Sixth Annual
HSCI STEM CELL SYMPOSIUM
Stem Cell Research in Diabetes and Metabolism
Friday, November 6, 2009

PROGRAM AND POSTER ABSTRACTS
WELCOME

Dear Friends and Colleagues,

The Harvard Stem Cell Institute marked its fifth anniversary in April, 2009. With this, our sixth annual symposium, we start our next five years with a continued commitment to our mission of fulfilling the promise of stem cell biology as the basis for cures and treatments for a range of chronic and degenerative diseases. As we look back over the last five years, time seems to have passed both slowly and with amazing rapidity. On the one hand, we are still trying to find that elusive cure for diabetes, to pick today’s topic. On the other hand, we can now create iPS cells from diabetics, a concept that wasn’t even in most people’s minds a few years ago. Moving forward in the field has taken the many contributions of our colleagues from around the world, including today’s outstanding roster of guest speakers. It is such sharing of ideas and collaborative research that have helped bring us to this exciting point where we are today.

The selection of the symposium theme for 2009, ‘Stem Cell Biology in Diabetes and Metabolism’, reflects the recent excitement and progress in the field. Whether it’s an academic lab’s research paper on direct reprogramming of pancreatic exocrine cells to insulin producing beta cells, a start-up company’s ability to turn ES cells into beta cells, or large pharma’s interest in diabetic iPS cells, the attention and imagination of stem cell scientists across the world have been energized. Today’s presentations will share new progress and new information but, as it typical with any scientific advance, raise additional questions at the same time. Our community needs to help answer those questions together.

The next five years will require a renewed commitment to excellence, cross-disciplinary collaboration, and rigor in scientific inquiry. We hope that we are all able to capitalize on a more encouraging federal research policy, so that we can continue to push the bounds of our assumptions and knowledge of development and disease. Our sincere thanks go to the three communities who come together in pursuit of this goal:

• our fellow scientists whose hearts and minds are engaged in this undertaking
• our philanthropic supporters whose enduring financial support makes this research possible
• our partners in the commercial sector whose help is critical to ultimately reaching patients.

We hope you enjoy this day of scientific exchange and interaction and we look forward to our work together.

Douglas Melton  
Co-Director HSCI

David Scadden  
Co-Director HSCI

Brock Reeve  
Executive Director HSCI
WELCOMING REMARKS:

David Scadden
Co-Scientific Director, HSCI

MODERATORS:

Gordon Weir, MD
Joslin Diabetes Center
HSCI Diabetes Program Leader

Amy Wagers, PhD
Joslin Diabetes Center
Harvard University Department of Stem Cell and Regenerative Biology
HSCI Executive Committee Member

FEATURED SPEAKERS:

“Signaling and Chromatin Regulation to Program Beta Cell Progenitors”
Kenneth S. Zaret, PhD
University of Pennsylvania School of Medicine

“Making Stem Cell-Generated Islets”
E. Edward Baetge, PhD
Novocell, Inc.

“Stem Cells to Make Beta Cells and Understand the Causes of Type I Diabetes”
Douglas A. Melton, PhD
Harvard University and HHMI

LUNCH BREAK

FEATURED SPEAKERS

“Developing New Models for Type 1 Autoimmune Diabetes Using Stem Cell-based Technologies”
Jacob Hanna, MD, PhD
Whitehead Institute for Biomedical Research

“Novel Stem / Progenitor Cells: Implications for Therapy”
Armand Keating, MD
University of Toronto

“Harnessing the Power of Stem Cells to Cure Obesity and Diabetes”
Jonathan M. Graff, MD, PhD
University of Texas Southwestern Medical Center

ROUNDTABLE with our guest speakers, moderated by Gordon Weir and Amy Wagers

POSTER SESSION AND COCKTAIL RECEPTION
**Kenneth S. Zaret, PhD**  
*University of Pennsylvania School of Medicine*

Kenneth S. Zaret is a Professor in the Department of Cell and Developmental Biology at the University of Pennsylvania School of Medicine. He is also the Associate Director of the UPenn’s Institute for Regenerative Medicine. Dr. Zaret is recognized for his research on liver and pancreas cell differentiation, gene regulation, and chromatin structure. His laboratory has determined how gene regulatory proteins can endow developmental competence upon multipotent progenitor cells and discovered a signaling network in embryonic cells that determines the cell type choice for liver or pancreas cell fates. His group also demonstrated a role for endothelial cells in promoting organogenesis.

Dr. Zaret received a Jane Coffin Childs fellowship in 1982 and a Searle Scholar faculty award in 1986. From 1986 to 1999 he was in the Biochemistry Section, and then in the Department of Molecular Biology, Cell Biology, and Biochemistry at Brown University Medical School, where he attained the rank of Professor. From 1999 to 2009 he was a Senior Member and Leader of the Cell and Developmental Biology Program at the Fox Chase Cancer Center, where he held the W.W. Smith Chair in Cancer Research. He also initiated the Epigenetics and Progenitor Cells Program at Fox Chase. Dr. Zaret is an editor of the journal Development and on the editorial board of Genes and Development. He has served as an editor of Molecular and Cellular Biology and as a guest editor of Current Opinions in Genetics and Development, Methods: A Companion to Methods in Enzymology, and Mechanisms of Development. He has served on the Molecular Biology, Cell and Developmental Function-1, and Molecular Genetics B Study Sections of the National Institutes of Health. He has also served on the Council for the National Institute of General Medical Sciences (NIH) and the Board of Scientific Counselors for the National Institute of Child Health and Human Development (NIH), including being Chair of the NICHD Board. In 1999 Dr. Zaret was a Fulbright Award Co-Investigator, in 2000 he was an awardee of the Human Frontiers Science Program, in 2002 he received the Hans Popper Basic Science Award from the American Association for the Study of Liver Diseases and the American Liver Foundation, in 2006 he was an awardee of the International Union Against Cancer and received a MERIT award from the National Institutes of General Medical Sciences (NIH), and in 2007 was elected to be a Fellow of the AAAS.

**E. Edward Baetge, PhD**  
*Novocell, Inc.*

E. Edward Baetge is currently Senior Vice President and Chief Scientific Officer of Novocell formed by the merger of CyThera and BresaGen Inc. in 2004. He was recruited to CyThera as Chief Scientific Officer in 2001 to head its then emerging human embryonic stem cell program.

Prior to joining CyThera, Dr. Baetge held the position of Chief Scientific Officer at Modex Therapeutics Ltd from 1997-2001, in Lausanne, Switzerland, which developed one of the first autologous adult stem cell products for the treatment of chronic skin ulcers. Before Modex, Dr. Baetge held management positions at CytoTherapeutics from 1992-1997 and at Bristol-Myers Squibb from 1987-1992.

He holds a Ph.D. in molecular neurobiology from Cornell University and completed postdoctoral work at Cornell University and the Howard Hughes Medical Institute. Dr. Baetge has published extensively in the field of cell therapy and has produced a number of cell technology patents.

**Douglas A. Melton, PhD**  
*Harvard University and HHMI*

Douglas Melton is the Thomas Dudley Cabot Professor in the Natural Sciences at Harvard University, an Investigator of the Howard Hughes Medical Institute, co-director of Harvard’s Stem Cell Institute and co-chair of Harvard’s new Department of Stem Cell and Regenerative Biology. He obtained his BS from the University of Illinois in 1975 and BA in History and Philosophy of Science from Cambridge University, England in 1977. He completed his PhD in Molecular Biology at Trinity College & MRC Laboratory of Molecular Biology, Cambridge University, England in 1980.

Dr. Melton is a member of the National Academy of Sciences (NAS) and a member of the Institute of Medicine with in the NAS. He is also a member of the American Academy of Arts and Sciences and an honorary member of the Japanese Biochemical Society. Dr. Melton has won many honors and awards, including the Eliot P. Joslin Medal, the Richard Lounsbery Award from the National Academy of Sciences, and the George Ledlie Prize.

The goal of his laboratory’s work is to make pancreatic tissue for transplantation into people with diabetes. To this end, his laboratory has significantly advanced the understanding of the genes and cells that normally make the pancreas during animal development and they are using that information to instruct cells to make pancreatic tissue. Dr. Melton’s lab has published over 160 peer-reviewed scientific articles.
Jacob Hanna obtained his MD/PhD degree from the Hebrew University - Hadassah Medical School in Jerusalem, studying the functions of human Natural Killer cells in health and disease. In 2007 he joined the laboratory of Rudolf Jaenisch in the Whitehead Institute - MIT as a postdoctoral fellow by the Helen Hay Whitney Foundation. His work focuses on understanding the mechanism of reprogramming somatic cells to induced pluripotent stem (iPS) cells and developing new iPS based genetic tools for modeling mammalian development and disease.

Armand Keating is Professor of Medicine, Director, Division of Hematology, and Epstein Chair in Cell Therapy and Transplantation at the University of Toronto in Toronto, Canada. He is also Director of the Cell Therapy Program at Princess Margaret Hospital.

He obtained the MD degree from the University of Ottawa, completed residencies in internal medicine and hematology at the University of Toronto and a research fellowship at the University of Washington, Fred Hutchinson Cancer Research Center, and VA Medical Center in Seattle, Washington. He was a Cancer Research Scientist of the National Cancer Institute of Canada for 10 years. For the past decade, he was Chief of Medical Services and Head of the Department of Medical Oncology and Hematology at Princess Margaret Hospital/Ontario Cancer Institute as well as Director of Hematology-Oncology at Mt. Sinai Hospital in Toronto. He was recently listed in Best Doctors in Canada.

Dr. Keating most recently served as an Officer and Secretary of the American Society of Hematology and previously was a Councilor. He is a past president of the American Society for Blood and Marrow Transplantation and currently serves as Chair of the Steering Committee for Cell-Based Therapy of the National Heart, Lung, and Blood Institute, US National Institutes of Health. He is also Chair of the Scientific and Clinical Affairs Committee of The Leukemia & Lymphoma Society (LLS) and an Officer of the LLS Board of Directors. He is on numerous editorial boards of scholarly journals, and is a Co-Editor of Bone Marrow Transplantation, Associate Editor of Biology of Blood and Marrow Transplantation, and a Guest Editor of Cancer.

Dr. Keating’s clinical and research interests focus on anti-cancer cell therapy, blood and marrow transplantation, leukemia, lymphoma, and regenerative medicine targeting cardiac and lung diseases. He has conducted laboratory, translational and clinical research in cell therapy, normal and leukemic hematopoiesis, and on the biology and clinical application of marrow mesenchymal stromal cells. He is the author of over 300 publications on these subjects.

Jonathan Graff is an Associate Professor, Department of Developmental Biology, Genetics and Development Program, at the University of Texas Southwestern Medical Center. He received his PhD from Duke University Graduate School and his MD from Duke University School of Medicine. His post-doctoral fellowship was served at Harvard University’s Department of Molecular and Cellular Biology in the laboratory of Dr. Douglas A. Melton. Since 1996, he has been a faculty member at the University of Texas Southwestern Medical Center. He is the author of numerous publications and is the recipient of several awards, including the Leukemia & Lymphoma Society Scholar Award, American Cancer Society Scholar Award and the American Society for Clinical Investigation.

Dr. Graff’s laboratory work integrates multiple experimental systems, ranging from invertebrates to mice to humans, to unravel the mechanisms that underlie the formation and function of adipose tissues. In particular, his lab has identified hundreds of genes important in metabolism, including novel conserved genes, transcription factors, and signaling cascade components. Employing ‘Evo-Physio’ studies, his lab has increased our understanding of metabolism and could lead to novel obesity and/or diabetes therapies. The lab also seeks to understand the development of the adipose lineage. The localization of the adipocytes progenitor to the vessel wall indicates the presence of a therapeutically-accessible vascular niche. Such studies may provide the basis for therapies for obesity, diabetes, and may be an excellent resource for regenerative medicine and tissue engineering.
1. Title: Functional relevance of lower-affinity cis-regulatory binding sites in mammalian development
   Presenting Author: Sheldon Rowan
   Affiliation(s): Brigham and Women’s Hospital Division of Genetics and Harvard Medical School
   Co-Author(s): Trevor Siggers1, Salil A. Lachke1, Yingzi Yue1, Martha L. Bulyk123, and Richard L. Maas1
   Affiliation(s): 1 Division of Genetics, Brigham and Women’s Hospital and Harvard Medical School, Boston, MA 02115, USA
                   2 Department of Pathology, Brigham and Women’s Hospital and Harvard Medical School, Boston, MA 02115, USA.
                   3 Harvard-MIT Division of Health Sciences and Technology (HST), Harvard Medical School, Boston, MA 02115, USA.
   Abstract:
   The unfolding of the developmental program is based on the changing spatial and temporal levels of key transcriptional regulators. What is not well understood is how regulators biophysically interpret the affinities and composition of their DNA binding sites to mediate quantitative responses. Pax6 is a dosage-sensitive transcriptional regulator that is essential for eye, brain, and pancreas development and is under precise transcriptional control during mammalian embryogenesis. Here we identify the Prep1 (pKnox1) homeodomain transcription factor as a critical and dose-dependent upstream regulator of Pax6 during lens formation. By utilizing protein binding microarrays, we identify a pair of phylogenetically-conserved lower-affinity Prep1 binding sites, each uniquely required for Pax6 lens enhancer activity during development. We show using both in vivo experimental data and mathematical modeling that Prep1 bound to the two sites works synergistically and not additively to regulate Pax6 transcription in response to the changing physiological concentration of Prep1. Furthermore, increasing the affinity of both sites led to premature high-level reporter activity, demonstrating that the affinity of the Prep1 binding sites dictates the timing of Pax6 lens enhancer activation. Thus, we demonstrate for the first time in a mammal an affinity-dependent mechanism, defined within the constraints of a simple biophysical model that controls the timing of gene expression. We propose that binding site affinity may be utilized more broadly as a tunable cis-autonomous device to interpret changing levels of key regulators in the developmental program.

2. Title: The role of sonic hedgehog signaling in adult skeletal muscle development
   Presenting Author: Michael Lin
   Affiliation(s): Joslin Diabetes Center, Wagers Lab
   Co-Author(s): Simone Hettmer, Junhao Mao, Amy Wagers
   Affiliation(s): Simone Hettmer and Amy Wagers - Joslin Diabetes Center
                   Junhao Mao - University of Massachusetts Medical School
   Abstract:
   Sonic hedgehog (Shh) signaling is essential in embryonic development, but evidence suggesting that Shh plays a role in regulating normal adult tissues is relatively scarce. Abnormal Shh signaling has previously been associated with the formation of muscle tumors, which led us to investigate Shh signaling in adult mouse skeletal muscle. Maintenance and repair of adult skeletal muscle begins with activation of normally quiescent muscle satellite cells, which are located beneath the basal lamina of mature muscle fibers. Combinatorial analysis of multiple cell surface markers allows direct discrimination and isolation by fluorescence-activated cell sorting of distinct subsets of satellite cells, including the highly regenerative skeletal muscle precursor cells (SMPs) capable of both differentiation and self-renewal (hallmark properties of tissue stem cells). We show that components of the Shh pathway are detectable by RT-PCR in all cells isolated from the myofiber-associated cell compartment of adult muscle, albeit at relatively low levels. Furthermore, inhibition of Shh signaling with KAADCyclopamine results in reduced proliferation and myogenic differentiation of SMPs, while treatment with Shh conditioned medium promotes their differentiation. However, fluorescence-activated cell sorting and immunohistochemistry of transgenic reporter mice demonstrate that no cells in regenerating muscle produce Shh pathway signals, and only very few cells receive them. Taken together, our findings suggest that it may be important to closely regulate Shh pathway activity in adult skeletal muscle, keeping Shh signaling at low levels in order to prevent abnormal proliferation or differentiation of skeletal muscle precursors, which could lead to tumor formation.

3. Title: Adipocytes Divide
   Presenting Author: Alessandra Rigamonti
   Affiliation(s): Department of Stem Cell and Regenerative Biology, Harvard Stem Cell Institute, Medical Research Institute and Harvard University
Co-Author(s): Alessandra Rigamonti(1,2), Kristen Brennand(3), Toyoaki Tenzen(1), Youn-Kyoung Lee(1), Filip Zembowicz(1), David Lum(1), Chad Cowan(1)

Affiliation(s):
1. Stowers Medical Research Institute and Harvard University, Department of Stem Cell and Regenerative Biology, Harvard Stem Cell Institute, 185 Cambridge Street CPZN 4, Boston MA, 02114
2. Doctorate of Prenatal Science, Fetal Diagnosis and Therapy, University of Milan, Italy
3. HHMI and Harvard University, Department of Molecular and Cellular Biology, Harvard Stem Cell Institute, 7 Divinity Avenue, Cambridge MA, 02138

Abstract:
Obesity is fast becoming a global health pandemic. Characterized by an increase in adipose tissue to the point where it is associated with adverse health effects, the prevalence of obesity has nearly tripled over the past fifty years. Obesity has an enormous economic burden and is the second leading cause of preventable death. The source of increased fat mass in obesity is currently attributed to two mechanisms: adipocyte hypertrophy, the process by which pre-existing fat cells increase in size due to an accumulation of lipids, and adipocyte differentiation from fat precursor cells. Here we show that in adult mice and humans, the adipocyte population is capable of substantial replication. In fact, 1% of murine adipocytes are in growth phase at any time. Furthermore, we find that adipocytes contribute equally to the growth and maintenance of fat tissue. Through three independent experimental approaches, Ki67 labeling, BrdU incorporation, and dilution of an inducible histone2B-green fluorescent protein (H2BGFP) through cell division, we show, at the level of individual fat cells and the adipocyte population as a whole, that adipocytes are capable of replication throughout adulthood. Additionally, we show that adipocyte replication is increased in obese mice. In light of these findings, we suggest that reducing adipocyte replication represents a possible new avenue of therapeutic intervention against obesity.

4. Title: Study of Pax3 Chromatin Modification in Mouse Embryonic Stem Cells During Differentiation and Oxidative Stress to Understand the Molecular Causes of Birth Defects in Diabetic Pregnancy
Presenting Author: Mary R. Loeken
Affiliation(s): Section on Developmental and Stem Cell Biology, Joslin Diabetes Center
Co-Author(s): Yong Zhang, Zaheer Sameermahmood

Abstract:
Pax3 encodes an embryonic transcription factor that is required for neural tube closure. We have previously shown that Pax3 expression is inhibited in embryos of diabetic mice, leading to increased neural tube defects (NTD) in diabetic pregnancies. Oxidative stress, resulting from maternal hyperglycemia, is responsible for inhibiting Pax3 expression.

We hypothesized that oxidative stress prevents modifications of Pax3-associated chromatin that are required for expression. To test this, we employed mouse embryonic stem cells (mESC) that were induced to form Pax3-expressing neuronal precursors. Chromatin immunoprecipitation (ChIP) was performed using chromatin from undifferentiated (U), differentiating (D), or differentiating mESC treated with antimycin A (AA), a drug that induces oxidative stress and inhibits Pax3 expression. Antibodies were against total histone H3 (Pan H3) or methylated or acetylated forms of histone H3. ChIP'd DNA was amplified by PCR using primers complementary to overlapping 200 bp sequences of the Pax3 transcription regulatory element. We found that histones were associated with Pax3 5' flanking DNA in U and D cells. The abundance of histones associated with some 200 bp regions changed with differentiation. In most cases, AA inhibited these changes. Histones associated with several 200 bp regions were modified upon differentiation, and AA inhibited most of these modifications. Moreover, inhibiting histone methylation or deacetylation inhibited Pax3 expression by D cells. These results suggest that changes in histone assembly and modification are necessary for induction of Pax3 during differentiation, but that interference with these processes by diabetic pregnancy-induced oxidative stress inhibits Pax3 expression, and thus, lead to NTD.

5. Title: Live cell imaging distinguishes bona fide human iPS cells from partially reprogrammed cells
Presenting Author: Sutheera Ratanasirintrawoot
Affiliation(s): Harvard University
Co-Author(s): Sutheera Ratanasirintrawoot, Elayan M Chan, In-Hyun Park, Philip D Manosi, Yuin-Han Loh, Hongguang Huo, Justine D Miller, Odelya Hartung, Junsung Rho, Tan A Ince, George Q Daley, Thorsten M Schaeger
Affiliation(s): 1Division of Pediatric Hematology/Oncology, Children’s Hospital Boston and Dana-Farber Cancer Institute, Boston, Massachusetts, USA. 2Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts, USA. 3Stem Cell Program, Children’s Hospital Boston, Boston, Massachusetts, USA. 4Biological and Biomedical Sciences, Harvard Medical School, Boston, Massachusetts, USA. 5Department of Pathology, Brigham and Women’s Hospital, Boston, Massachusetts, USA. 6Harvard Stem Cell Institute, Cambridge, Massachusetts, USA. 7Howard Hughes Medical Institute at Children’s Hospital Boston, Boston, Massachusetts, USA. 8Division of Hematology/Oncology, Brigham and Women’s Hospital, Boston, Massachusetts, USA. 9Manton Center for Orphan Disease Research, Children’s Hospital Boston, Boston, Massachusetts, USA. 10 These authors contributed equally to this work.

Abstract:
Somatic cells can be reprogrammed into induced pluripotent stem (iPS) cells by enforced expression of transcription factors. Using serial live imaging of human fibroblasts undergoing reprogramming, we identified distinct colony types that morphologically resemble embryonic stem (ES) cells yet differ in molecular phenotype and differentiation potential. By analyzing expression of pluripotency markers, methylation at the OCT4 and NANOG promoters and differentiation into teratomas, we determined that only one colony type represents true iPS cells, whereas the others represent reprogramming intermediates. Proviral silencing and expression of TRA-1-60, DNMT3B and REX1 can be used to distinguish the fully reprogrammed state, whereas alkaline phosphatase, SSEA-4, GDF3, hTERT and NANOG are insufficient as markers. We also show that reprogramming using chemically defined medium favors formation of fully reprogrammed over partially reprogrammed colonies. Our data define molecular markers of the fully reprogrammed state and highlight the need for rigorous characterization and standardization of putative iPS cells.

6. Title: Defining Mx1+ osteogenic stem/progenitor cells by tracing the fate of osteoblasts in live animals using stage-specific markers
Presenting Author: Dongsu Park
Affiliation(s): Massachusetts General Hospital & Harvard Stem Cell Institute
Co-Author(s): Bong Ihn Koh, Joji Fujisaki, Charles P. Lin, Henry M. Kronenberg, David T. Scadden
Affiliation(s): Massachusetts General Hospital & Harvard Stem Cell Institute
Abstract:
Maintenance of mature cells is accomplished by either proliferation of existing mature cells or differentiation from less mature, proliferating progenitors. In the mature skeleton, it is unclear which strategy is used during bone remodeling. Despite the concept that multipotent mesenchymal stem cells (MSCs) can be isolated and expanded ex vivo, it is unclear if such cells contribute to musculoskeletal regeneration in vivo. We sought to address this question using inducible fluorescent markers activated at particular stages of osteolineage differentiation and tracked their contribution to endosteal osteoblasts in living animals. Long-term in vivo tracing of labeled osteoblasts using inducible osteoblast reporter (osteocalcin-Cre-ERT;Rosa-EYFP) and inducible osteoblast-ablation (osteocalcin-Cre-ERT;Rosa-EYFP;Rosa-DTR) models revealed that mature osteoblasts have a short lifespan (~60 days) and do not proliferate under either homeostatic or injury conditions but can relocate to injury sites. In contrast, Mx1-Cre induction persistently marked the majority of osteogenic progenitors (CD45-CD31-CD105+CD140a+) and osteoblasts in Mx1-Cre;Rosa-EYFP and Mx1-Cre;Rosa-mTomato/mEGFP mice. The purified Mx1-Cre+ osteogenic progenitors showed clonogenic and ex vivo multi-lineage differentiation potentials, suggesting Mx1-Cre can mark multipotent MSCs. On transplantation, however, Mx1-Cre+ osteogenic progenitors only formed osteoblastic clusters on the endosteal surface. Moreover, in vivo tracking over 6 months of the endogenous progenitors marked by induction of Mx1-Cre revealed that they provided the vast majority of osteoblasts (~80%) with rare adipocytes (>5%) but not chondrocytes, endothelial cells or other mesenchymal elements in bone. These data indicate that osteoblastic cells in the adult mouse skeleton are short-lived and maintained by replacement from Mx1+ osteogenic stem/progenitors rather than expansion of mature osteoblastic cells.

7. Title: Mouse and human amniocytes are highly efficient sources of iPS cells and serve as feeder layers for the propagation of ES cells
Presenting Author: Raymond Anchan, MD, PhD
Affiliation(s): Division of Reproductive Endocrinology and Infertility, Obstetrics and Gynecology, Brigham and Women’s Hospital
Co-Author(s): Philipp Quaas(b), Behzad Gerami-Naini(b), Daniel Day (c), Hrishikesh Bartake (b), Adam Griffin (a), Jennifer L. Eaton (b), Liji L. George (b), Catherine Naber (b), Annick Turbe-Doan (b), Peter J. Park (d), Mark D. Hornstein (a) and Richard L. Maas (b)
Pax3 Regulates Neural Tube and Neural Crest Development by Stimulating p53 Degradation in Neuronal Precursors

Presenting Author: Xiao Dan Wang
Affiliation(s): Section on Developmental and Stem Cell Biology, Joslin Diabetes Center, One Joslin Place, Boston, MA 02215, USA
Co-Author(s): James H. Chappell Jr., Sarah C. Morgan, Mary R. Loeken
Affiliation(s): Section on Developmental and Stem Cell Biology, Joslin Diabetes Center, One Joslin Place, Boston, MA 02215, USA

Abstract:

Pax3 is a transcription factor that is expressed during early embryogenesis in the neural tube, neural crest, and somites. Splotch embryos, which carry mutant Pax3 alleles, develop neural tube and neural crest defects due to neuroepithelial and neural crest apoptosis. p53 knockout mutations prevent apoptosis and neural tube and neural crest defects in Splotch embryos, suggesting that Pax3 suppresses p53-dependent apoptosis. In mouse embryos, steady state p53 protein levels (but not mRNA) are inversely related to numbers of wt Pax3 alleles, suggesting that Pax3 inhibits p53 protein synthesis, stability, or both.

To study this, murine embryonic stem cells (mESC) were induced to differentiate to form neuronal precursors. Pax3, was not expressed in undifferentiated (UD) mESC, but it was expressed in differentiating (D) mESC. In contrast, p53 protein, which
was abundant in UD mESC, was significantly reduced in D mESC. p53 mRNA remained unchanged. The decreased p53 protein upon differentiation was due to Pax3, because increasing expression of Pax3 by stably or transiently transfected expression plasmids by UD mESC decreased p53 protein in a dose-dependent fashion. However, transfection of a plasmid encoding Splotch Pax3 did not decrease p53. Pulse and pulse-chase labeling showed that the rate of p53 protein synthesis did not change between UD and D mESC, but that the half-life of p53 was reduced about 3-fold in D, compared to UD, mESC, suggesting that Pax3 stimulates p53 degradation.

These results indicate that during embryogenesis, Pax3 suppresses p53-dependent processes, thereby maintaining viability and self-renewing capabilities of neuroepithelium and neural crest.

10. Title: Identification of renal stem/progenitor cells during kidney regeneration in zebrafish
Presenting Author: Cuong Q. Diep
Affiliation(s): MGH/HMS
Co-Author(s): Dongdong Ma(2), Frank Bollig(3), Takanori Ikenaga(4), Fumihito Ono(4), Christoph Englert(3), Robert I. Handin(2), Alan J. Davidson(1)
Affiliation(s): (1) Center for Regenerative Medicine, Massachusetts General Hospital, Boston, MA 02114, USA (2) Hematology Division, Brigham and Women’s Hospital, Boston, MA 02115, USA (3) Friedrich-Schiller-University, Jena, Germany (4) Section on Model Synaptic Systems, Laboratory of Molecular Physiology, NIH/NIAAA, Bethesda, MD 20892, USA

Abstract:
The potential of renal regeneration as a therapy for kidney disease has gained intensive interest due to growth in the prevalence of chronic kidney disease and the limitations of current treatments. To date, it is controversial as to whether the mammalian kidney contains renal stem/progenitor cells (RSCs) capable of replacing damaged nephrons. In contrast, the zebrafish kidney displays remarkable regenerative capabilities. Using transgenic zebrafish where specific renal cell populations are fluorescently tagged, combined with gentamicin-induced injury and transplantation experiments, we have identified the existence of a population of RSCs that when infused into circulation can home back to the kidney and generate new functional nephrons. Following kidney injury or during formation of the adult kidney in larval fish, RSCs proliferate to form clusters of mesenchymal cells that express the renal transcription factors wt1b and pax2, supporting the notion that regeneration recapitulates development. Taken together, we conclude that the zebrafish kidney contains RSCs capable of de novo nephron formation during kidney regeneration. Our work provides greater insight into the mechanism of renal regeneration and may lead to the future development of renal regenerative therapies.

11. Title: PGE2-regulated wnt signaling and N-acetylcysteine are synergistically hepatoprotective in zebrafish acetaminophen injury
Presenting Author: Lea Vedder
Affiliation(s): BIDMC
Co-Author(s): Trista E. North1*, Lea M. Vedder1, Allegra M. Lord3, I. Ramesh Babu2, John S. Wishnok2, Steven R. Tannenbaum2,4, Leonard I. Zon3, and Wolfram Goessling5*
Affiliation(s): 1Department of Pathology, Beth Israel Deaconess Medical Center, Boston, MA 02115; 2Stem Cell Program, Children’s Hospital, Harvard Stem Cell Institute, Harvard Medical School, Boston, MA 02115; 3Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139; 4Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139; 5Genetics and Gastroenterology Divisions, Brigham and Women’s Hospital, Gastrointestinal Cancer Center, Dana-Farber Cancer Institute, Boston, MA 02115
*equal contribution

Abstract:
Acetaminophen (APAP) toxicity is the most common drug-induced cause of acute liver failure in the U.S. The only available treatment, N-acetylcysteine (NAC), has a limited time-window of efficacy, indicating a need for additional therapeutic options. Zebrafish have emerged as a powerful tool for drug discovery. Here, we developed a clinically relevant zebrafish model of APAP toxicity. APAP depleted glutathione stores, elevated aminotransferase levels, increased apoptosis and caused dose-dependent hepatocyte necrosis. These outcomes were limited by NAC and conserved in zebrafish embryos. In a pilot embryonic chemical screen, prostaglandin E2 (PGE2) was identified as a potential therapeutic agent; in the adult, PGE2 similarly decreased APAP-associated toxicity. Significantly, when combined with NAC, PGE2 extended the time-window of therapeutically beneficial intervention, synergistically reducing apoptosis, improving liver enzymes, and preventing death. Use of a wnt reporter zebrafish line and chemical genetic epistasis demonstrated that the effects of PGE2 are mediated through the wnt signaling pathway. Zebrafish can be used as a clinically relevant toxicological model amenable to the identification of additional therapeutics and biomarkers of APAP injury, our data suggest combinatorial PGE2 and NAC treatment would be beneficial for patients with APAP-induced liver damage.
Human Umbilical Cord Blood Stem Cell Function Is Augmented by Exposure to Prostaglandin E2

**Abstract:**

Hematopoietic stem cells (HSCs) comprise the base of the entire hematopoietic system and alone possess the ability to both self-renew and differentiate into all mature blood lineages. Prostaglandin (PG) E2 has been shown to enhance HSC engraftment in murine transplantation models. We sought to determine the safety and efficacy of ex vivo dmPGE2 exposure in human cord blood (hCB) stem cells. To determine whether dmPGE2 treatment affected hCB cell viability, apoptosis was measured by FACS analysis for 7AAD and AnnexinV in pooled CD34-enriched (CD34+) hCB samples; at 6 and 9 hours post exposure, cells treated with dmPGE2 showed a significant reduction in apoptosis. Cell proliferation assays demonstrated that dmPGE2 also enhanced HSC self-renewal. To determine if dmPGE2 exposure altered the functional characteristics of hCB, in vitro culture assays were conducted; a 2-fold enhancement in total colony number (p<0.001) was found following dmPGE2 treatment. dmPGE2 likewise caused a dose-dependent increase in cAMP concentration. qPCR analysis of dmPGE2-treated CD34+ hCB cells demonstrated upregulation of RUNX1, the homing effector CXCR4, the anti-apoptotic gene BCLXL and cyclinE1. To mimic clinical transplantation protocols, fresh whole cord blood samples were employed for the xenotransplantation studies; ex vivo dmPGE2 treatment significantly enhanced the number of engrafted recipients compared to matched controls (27/46 vs 13/42; *p=0.01). These data suggest that treatment of hCB with dmPGE2 will be both safe and effective in achieving expansion of HSCs for clinical use; an FDA-approved phase 1 clinical trial evaluating dmPGE2 for this purpose is currently accruing patients at Dana-Farber Cancer Center.
Title: Metabolic endocrine regulators of hematopoietic stem cell formation and function

Presenting Author: James Harris

Affiliation(s): BIDMC

Co-Author(s): Harris J1, Cutting CC2, Goessling W2*, North TE1*

Affiliation(s): 1Department of Pathology, Beth Israel Deaconess Medical Center, Boston, MA 02115 2Genetics and Gastroenterology Divisions, Brigham and Women’s Hospital, Gastrointestinal Cancer Center, Dana-Farber Cancer Institute, Boston, MA 02115

*equal contribution

Abstract:

Obesity and diabetes have emerged as major health problems in the US, however, the direct effects of elevated blood glucose levels on the hematopoietic system are not well characterized. To evaluate the effect of glucose on hematopoietic stem cell (HSC) production, zebrafish embryos were exposed to increasing doses of D-glucose from 5 somites to 36 hours post fertilization (hpf); runx1+ HSCs increased over a range of 0.5 to 2% glucose. FACS confirmed a 2-3-fold enhancement. BrdU incorporation in the AGM was elevated after 1% glucose treatment, while acridine orange staining was reduced. To identify potential mediators of this effect, embryos were injected with morpholino oligonucleotides (MO) against the insulin (insr), and insulin-like growth factor receptors (igfr); MO knockdown of insr or igfrb, but not igfra, influenced HSCs. D-glucose completely reversed these effects, implying functional redundancy, or a multi-effector process of HSC regulation by endocrine factors. To clarify when insr- and/or igfr-mediated activity altered HSC formation, embryos were treated for defined periods with 1% glucose, insulin, or IGF. IGF regulated HSC production only after the establishment of the hematopoietic niche (>24hpf). Glucose positively influenced HSC formation at all time points examined. MO knockdown of the glucose transporter glut1 diminished HSCs, confirming a direct role of glucose in this process. Cyanide and oxaloacetate, inhibitors of oxidative phosphorylation, reversed the beneficial effects of D-glucose, indicating that energy production directly modulates HSC formation. These results could unveil specific risks of obesity and diabetes for hematopoiesis and HSC homeostasis during gestation and in the adult.

Title: Efficient generation of iPS cells from skeletal muscle stem cells

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Abstract:

Reprogramming of somatic cells into inducible pluripotent stem (iPS) cells has generally been found to occur at low efficiency. Initial experiments with mouse embryonic fibroblasts indicated efficiencies of reprogramming less than 0.1%. Inefficient reprogramming could result from multiple causes, including the tissue of origin and differentiation state of the target cell. Recently, hematopoietic stem cells (HSCs) were shown to reprogram at high efficiency (up to 28%), compared to their terminal differentiated progeny. We set out to test the hypothesis that other adult stem cells, specifically skeletal muscle precursor (SMP) cells, might likewise reprogram more efficiently than other, more differentiated cell types in the myogenic lineage. Here we report that, like HSCs, mouse SMPs reprogram with remarkably high efficiency (up to 28%) in contrast to their more differentiated daughter cells (myoblasts). Likewise, multipotent mesenchymal precursor cells also exhibit highly efficient reprogramming in clonal assays. Our data support the notion that adult stem cells, in general, may reprogram more efficiently than cells that have undergone terminal differentiation. Identifying the molecular differences between immature and mature cell populations should reveal barriers inherent to the reprogramming process. Moreover, adult progenitors might be an ideal source for generating human iPS cells at high efficiency.

Title: Uroepithelial differentiation of mouse embryonic stem cells is mediated by retinoic acid and dependent on GATA-4 and GATA-6

Presenting Author: Carlos R. Estrada, M.D.

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Abstract:
In this study, we describe a novel in vitro cell culture system for derivation of bladder-like uroepithelial (UE) cells from mouse embryonic stem cells (ESCs) in response to all trans retinoic acid (RA) and demonstrate a role for GATA4/6 signaling mechanisms in this process.

ESCs were seeded on collagen films and cultured for up to 9 days in DMEM/15%FCS in the absence or presence of RA (0.01-10 microM). The extent of differentiation was assessed by quantitative RT-PCR (qPCR), immunocytochemical (ICC), and immunoblot analyses for markers of pluripotency: OCT-4; pan-endoderm: SOX17, FOXA2, GATA4/6; extra-embryonic endoderm (ExE): α-fetoprotein, SOX7, PEM; definitive hindgut endoderm (DHE): p63, CXCR4, HOXA13; and UE lineage: uroplakins (UP) and cytokeratins (CK). qPCR and ICC analyses demonstrated that RA (10µM) treatment resulted in significant upregulation of all UPs, CK18 and CK20 as well as loss of OCT-4 expression in comparison to controls. In addition, RA enrichment of UP+ populations coincided with peak expression of pan-endoderm and DHE markers as well as downregulation of ExE specification.

qPCR analysis revealed that RA-treated GATA6-/- ESCs failed to upregulate UP expression while GATA4-/- ESCs displayed significantly attenuated UP levels in comparison to WT controls. In addition, electromobility shift assays (EMSA) were performed on 2kb UP1B and UP2 promoter fragments containing putative GATA binding sites. Competition and supershift EMSA analysis revealed specific transcriptional complex formation at each GATA binding site within the murine UP1B and 2 promoters which consisted of both GATA4/6 following RA stimulation.

Title: Embryonic stem cell autocrine signaling in growth, metabolism, and self-renewal
Presenting Author: Laralynne Przybyla
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Abstract:
Signaling between mouse embryonic stem cells (mESCs) plays a significant role in most regulatory processes, including survival, proliferation, differentiation, and self-renewal. An initial step in alteration of a cell’s growth patterns or metabolic state is modulation of the external cellular microenvironment. While this is traditionally achieved with neutralizing antibodies or specific inhibitors, the lack of specificity of such molecules and crosstalk between pathways can make interpretation difficult. An alternate approach to modulation of external signaling is to downregulate all diffusible signaling pathways in an unbiased manner by using microfluidic perfusion to sweep away cell-secreted soluble factors. Using this technique, we have shown the ability to remove mESC-secreted proteins in defined self-renewal media and we have found that downregulation of diffusible signaling affects the growth and metabolic processes of mESCs. Cell growth is slower and levels of functionally important proteins such as Stat3 and Dnmt3b are altered, indicating that autocrine signaling plays a specific role in maintenance of these core cellular processes.

In addition, we have shown that the self-renewal potential of mESCs is impeded upon diminishment of diffusible autocrine signaling. We have evidence for lower expression levels of self-renewal markers and higher levels of early ectoderm differentiation markers in cells that were cultured under perfusion compared to those in conventional static culture. These cells also have a 10-fold reduction in replating efficiency and diminished differentiation potential in embryoid body culture.

Taken together, these results suggest that previously unidentified autocrine factors are vital for maintaining the characteristic properties of mESCs.

Title: Identification of Brown Fat Progenitors in Mouse Skeletal Muscle
Presenting Author: Tim J. Schulz
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Abstract:
Obesity develops when energy intake exceeds energy expenditure. Recent rediscovery of functional brown fat in adult humans provides potential new therapeutic approaches for obesity. Cellular lineage specification to determine brown fat progenitor cell differentiation remains poorly understood. We have identified that bone morphogenetic protein (BMP) 7 functions as an inductive signal for brown fat development. In this study, we prospectively isolated a subpopulation of adipocyte progenitor cells (Sca-1+, CD45-, Mac1-) residing in the population of myofiber-associated cells. Skeletal muscle resident Sca-1+ progenitor
cells (ScaMPCs) are non-myogenic and non-osteogenic, suggesting that this population of cells represents a pool of committed adipose progenitors. Treatment of these ScaMPCs with BMP7 prior to adipogenic differentiation is sufficient to generate bona fide brown adipocytes, marked by induction of early regulators of brown fat fate, PRDM16 and PGC-1, and brown fat-specific markers, UCP1 and Cidea. When engrafted into skeletal muscle after exposure to BMP7, ScaMPCs develop into brown fat-like multilocular cells mixed with some unilocular adipocytes. The ability of ScaMPCs to differentiate into UCP1 positive brown adipocytes is impacted by genetic background. While the brown adipogenic capacity of ScaMPCs is unchanged in animals maintained on high fat diet, progenitors isolated from skeletal muscle of obesity-resistant strains of mice show significantly increased UCP1 expression upon BMP7 treatment compared to cells isolated from muscle of the obesity-prone control mice. Together, these data reveal the identification of committed adipocyte progenitors within skeletal muscle, and the interplay between these cells and BMP-7 drives them to become genuine brown adipocytes.

19. Title: EphrinA3 inhibits the proliferation and neurogenic potential of retinal stem cells in adult mouse  
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Affiliation(s): Schepens Eye Research Institute, Harvard medical school, Department of Ophthalmology, Harvard Medical School, Boston MA

Abstract: Retinal stem cells in adult mammalian retina exhibit limited proliferation and differentiation ability comparing to lower vertebrates, but the mechanism is unknown. Recently, ephrinA2 and ephrinA3 have been identified as the negative regulators of neurogenesis in the adult central nervous system. This project was aimed at exploring the roles of ephrinA2 and ephrinA3 in the regulation of retinal stem cells. Data of Western blot analysis showed that the expression of ephrinA2 and ephrinA3 was detected beginning from postnatal day 10 (P10) in the mouse retina and increased in the adult. The expression of ephrinA2 and ephrinA3 were widely detected in the mouse eye, which include the ciliary body epithelial cells and cornea of adult mouse. Using BrdU pulse labeling method, we showed that mouse deficient for ephrin-A2 and -A3 exhibited increased proliferation of ciliary body stem cells and retinal stem cells as compared to wild-type controls. In culture, cells dissociated form the retina and ciliary body of ephrin-A3-/- or ephrin-A2/-ephrin-A3-/- mice grew more neurospheres than that from WT mice. These sphere-derived stem cells also exhibited higher proliferative potential as compared to those from wild-type mice. Most interestingly, RT-PCR results indicated increased expression of neural stem cell markers and decreased pigmented epithelial cell markers in sphere-derived cells taken from ephrinA3 KO mouse as compared to that from WT mouse. Conclusion: EphrinA2/A3 negatively regulated the proliferation and neurogenic potential of retinal stem cells in adult mouse.

The initial steps in the cascade of molecular events that result in acute leukemia remain incompletely understood. The TEL-AML1 fusion gene, the hallmark translocation in Childhood Acute Lymphoblastic Leukemia and the first genetic hit, occurs years before the onset of clinical disease, most often in utero. We have generated mice in which TEL-AML1 expression is driven from the endogenous promoter and can be targeted to specific populations. We show that TEL-AML1 renders mice prone to malignancy after chemical mutagenesis when expressed in hematopoietic stem cells (HSCs), but not in early lymphoid progenitors. We reveal that TEL-AML1 markedly increases the number of HSCs in the quiescent (G0) stage of the cell-cycle. TEL-AML1+ HSCs retain self-renewal and contribute to normal hematopoiesis, but fail to out-compete normal HSCs. Our work shows that stem cells are susceptible to subversion by weak oncogenes that can subtly alter their molecular program to provide a latent reservoir for the accumulation of further mutations.
Notes