs (multimodal precursor-derived astrocytes). Our findings suggest that MPAC-Therapy not only supports cell replacement, but may reprogram the lesion towards a regenerative environment.

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33: EFFICIENT TRANSCRIPTIONAL REPROGRAMMING OF MATURE AMNIOTIC CELLS INTO VASCULAR NICHE CELLS THAT SUPPORT HEMATOPOIESIS

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Transplantation of *ex vivo* expanded human umbilical cord blood cells (hCBs) only partially enhances the hematopoietic recovery after myelosuppressive therapy. Incubation of hCBs with optimal combinations of cytokines and niche cells, such as endothelial cells (ECs), could augment the efficiency of hCB expansion. We have recently demonstrated that human ECs support the long-term expansion of engraftable human hematopoietic stem and progenitor cells (HSPCs). However, obtaining efficient numbers of human ECs for clinical scale expansion of hCBs can be cumbersome. In this report, we show that transduction of human mid-gestation c-Kit- lineage-committed amniotic cells (ACs) with the ETS transcription factors ETV2, FLI1 and ERG1 concomitant with TGFβ inhibition can efficiently reprogram ACs into vascular ECs (rAC-VECs) without transitioning through a pluripotent state. Genome-wide transcriptional analyses showed that rAC-VECs are similar to adult ECs in which vascular-specific genes are expressed and non-vascular genes are silenced. Functionally, rAC-VECs form stable vasculature in Matrigel plugs and can engraft into regenerating livers. Our novel technology allows for the generation of large quantities of bona fide ECs that could be used in a wide array of clinical applications. Here we tested whether rAC-VECs can support the *ex vivo* expansion of hCBs. We show that rAC-VECs are endowed with a pro-hematopoietic angiocrine repertoire that enables the expansion of CD34+CD38- human HSPCs that have the potential to rapidly reconstitute the hematopoietic system of NSG mice. rAC-VECs may prove to be an ideal cellular platform to expand hCB and allow identification of new growth factors that could regulate the expansion, maintenance, and differentiation of human HSPCs. Additionally, banking of HLA-typed rAC-VECs establishes a vascular inventory that can potentially be used with HLA-matched cord blood cells for the treatment of diverse hematological disorders.

34: TWO SMALL RNAS DERIVED FROM ncRNA, RMRP, HAVE GENE REGULATORY ACTIVITY RELEVANT TO HUMAN CARTILAGE HAIR HYPOPLASIA (CHH) SYNDROMES

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The ENCODE transcription database has shown that small nuclear(sn) RNAs with unknown functions are predominantly represented by three read peaks corresponding to the 5' end, the middle of the RNA, and the 3' end of the RNA, indicating fragmentation of some snRNAs [Djebali, 2012]. We report that the 268 nt, intronless, non-polyadenylated non-coding RNA component of Mitochondrial RNA Processing endoribonuclease, (RMRP) is the source of at least two short (~20nt) RNAs originating from the 5' end and the middle of the RNA (RMRP-S1 (5' end) and RMRP-S2 (middle)). Point mutations in RMRP cause CHH and some of the mutations map to RMRP S1 and S2. Using custom TaqMan probes, we show that RMRP S1 and S2 are significantly reduced in two fibroblast cell lines and a B cell line derived from CHH patients. Published CLIP data and our IP data establish association of RMRP S1 and S2 with Argonaute ribonucleoprotein complexes. Transfection of custom synthesized inhibitors of RMRP S1 and S2 specifically reduced the growth of normal human dermal fibroblasts. Gene regulatory activity of RMRP S1 and S2 was tested in Hek293 cells followed by Affymetrix array analysis. We identified over 900 genes that were significantly regulated. Strikingly, over 75% were down regulated and of these over 90% contained seed complement target sites for RMRP S1 or S2, located predominantly in the 3' UTRs. Furthermore, IPA analysis of the regulated genes identified highly enriched categories in skeletal and muscular disorders, hematological system development, and cancer, consistent with the dwarfism, hair loss and blood disorders of CHH. These data suggest that RMRP S1 and S2 mediate newly identified functions of RMRP that affect the CHH syndrome.

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35: DIRECT REPROGRAMMING OF HUMAN ENDOTHELIAL CELLS INTO FUNCTIONAL HEMATOPOIETIC MULTI-LINEAGE PROGENITORS BY DEFINED FACTORS

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Generation of unlimited quantities of patient matched transplantable hematopoietic stem cells/progenitors (HSPCs) is of paramount importance for the treatment of multiple blood malignancies. Directed differentiation of human pluripotent cells into transplantable HSPCs is limited by our understanding of the hematopoietic system development and is yet to produce sufficient number of engraftable HSPCs.

We screened differentially expressed transcription factors (TFs) from human umbilical vein endothelial cells (HUVECs) and $CD34^{+}Lin^{-}$ human cord blood hematopoietic progenitors (CBHPs) and identified four transcription factors, FGRS, which efficiently reprogram human endothelial cells (ECs) into human multi-lineage progenitors (rEC-HMLPs). Co-culture of the transduced ECs with vascular niche-like feeder layer in serum-free environment was essential for efficient clonal conversion into rEC-HMPLs. These rEC-HMLPs are able to proliferate and spontaneously differentiate into myeloid hematopoietic lineages *in vitro.* Phenotypic analysis of rEC-HMLPs revealed populations of cells that were either $CD45^{+}Lin^{-}CD45RA^{-}CD90^{+}CD38^{-}CD34^{+/low}$ or $CD45^{+}Lin^{-}CD45RA^{-}CD90^{-}CD38^{-}CD34^{+/low}$ satisfying criteria for hematopoietic stem-like cells or multi-potent progenitors, respectively. When transplanted into immune-deficient mice, rEC-HMLPs were capable of multi-lineage hematopoietic *in vivo* engraftment populating bone marrow and spleen and generating a small number of bone-marrow residing cells phenotypically resembling multi-potent progenitors ($Lin^{-}CD34^{+}CD38^{-}CD90^{-}CD45RA^{-}$). Genome-wide transcription profiles of rEC-HMLPs before transplantation and after 22 weeks of *in vivo* education were more similar to the human $CD34^{+}Lin^{-}$CBHPs than to HUVECs, with tighter clustering of *in vivo* educated rEC-HMLPs phenocopying CBHPs.

Global analyses of binding sites of two major transcription factors, suggests that one mainly binds to active promoters and enhancers in HUVECs, while the other functions as transcription repressor with special favor to angiogenesis regulators.

Direct reprogramming of somatic cells into hematopoietic progenitors will advance our understanding of the hierarchy of the transcription factors and micro-environmental cues that orchestrate the development of hematopoietic system and may prove useful for regenerative medicine.

36: HOW E2A REGULATES PAX5 AND/OR CD19 EXPRESSION DURING B CELL DIFFERENTIATION FROM HEMATOPOIETIC STEM CELLS

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The development of an *in vitro* system for the generation of B cells is critical for our studies on common variable immunodeficiency (CVID), a late-onset condition that impairs B cell development in the bone marrow (BM). Our current transcriptome and quantitative RT-PCR data obtained from BM of CVID-affected and control horses revealed that B cell production is halted at the pre-pro and pro-B cell developmental stage. Specifically, the expression of the transcription factor E2A is significantly lower in CVID-affected horses, in comparison to healthy horses, and so are PAX5 and CD19, but not EBF1. This finding creates the possibility for a direct interaction of E2A and PAX5 and/or CD19. We have developed a B cell differentiation culture system using BM-derived HSCs, the stromal cells that naturally develop along with them *in vitro*, and a cytokine cocktail. The presence of stromal cells supports the differentiation of small cells that expand in number with time. We harvested these cells and transferred them to adhesive slides for immunofluorescence staining. Using Integrated Morphometric Analysis, we measured a high percentage of $CD19^+$ cells, resulting in a high absolute $CD19^+$ cell number ($4x10^4$ cells/well/culture). We also noticed that the majority of cells expressed both E2A/CD19 (48.7%) or only E2A (36.1%), while fewer cells expressed high level of E2A but low level of CD19 protein. Thus, our culture system is an ideal tool to define early B cell developmental stages, which in future experiments will be sorted for quantitative analysis of E2A, EBF1, PAX5 and CD19 protein and gene expression, and for their methylation status to understand how E2A regulates PAX5/CD19. The same system will then be applied to support B cell differentiation from HSCs harvested from horses with CVID, and investigate the mechanism that impairs early B cell differentiation in disease.

37: DYNAMIC COOPERATION BETWEEN CELLS AND SUBSTRATE DETERMINE DIFFERENTIATION

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Although regenerative medicine approaches using stem cell therapies are well established for soft tissue, they have not been widely implemented in dental medicine. The tooth is a complex structure where a high level of organization must occur within a confined region and with minimal perfusion. One of the major obstacles to differentiation *in vitro*, in the absence of chemical inducers, has been a lack of substrates that mimic the organization of the *in vivo* matrix.

Entangled elastomer surface confinement has allowed us to produce substrates whose modulus varies in a continuous and differential manner. These polymers support dental pulp stem cell (DPSC) growth and allow us to simultaneously probe the mechanical properties of substrates and cells. Using this principal to produce multi-scaled, topographically flat, mechanically heterogeneous patterned surfaces, we have also been able to evaluate how DPSC respond to complex gradients, and organize focal adhesions and their associated signals. We found that the length scale of the mechanical gradient is a critical determinant and that micro-scale patterns suppressed biomineralization, even when chemical inducers (dexamethasone) were present, whereas nanoscale patterns increased hydroxyapatite deposition.

On differentiation promoting hard surfaces, differentiation markers were observed in all layers of a multilayered tissue although only one layer was in contact with the substrate. However, when the high modulus surface was substituted with a non-inductive (soft) substrate, or when focal adhesion functions are reduced by Rho kinase inhibition, hydroxyapatite formation ceased until contact with the inductive surface was resumed. The sensitivity of the cells to surface mechanics was severely reduced when dexamethasone was introduced, suggesting that cellular responses to the niche are hindered when nonphysiological quantities of inducers are present. This dynamic cooperation between the cell and substrate mechanics help us understand the process by which stem cells in-vivo respond to continual changes in their environment.

[Funded in part by the NSF-MRSEC Program]

38: IMMERSIVE TRAINING FOR WORK UNDER cGMP

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We have developed a novel method to train multiple students in the design of protocols, batch records and flow diagrams for work within a clean room environment (under cGMP). The instruction enhances traditional techniques of classroom and laboratory training in regulations and instrument utilization and was developed in partnership with the Stony Brook **Reality Deck Project**. The method includes a new way to view, learn, and train using the "Reality Deck" (see http://www.cs.stonybrook.edu/~realitydeck/) to provide an immersive gigapixel resolution display, in which the viewer is surrounded with high resolution life-like images of the facility. It allows one to walk around the room, become familiar with the layout, and feel like he/she can actually move from room to room or within each room and between incubators and biosafety cabinets, or to look into the microscopes with digital displays of cultures. This approach is designed to allow trainees to better plan work flow and hence rapidly develop and implement batch records that will be used in cell therapy manufacturing without potentially interrupting facility usage.

The new "GMP facility" at Stony Brook University is designed to manufacture clinical grade cell and cell based products for the treatment of chronic and acute injury. There are two spaces available, one for large scale production equipped with four biosafety cabinets and incubators and one for small scale production equipped with one biosafety cabinet and one incubator. The incubators are tri-gas enabling low oxygen environments to be maintained and the microscopes are linked to the web providing the option for remote viewing of cell cultures during expansion and production processes. The Reality Deck is a unique visualization facility, located at the Stony Brook University Center of Excellence in Wireless and Information Technology and supported by the National Science Foundation and the State of New York.

39: PARALLEL TRANSCRIPTIONAL ANALYSIS OF MULTIPLE STEM AND PROGENITOR POPULATIONS IDENTIFIES NOVEL COMMONLY DYSREGULATED AND FUNCTIONALLY RELEVANT TARGETS IN ACUTE MYELOID LEUKEMIA

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Acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) are, in the majority of the cases, incurable hematological malignancies. Experimental evidence suggests that AML and MDS originate from hematopoietic stem and progenitor cells (HSPC) following the acquisition of genetic or epigenetic changes giving rise to leukemia stem cells (LSC). Eradication of LSC holds the promise of curing the disease. To find molecular characteristics of LSC that can be used as therapeutic targets to eradicate these cells, we employed parallel transcriptional analysis on sorted phenotypic long-term hematopoietic stem cells (HSC), short-term HSC, and granulocyte-monocyte progenitors (GMP) from individuals with AML with normal karyotype (N=5), -7/7q- (N=6) and complex karyotype (N=5) and compared them with the corresponding cell populations from aged-matched healthy controls. Specifically, we sorted $Lin^-/CD34^+/CD38^-/CD90^+$ (LT-HSC), $Lin^-/CD34^+/CD38^-/CD90^-$ (ST-HSC), and $Lin^-/CD34^+/CD38^+/CD123^+/CD45RA^+$ (GMP), and hybridized RNA to Affymetrix GeneST 1.0 expression arrays. Differential gene expression was determined within each compartment by direct comparison of AML LT-HSC vs. HC LT-HSC, AML ST-HSC vs. HC ST-HSC, and AML GMP vs. HC GMP. This analysis revealed overexpression at the gene and protein level of IL-1 receptor accessory protein (IL1RAP) in all leukemic stem and progenitor cell compartments. IL1RAP expression marked cells with the -7/7q- anomaly in AML patients. IL1RAP was also overexpressed on HSC of patients with high-risk MDS, suggesting a pervasive role in different disease subtypes. High IL1RAP expression was independently associated with poor overall survival in 3 independent cohorts of AML patients. Knockdown of IL1RAP decreased clonogenicity and increased cell death of AML cells *in vitro* and reduced infiltration of hematopoietic organs *in vivo*. Our study provides a map of consistently dysregulated transcripts across multiple fractionated stem and progenitor cell types from patients with AML, and identifies IL1RAP as a putative new therapeutic and prognostic target in stem cells in AML and MDS.

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40: REPURPOSING FDA-APPROVED DRUGS TO RESTORE C-CBL FUNCTION IN GLIOBLASTOMA AND OTHER CANCERS

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Two central goals in cancer research are to identify molecular mechanisms that distinguish between tumor cells and normal cells, and to discover changes in cellular function required for maintenance of tumor-initiating competence in transformed cells. Moreover, the general failure in improving survival of people with malignant tumors emphasizes the urgency of discovering therapies that can be rapidly translated into the clinic. In addressing these challenges we found that inhibition of the function of the c-Cbl ubiquitin ligase is of critical importance in multiple cancers. Glioblastomas (GBMs) and basal-like breast cancers (BLBCs) both inhibit normal regulation of c-Cbl function via the redox/Fyn/c-Cbl pathway, thus providing a novel means of escaping consequences of increased oxidative status. Although GBMs and BLBCs utilize expression of different proteins (Cool-1 and Cdc42, respectively) to inhibit c-Cbl function, in both cases inhibition of the inhibitory proteins inhibited function of tumor initiating cells and of bulk tumor cells. Restoration of normal RFC pathway activity by genetic inhibition of Cool-1 in GBM cells also increased sensitivity to temozolomide and BCNU. Genetic or pharmacological inhibition of the Cdc42 function BLBCs enabled us to render these tumors sensitive to secondary, and estrogen receptor-independent, pro-oxidant activities of tamoxifen. Applying our knowledge of RFC pathway function to repurposing of FDA-approved drugs has led to discovery of new uses of known compounds that enable us to more effectively target both GBMS and BLBCs and holds promise for treatment of multiple other types of cancer. These studies have also provided lead molecular candidates for enhancing sensitivity to secondary activities of a major existing approach to cancer treatment. Moreover, these approaches appear to be selective for tumor cells. These, these studies identify a novel and convergent molecular mechanism that modulates tumor generation and sensitivity to treatment, and provided the clues needed to discover new therapeutic approaches, some of which hold promise for rapid clinical translation.

41: THE DEUBIQUITINATING ENZYME PSMD14 REGULATES EMBRYONIC STEM CELL SELF-RENEWAL AND PLURIPOTENCY

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Pluripotent embryonic stem (ES) cells have the unique ability to both self-renew and differentiate into all three germ layers. The mechanisms by which ES cells maintain pluripotency allow us to understand how the dynamic alterations that underlie developmental transitions are dictated. The role of protein networks and their associated cellular interactions has emerged in the recent years to provide a novel layer of regulation on cell fate decisions in the field of stem cell biology. The relationships between the ubiquitin family of enzymes with their cognate substrates in particular, that participate in key functions of cell processes such as sub-cellular localization, protein turnover, epigenetic regulation and cell cycle control, harbor the ability to regulate self-renewal, differentiation and lineage specification decisions. In our laboratory, we have strong evidence suggesting that inhibition of the proteasome function affects patterns of ES cell differentiation. Moreover, we have mapped in detail the ubiquitinated protein landscape during ES cell self-renewal and differentiation, characterizing a considerable amount of novel substrates that are differentially regulated by ubiquitination. Additionally, we have identified several new ubiquitin E3 ligases and deubiquitinating enzymes to be essential for ES cell pluripotency, homeostasis and differentiation. One of these deubiquitinating enzymes, Psmd14, is of particular interest since it acts as the “gatekeeper” of the proteasome, regulating the limiting step of ubiquitin removal prior to protein degradation. Our data provide the first line of evidence that deubiquitination and proteasomal degradation are key determinant processes of ES cell function, opening the way for further functional studies in order to provide a detailed understanding of the intricate crosstalk between ubiquitination and pluripotency.

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42: EFFECTS OF MILD HEATING ON THE OSTEOGENESIS OF MESENCHYMAL STEM CELLS DURING INFLAMMATION

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Mesenchymal stem cells (MSCs) from bone marrow are multipotent cells that can replicate as undifferentiated cells and differentiate to lineages of mesenchymal tissues including bone, cartilage, fat, tendon, muscle, and marrow stroma. These cells have generated a great deal of interest because of their potential use in regenerative medicine. Cytokines likely affect the phenotype of MSCs; therefore, increasing the understanding of the cytokines that affect MSC function and differentiation may lead to invaluable information for future stem cell-based therapies. The influence of pro-inflammatory cytokines on osteogenic differentiation during mild-elevated temperature conditions, similar to what occurs during *in vivo* inflammation, remains to be fully explored. In this study, human MSCs (hMSCs) were cultured with Tumor Necrosis Factor-alpha (TNF-α), an important mediator of the acute phase response, and Interleukin-6 (IL-6) which plays a role in damaging chronic inflammation, in both osteogenic medium and hMSC growth medium. These cells were heat shocked at 39°C, and then alkaline phosphatase (AP) activity and calcium deposition was evaluated and compared to controls. TNF-α appears to induce significantly more mineral precipitation than IL-6 (Figures 1A&B). Regular hyperthermia appears to mitigate the inhibitory effects of IL-6 and TNF-α in the early days of differentiation. DNA data (Figure 1C) show that periodic mild heating induced proliferation of hMSCs and inhibited TNF-α-induced apoptosis. Quantitative assays show significantly higher levels of AP activity and calcium precipitation in osteogenic cultures treated with TNF-alpha compared to IL-6 and controls. These results demonstrate that mild hyperthermia may potentially be used to facilitate bone regeneration in the joint using hMSCs, and therefore may influence the design of heat-based therapies to be used *in vivo*.

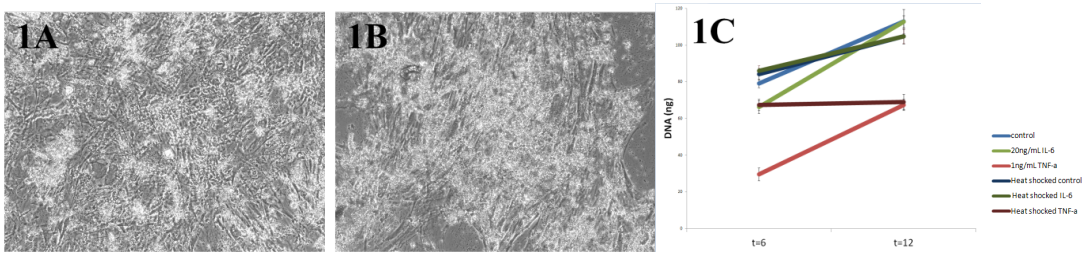

Figure 1. (A) MSCs in osteogenic medium, 20ng/mL IL-6, t=12 days. (B) MSCs in osteogenic medium, 1ng/mL TNF-α, t=12 days**,** (C) DNA content in osteogenic cultures measured on days 6 and 12.

43: GENERATION OF INDUCED PLURIPOTENT STEM CELLS FROM REVERTANT MOSAIC KERATINOCYTES AND FIBROBLASTS

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Revertant mosaicism (RM) is a naturally occurring phenomenon involving spontaneous correction of a pathogenic mutation in a somatic cell. It has been observed in several genetic diseases, including epidermolysis bullosa (EB), a group of inherited skin disorders characterized by blistering and scarring. The therapeutic potential of induced pluripotent stem cells (iPSCs) has recently been demonstrated from patients with various diseases. Despite efforts toward developing gene correction of iPSCs using approaches such as homologous recombination, there remain technical difficulties including low efficiency and excisional footprints after gene correction. Here, we developed a novel strategy of "natural gene therapy" by generating patient-specific revertant mosaic iPSCs (RM-iPSCs) in a subset of EB patients with revertant mosaic patches of skin.

First, RM skin was identified in a clinically and immunohistochemically healthy area in a junctional EB patient with compound heterozygous *COL17A1* mutations. RM-iPSCs were generated from keratinocytes cultured from this region, and we confirmed the authenticity of RM-iPSCs by RT-PCR and immunostaining for stem cell markers. Sequence analysis of RM-iPSCs showed the paternal *COL17A1* mutation in exon 51 (p.R1226X), but the maternal mutation in exon 18 (c.1706delA) was corrected by mitotic gene conversion. RM-iPSCs were then differentiated into keratinocytes and fibroblasts to generate complete gene-corrected skin. Importantly, keratinocytes derived from RM-iPSCs expressed type XVII collagen (COL17) by immunostaining. We also generated fibroblasts derived from RM-iPSCs via embryoid body formation using ascorbic acid and transforming growth factor β2 (TGFβ2). Finally, we generated *in vitro* 3D skin equivalents using keratinocytes derived from RM-iPSCs, in which COL17 was strongly expressed in the basal layer. We have demonstrated the feasibility of utilizing revertant keratinocytes as a source of spontaneously corrected cells for developing RM-iPSC-based therapeutic approaches in EB, for both topical and systemic administration.

44: ASYMMETRICALLY MODIFIED NUCLEOSOMES AND THEIR FUNCTION IN THE ESTABLISHMENT OF BIVALENT DOMAINS IN EMBRYONIC STEM CELLS

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Histone posttranslational modifications (PTMs) are key elements in the regulation of gene expression. Mononucleosomes, the basic building blocks of chromatin, contain two copies of each core histone. Despite extensive research on histone PTMs, it remained unknown whether both copies carry identical PTMs *in vivo*. The symmetry state of modifications on sister histones bears directly on their potential inheritance, readout by effector proteins, and the range of mark combinations achievable at a given locus. Bivalent domains contain the activating mark histone H3 lysine 4 trimethylation (H3K4me3) and the repressive mark H3K27me3. Both their nucleosomal conformation and mechanism of establishment have remained elusive. To assess histone PTM symmetry, we devised an approach based on modification-specific antibodies and quantitative mass spectrometry (MS). We show that chromatin consists of both symmetric and asymmetric nucleosome populations *in vivo*. The H3K27me2/3 marks were mostly present on in symmetric fashion in ES cells. For H4K20me1, we likewise observed a combination of symmetric and asymmetric nucleosomes for ES cells, HeLa cells, and MEFs. These data suggest that asymmetry might be a general feature of nucleosomes. To explore its implications, we analyzed co-occurrence of histone marks and obtained evidence for bivalent nucleosomes carrying H3K4me3 or H3K36me3 along with H3K27me3. Supporting the MS-based findings, sequential ChIP indicated that bivalent nucleosomes map to gene promoters and resolve upon differentiation. Interestingly, PRC2-mediated methylation of H3K27 was inhibited when nucleosomes contain H3K4me3 or H3K36me3 in symmetrical, but not asymmetrical fashion. In line with these findings, MS analysis revealed that H3K27me2/3 and H3K4me3/H3K36me3 asymmetrically reside on opposite tails in bivalent nucleosomes *in vivo*. These findings uncover a potential mechanism for the incorporation of bivalent features into nucleosomes and show how asymmetry might diversify functional nucleosome states. The readout of such bivalent nucleosomes is the focus of current efforts, and recent data will be presented.

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45: NANOG-DEPENDENT FUNCTION OF TET1 AND TET2 IN ESTABLISHMENT OF PLURIPOTENCY

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Molecular control of the pluripotent state is thought to reside in a core circuitry of master transcription factors including the homeodomain-containing protein Nanog, which plays an essential role in establishing ground state pluripotency during somatic cell reprogramming. While the genomic occupancy of Nanog has been extensively investigated, comparatively little is known about Nanog-associated proteins and their contribution to the Nanog-mediated reprogramming process. Using enhanced purification techniques and a stringent computational algorithm, we identified 27 high-confidence protein interaction partners of Nanog in mouse ES cells. These consist of 19 novel partners of Nanog that have not been reported before including the Ten eleven translocation (Tet) family methylcytosine hydroxylase Tet1. We confirmed physical association of Nanog with Tet1, and demonstrated that Tet1, in synergy with Nanog, enhances the efficiency of reprogramming. We also found physical association and reprogramming synergy of Tet2 with Nanog, and demonstrated that knockdown of Tet2 abolishes the reprogramming synergy of Nanog with a catalytically deficient mutant of Tet1 (Tet1Mut). These results indicate that the physical interaction between Nanog and Tet1/2 proteins facilitates reprogramming in a manner that is dependent on Tet1/2's catalytic activity. Tet1 and Nanog co-occupy genomic loci of genes associated with both maintenance of pluripotency and lineage commitment in ES cells, and Tet1 binding is reduced upon Nanog depletion. Co-expression of Nanog and Tet1 results in expression priming of and increased 5hmC levels at top ranked common targets Esrrb and Oct4 before reprogramming to naïve pluripotency. We propose that Tet1 is recruited by Nanog to enhance the expression of a subset of key reprogramming target genes. These results provide an insight into the reprogramming mechanism of Nanog and uncover a novel role for 5mC hydroxylases in the establishment of naïve pluripotency.

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46: SEQUENTIAL TARGETING OF DEVELOPMENTALLY EXPRESSED RECEPTORS OF THE FETAL HUMAN STRIATUM PERMITS THE EFFICIENT INSTRUCTION OF HUMAN MEDIUM SPINY NEURONS FROM HUMAN ESCs

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Medium spiny neurons (MSNs) of the striatum are a principal cell type lost in Huntington disease (HD). Isolated preparations of human MSNs are needed for drug screening as well as for potential cellular therapeutics. To develop an efficient protocol for generating MSNs from pluripotential stem cells, we first isolated human MSNs from fetal forebrain tissue, using DLX5 enhancer-driven EGFP-directed fluorescence activated cell sorting to isolate striatal progenitor cells from their source in the second trimester human fetal lateral ganglionic eminence (LGE). We cultured the resultant progenitor isolates, and determined that virtually all Dlx5:EGFP$^+$ neuronal daughters differentiated as DARPP32$^+$ MSNs. We then used Affymetrix microarrays to define the expression profiles of these MSNs, and of their parental Dlx5:EGFP$^+$ LGE progenitors; by comparing these profiles to those of SOX2$^+$ human fetal neural progenitors, we identified a set of genes differentially expressed during human MSN differentiation. This analysis revealed a set of receptors selectively expressed by developing MSNs, and allowed us to predict the ligands to which MSNs were differentially exposed during ontogenesis. Among these sequentially expressed MSN-lineage receptors and their predicted ligands or modulators thereof were NTRK2/BDNF, ACVR/Activin, LPR5:FZD/DKK1, PLXNA2:3/SEMA3, THRA:B/T3, INSR/insulin, and IGF1R/IGF1. Using this information, we designed a high efficiency protocol for generating MSNs from both normal (H9/WA09 and GENEA19) and *huntingtin* mutant (GENEA 20) human embryonic stem cells. We found that induction of the MSN phenotype was significantly potentiated by the sequential and combinatorial application of these agents, relative to extant protocols for MSN production, such that >80% of all neurons in the resultant cultures expressed a HuD$^+$/GAD67$^+$/DARPP32$^+$ antigenic phenotype characteristic of MSNs. Thus, human medium spiny neurons may now be efficiently generated from human ESCs, including both normal and HD mutant hESC lines; these cells should permit detailed molecular investigation of both normal human MSN biology, and its disruption in HD.

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47: HISTONE VARIANT H3.3 IS AN ESSENTIAL MATERNAL FACTOR FOR SOMATIC CELL REPROGRAMMING

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Differentiated cells can be reprogrammed to pluripotency by nuclear transfer (SCNT), cell fusion, or over-expression of a set of defined transcription factors. The reprogramming processes involve the exchange of proteins between donor nucleus and ooplasm, chromatin remodeling, and reactivation of pluripotency genes; however, the mechanism of this overall processes has largely remained elusive. Here, we report that the histone variant H3.3 is an essential maternal factor for somatic cell reprogramming. Our RT-qPCR results showed that mouse mature oocytes are abundant with maternal H3.3 mRNAs. Injection of siRNAs against H3.3 (H3.3KD) could sufficiently and specifically deplete maternal H3.3 mRNAs (>90%) and its derivative protein in early embryos. Using H3.3KD oocytes for nuclear transfer, the developmental potential of cumulus SCNT embryos to blastocyst was significantly decreased (2.2%) comparing to the luciferase siRNA-injected control oocytes (36.1%); injecting H3.3mRNA, but not H3.2mRNA into the H3.3KD oocytes, significantly increased the blastocyst rates (25.1% vs 4.5%). RNA-sequencing of SCNT embryos at the 4-cell stage revealed that many key pluripotency genes, including Pou5f1(Oct4), Nanog, Tbx3 and Id3, are down-regulated in H3.3KD SCNT embryos; injecting H3.3mRNA, but not H3.2mRNA into H3.3KD oocytes, could increase the expression of these pluripotency genes. Using H3.3B-HA tagged mice, we found that H3.3 is involved in reprogramming through replacement of the donor cell-derived H3 (both canonical H3 and H3.3 protein) with *de novo* synthesized maternal H3.3, and the replacement is associated with removal of repressive modifications in the donor nuclei, such as tri-methylation of lysine 27 on histone H3 (H3K27me3). Our work has identified that H3.3 is a crucial maternal factor required for somatic cell reprogramming and shed important light on this field of study. Our data also suggest a previously unrecognized function of H3.3, an H3.3-dependent pathway in removing H3K27me3 in the nuclei of SCNT embryos.

48: HUMAN IPSC-DERIVED OLIGODENDROCYTE PROGENITOR CELLS CAN EFFICIENTLY AND COMPLETELY RESTORE CENTRAL MYELIN IN ANIMAL MODELS OF ACQUIRED AS WELL AS CONGENITAL DEMYELINATION

Martha Windrem[1,2], Janna Bates[1], Steven Schanz[1], Devin Chandler-Militello[1], Lorenz Studer[3], Konrad Hochedlinger[4], Su Wang[1,2], Steven A. Goldman[1,2]

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Neonatal engraftment by human fetal tissue-derived oligodendrocyte progenitor cells (OPCs) permits the myelination of congenitally dysmyelinated brain (Nature Medicine, 2004; Cell Stem Cell, 2008). To establish a potential autologous source of these cells for therapeutic implantation into adults with acquired demyelination, we developed a strategy by which to differentiate human induced pluripotential stem cells (hiPSCs) into OPCs. From 3 hiPSC lines, as well as from human embryonic stem cells (hESCs), we generated highly enriched OLIG2$^+$/PDGFRα$^+$/NKX2.2$^+$/SOX10$^+$ hOPCs, which could be further purified using fluorescence-activated cell sorting. hiPSC OPCs efficiently differentiated into both myelinogenic oligodendrocytes and astrocytes, *in vitro* and *in vivo*. In myelin-deficient shiverer mice, neonatally engrafted hiPSC OPCs robustly myelinated both the brain and spinal cord, and substantially increased the survival of these mice. In adults demyelinated by cuprizone ingestion, resident hiPSC OPCs that had been introduced neonatally were recruited to generate new oligodendrocytes, which efficiently remyelinated the demyelinated host axons. These mobilized hiPC-derived OPCs ultimately mediated the complete remyelination of the adult-demyelinated brain, by oligodendroglia newly generated from the activated pool of resident hiPSC OPCs. In each model of neonatally-engrafted hiPSC OPC mice, including both congenitally hypomyelinated shiverers and adult cuprizone-demyelinated adults, the speed and efficiency of myelination by hiPSC OPCs was at least as high as that previously observed using fetal tissue-derived OPCs; critically, no donor-derived tumors were ever noted in either model, in animals studied as long as 9 months after neonatal transplant. These results strongly suggest both the safety and efficacy of human iPSC-derived OPCs in treating adult as well as congenital disorders of myelin loss.

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49: NFIX REGULATES THE FATE OF NEURAL STEM CELLS IN POSTNATAL MOUSE BRAIN

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Nuclear Factor I X (NFIX) is a transcription factor that has been shown to play an important role in pre- and postnatal brain development. Previously we showed that the cerebral cortex of postnatal *Nfix*$^{-/-}$ mice is significantly larger than those of wild-type (WT) mice and the hippocampi are distorted. In addition, excess PAX6+ and DCX+ cells are present in the postnatal *Nfix*$^{-/-}$ subventricular zone (SVZ) of *Nfix*$^{-/-}$ mice, indicating more neural stem and/or progenitor cells and migrating neuroblasts in *Nfix*$^{-/-}$ brains. These defects indicate that *Nfix* may have a role in regulating the fate of neural stem cells (NSCs).

We show here that NFIX is expressed in some putative NSCs and most neuronal progenitors and neuroblasts in the dentate gyrus (DG) of the hippocampus. By comparing the percentage of proliferating NSCs in WT and *Nfix*$^{-/-}$ DG, we found evidence that *Nfix* may block the proliferation of NSCs *in vivo*. Also, in both neurosphere assay and monolayer culture, we found a higher percentage of neurospheres or monolayer cells derived from *Nfix*$^{-/-}$ brains can differentiate into oligodendrocytes compared to WT brains. qPCR shows higher transcript levels of markers of oligodendrocytes and their progenitors in early postnatal *Nfix*$^{-/-}$ brains. We are now generating induced *Nfix* conditional KO mice driven by NestinCreERT2 so that we can study the direct effect of NFIX on NSCs proliferation and neurogenesis and trace the fate of *Nfix*$^{-/-}$ NSCs *in vivo*.

Understanding the mechanism by which *Nfix* regulates neurogenesis may allow the development of improved therapies for neuro-degenerative diseases, ischemic stroke and brain injury. Also, as *Nfix* has been strongly implicated as a putative oncogene or tumor suppressor gene in a mouse model of glioblastoma, studying the role of *Nfix* in neurogenesis may yield insights into the generation and proliferation of glioblastoma cells.

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PROGRAM AT-A-GLANCE

WEDNESDAY, MAY 22, 2013: Concourse Lobby, Proshansky Auditorium

NOON	REGISTRATION and POSTER SET-UP
1:00 - 1:10	OPENING REMARKS
1:10 - 2:10	PLENARY I: SINGLE CELL ANALYSES
2:10 - 2:40	BREAK and POSTER VIEWING
2:40 - 4:00	PLENARY II: STEM CELLS IN CANCER
4:00 - 6:00	POSTER SESSION I and RECEPTION

THURSDAY, MAY 23, 2013: Concourse Lobby, Proshansky Auditorium

8:00	REGISTRATION and CONTINENTAL BREAKFAST
9:00 - 11:00	PLENARY III: STEM CELL BIOLOGY
11:00 - NOON	POSTER SESSION II
NOON - 1:00	LUNCH and POSTER VIEWING
1:00 - 1:10	COMMISSIONER'S REMARKS, INTRODUCTION OF KEYNOTE
1:10 - 2:00	KEYNOTE ADDRESS
2:00 - 3:15	PANEL: NAVIGATING THE MAZE OF STEM CELL THERAPIES
3:15 - 3:45	BREAK and POSTER VIEWING
3:45 - 4:45	PLENARY IV: NYSTEM SUCCESS STORIES
4:45	CLOSING REMARKS
5:00	ADJOURN