University at Buffalo
The State University of New York

# 2nd Annual
## Stem Cells in Regenerative Medicine Symposium
## (SCiRM)

PRESENTED BY :
THE SCIRM TRAINING PROGRAM

NYSTEM NEW YORK STATE STEM CELL SCIENCE

Clinical and Translational Research Center,
University at Buffalo

# June 11th, 2018

# ACKNOWLEDGMENTS

The Stem Cells in Regenerative Medicine (SCiRM) was established Fall 2016 with funding from the New York Stem Cell Board. SCiRM brings together 18 faculty from the School of Engineering and Applied Sciences, the Jacobs School of Medicine and Biomedical Sciences and Roswell Park Comprehensive Cancer Center working in various aspects of stem cell biology and bioengineering with emphasis on applications of stem cells in regenerative medicine. SCiRM is directed by Stelios Andreadis (SUNY Distinguished Professor and Chair, Chemical and Biological Engineering) and co-directed by Sriram Neelamegham (Professor, Chemical and Biological Engineering) and Richard Gronostajski (Professor, Genetics, Genomics and Bioinformatics).

buffalo.edu/stem-cells-in-regenerative-medicine.html

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# WELCOME FROM THE DIRECTOR

The Stem Cells in Regenerative Medicine (SCiRM) Training Program was launched in Fall of 2016 with $1.85M in funding from the New York State Stem Cell Science Board (NYSTEM). The UB School of Engineering and Applied Sciences, the UB Jacobs School of Medicine and Biomedical Sciences and Roswell Park Cancer Institute (RPCI) Graduate Division joined forces to develop an interdisciplinary program aiming at fostering stem cell science and engineering to accelerate clinical translation of stem cell research and to train the future leaders in stem cell science and engineering.

Our faculty are involved in research with various types of stem cells including embryonic and induced pluripotent stem cells, as well as adult stem cells including mesenchymal, cardiac, skeletal, hematopoietic, neural and neural crest stem cells among others. Our research ranges from basic aspects of stem cell maintenance and differentiation to translational work that aims at using stem cells for treatment of cardiovascular, neurologic or metabolic disorders. SCiRM fellows are co-advised by faculty from Medicine and Engineering, thereby promoting multidisciplinary collaborations and interdisciplinary approaches to research and education. Excellent facilities are available for cutting-edge research including the Western New York Stem Cell Culture and Analysis Center (also funded by NYSTEM), the Next-Generation Sequencing and Expression, Proteomics/Mass Spectrometry, Confocal Microscopy & Flow Cytometry, Cleanroom, Materials Characterization Laboratory and Digital Manufacturing Laboratory among others.

The SCiRM training program is greatly facilitated and enriched by numerous biomedical research and education institutions in the newly built Buffalo-Niagara Medical Campus. These include the new Jacobs School of Medicine and Biomedical Sciences, the new John R. Oishei Children's Hospital, the Clinical and Translational Research Center, the Center of Bioinformatics and Life Sciences, the Hauptman-Woodward Medical Research Institute and the new RPCI Clinical Research Center.
This rich, interdisciplinary environment fosters the integration of scientific discoveries and engineering breakthroughs with the ultimate goal to develop stem cell therapies that can be translated into clinical practice. The combination of a highly trained science and engineering workforce, and the potential for clinical translation and commercialization of research findings, are expected to have significant economic impact in Western New York, as well as throughout NY State. We look forward to the coming years in the program.

Thank you for your interest.

Stelios Andreadis
Director

# Stem Cell in Regenerative Medicine Faculty

## Stelios Andreadis, SUNY Distinguished Professor and Department Chair, Chemical Engineering, UB

*Ph.D., University of Michigan Chemical Engineering*

Research Interests: Stem cells for vascular tissue engineering; signaling pathways in cell-cell adhesion and wound healing; lentiviral vectors and lentiviral microarrays for high-throughput gene expression analysis and gene discovery

## John Canty Jr., SUNY Distinguished Professor and Chief, Division of Cardiovascular Medicine UB

*MD, University at Buffalo*

Research Interests: Apoptosis and cell death; Cardiac pharmacology; Cardiology; Cardiovascular Disease; Gene therapy; Genomics and proteomics; Molecular Basis of Disease; Stem Cells

## Thomas Cimato, Associate Professor, Medicine, UB

*MD, Ph.D., University at Buffalo*

Research Interests: Cardiology; Critical Care Medicine

## Jian Feng, Professor, Physiology and Biophysics, UB

*Ph.D., University of Tennessee*

Research Interests: Apoptosis and cell death; Cytoskeleton and cell motility; Gene Expression; Molecular and Cellular Biology; Molecular genetics; Neurobiology; Neurodegenerative disorders; Neurology; Pathophysiology; Protein Folding; Signal Transduction; Toxicology and Xenobiotics; Transcription and Translation

## David Goodrich, Professor Oncology, Roswell Park Comprehensive cancer Center

*Ph.D., University of California, Berkeley*

Research Interests: Understanding molecular mechanisms underlying tumor suppression mediated by the RB1 and TP53 genes. Identifying genes involved in prostate cancer metastasis. Discerning how transcriptionally formed R-loops contribute to cancer initiation and progression.

## Gen Suzuki, Associate Professor, Medicine, UB

*M.D., Ph.D., Akita University School of Medicine*

Research Interests: Apoptosis and cell death; Cardiology; Cardiovascular Disease; Cell Cycle; Cell growth, differentiation and development; Gene therapy; Internal Medicine; Stem Cells

# Stem Cell in Regenerative Medicine Faculty

## Richard Gronostajski, Professor Biochemistry, UB; Director, Genetics, Genomics & Bioinformatics Program

**Director, Western NY Stem Culture & Analysis Center (WNYSTEM)**

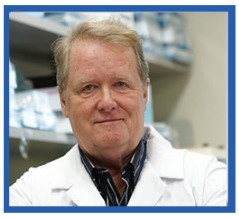
*Ph.D., Harvard University*

Research Interests: Bioinformatics; Cell growth, differentiation and development; Gene Expression; Genomics and proteomics; Molecular and Cellular Biology; Molecular Basis of Disease; Molecular genetics; Neurobiology; Stem Cells; Transgenic organisms

## Kenneth Gross, Emeritus Faculty, Molecular & Cellular Biology, Roswell Park Comprehensive Cancer Center

*Ph.D., M.I.T., Cambridge*

Research Interests: The Renin-expressing Cell and Development of the Renal Vasculature



## Michael Higgins, Associate Professor, Molecular & Cellular Biology, Roswell Park Comprehensive Cancer Center

*Ph.D., Queen's University*

Research Interests: Molecular genetics of Beckwith-Wiedemann syndrome (BWS); epigenetic imprinting and cancer.

## Te-Chung Lee, Associate Professor, Biochemistry, UB

*Ph.D., Virginia Commonwealth University*

Research Interests: Cardiology

## Jonathan Lovell, Assistant Professor, Biomedical Engineering, UB

*Ph.D., University of Toronto*

Research Interests: Nanomedicine and Phototherapy

# Stem Cell in Regenerative Medicine Faculty

**Sriram Neelamegham, Professor, Chemical & Biological, Biomedical Engineering, UB**

*Ph.D., Rice University*

Research Interests: Biomedical Engineering; cell biomechanics; vascular engineering

**Michael Nemeth, Assistant Member, Medicine, Roswell Park Comprehensive Cancer Center**

*Ph.D., Dartmouth College*

Research Interests: Identifying the mechanisms that regulate the development and maintenance of adult stem cells. Developing therapeutic strategies that can target the cancer stem cell population.

**Natesh Parashurama, Assistant Professor, Chemical & Biological Engineering, UB**

*MD, Ph.D., Rutgers University*

Research Interests: Liver stem cell biology; differentiation; cell therapy; organogenesis; disease modeling; tissue engineering; multimodality molecular imaging; monitoring molecular events in living subjects

**Steven Pruitt, Professor, Oncology, Molecular & Cellular Biology, Roswell Park Comprehensive Cancer Center**

*Ph.D., University of Virginia*

Research Interests: Understanding the mechanism by which somatic stem cells maintain tissue homeostasis and the consequences of dysfunction in these mechanisms for age related disease.

**Fraser Sim, Associate Professor, Pharmacology & Toxicology, UB**

*Ph.D., University of Cambridge*

Research Interests: Genomics and proteomics; Neurobiology; Neurodegenerative disorders

**Satrajit Sinha, Associate Professor, Biochemistry, UB**

*Ph.D., University of Texas Health Science Center*

Research Interests: Gene Expression; Genomics and proteomics; Molecular and Cellular Biology

**Daniel Swartz, CEO, Angiograft LLC**

# AGENDA

## Monday, June 11th

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| 8:00 - 8:55 a.m. | Continental Breakfast and Registration |
| 9:00 a.m. | Welcome<br><br>Dr. Stelios Andreadis, SCiRM Director and SUNY Distinguished Professor, University at Buffalo |
| 9:00 - 9:30 a.m. | Nenad Bursac, PhD, Duke University<br><br>"Engineering of Biomimetic Skeletal Muscle" |
| 9:30 - 10:00 a.m. | Sean Wu, MD, PhD, FACC, Stanford University<br><br>"Stem Cell-based Approaches to Cardiac Development and Regeneration" |
| 10:00 - 10:15 a.m. | Coffee Break |
| 10:15 - 10:45 a.m. | Juliane Nguyen, PharmD, PhD, University at Buffalo<br><br>"Utilizing miRNA-enriched MSC Exosomes for Cardiac Regeneration: An Activity Composition Analysis" |
| 10:45 - 11:15 a.m. | Christian Gluck, MD/PhD Candidate, University at Buffalo<br><br>"Exploring the link between ETS1 and Tumor Heterogeneity: Identifying a novel regulator in EMT enriched tumor subtypes" |
| 11:15 - 11:45 a.m. | Jason Spence, PhD, University of Michigan<br><br>"Modeling human development using organoids and embryos" |

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| 2:00 - 2:30 p.m. | Amelia Eisch, PhD, University of Pennsylvania<br><br>"New neurons in the postnatal hippocampus:<br>What are they good for?<br>And what is good for them?" |
| 2:30 - 3:00 p.m. | Jian Feng, PhD, University at Buffalo<br><br>"Decoding Parkinson's Disease with Patient-Specific Neurons" |
| 3:00 - 3:15 p.m. | Coffee Break |
| 3:15 - 3:45 p.m. | Xiuqian Mu, MD, PhD, University at Buffalo<br><br>"Multi-levels of gene regulation control retinal cell differentiation" |
| 3:45 - 4:15 p.m. | Lorenz Studer, MD, Memorial Sloan Kettering<br><br>"Human pluripotent stem cells to model and treat neurological disease" |
| 4:30 p.m. | Closing remarks and poster winners announced<br><br>** Students must be present in order to receive an award. |

# SPEAKERS

## Nenad Bursac, Ph.D.

Professor of Biomedical Engineering, Medicine and Cell Biology, Member of the Duke Cancer Institute and Co-Director of the Regeneration Next Initiative, Duke University

Dr. Nenad Bursac is a Professor of Biomedical Engineering, Medicine, and Cell Biology at Duke University. As a PhD student in Robert Langer's group at MIT, he demonstrated the first engineering of functional heart tissues using mammalian cells. As a postdoctoral fellow in Leslie Tung's group at Johns Hopkins University, he developed novel methods to control architecture and function of cardiomyocyte cultures for studies of cardiac arrhythmias. Currently, Dr. Bursac's research involves development of novel cell, tissue, and genetic engineering therapies for heart and skeletal muscle disease. Examples of this work include engineering of first human contractile skeletal muscle tissues from primary and induced pluripotent stem cells, first fabrication of human cardiac tissue patches with clinically relevant dimensions, and use of engineered prokaryotic sodium channels as a platform for control of tissue excitability. Dr. Bursac has authored more than 90 scientific articles and has mentored more than 30 PhD students and postdoctoral and medical fellows. He co-directs Regeneration Next Initiative at Duke University. He is a recipient of the Stansell Family Distinguished Research Award and Stem Cell Innovation Award. In 2014, Dr. Bursac was the president of the North Carolina Tissue Engineering and Regenerative Medicine Society. Since 2015, Dr. Bursac has been a Fellow of American Institute for Medical and Biological Engineering.

## Amelia Eisch, Ph.D.

Professor of Anesthesiology and Critical Care, Perelman School of Medicine University of Pennsylvania

Dr. Eisch is a tenured Professor in the Department of Anesthesiology and Critical Care at The Children's Hospital of Philadelphia and Department of Neuroscience at the University of Pennsylvania Perelman School of Medicine (CHOP/PennMed). Dr. Eisch grew up in upstate NY (Vestal, NY), and received her undergraduate degree in Psychology (Biology Track) from Yale University. She earned her PhD in Biology from the University of California at Irvine where she worked with Dr. John Marshall in the Department of Psychobiology to probe the neural underpinnings of psychostimulated-induced neurotoxicity of dopaminergic neurons. During her postdoctoral studies at Yale University Medical School in the lab of Dr. Eric Nestler, Dr. Eisch first identified the negative consequences of drugs of abuse for the birth of new neurons in the adult brain. This finding opened the possibility that new neurons in the adult brain were involved in addiction, and that harnessing the regenerative power of adult neurogenesis may be helpful in treating addiction. Since starting her independent lab in the Department of Psychiatry at the University of Texas Southwestern Medical Center in Dallas in 2000 through her move to CHOP/PennMed in 2016, Dr. Eisch and her team have probed the relationship between adult neurogenesis and psychiatric disorders – like addiction, depression, and post-traumatic stress disorder – with the ultimate goal being to translate these findings into clinical relevance. She has generated new tools, like inducible transgenic mouse lines that allow fate tracking and genetic manipulation of adult neural stem cells in vivo. She has used these tools to define the cell autonomous regulation of adult stem cells by a variety of molecules, including Cdk5, FMRP, Reelin, Ascl1, NeuroD1, Notch1, and Mef2. More recently, she has used behavioral, FACS, microarray, and neurosphere approaches to identify how a novel small molecule promotes neurogenesis and memory in mice, and used a circuit-based approach to uncover a novel pathway in mice to regulate mood. Dr. Eisch has received numerous honors, including being named the inaugural Seymour Benzer Lecturer by the US National Academy of Sciences, and many teaching and mentoring awards.

## Jian Feng, Ph.D.

Professor, Department of Physiology and Biophysics, Jacobs School of Medicine & Biomedical Sciences, University at Buffalo

Dr. Feng received his B.Sc. in biochemistry from Nanjing University in China and Ph.D. in biochemistry from University of Tennessee in the laboratory of Dr. James Ihle at Howard Hughes Medical Institute, St. Jude Children's Research Hospital. His postdoctoral research was performed at the Rockefeller University with Dr. Paul Greengard, who received the Nobel Prize in Physiology or Medicine in 2000. Dr. Feng joined the faculty of the State University of New York at Buffalo in 2000 and became a full professor in 2010. He is the Director of the Induced Pluripotent Stem Cell Facility at the University since 2010. Dr. Feng has been studying the molecular and cellular mechanisms of Parkinson's disease for over 20 years. He is an expert on using stem cells to study Parkinson's disease. Dr. Feng has published 75 papers with an h-index of 47. His laboratory was the first to generate induced pluripotent stem cells from Parkinson's disease patients with parkin mutations. He has discovered critical functions of the Parkinson's disease gene parkin in human midbrain dopaminergic neurons. Dr. Feng is a member of Faculty of 1000 and serves as the Associate Editor in the Stem Cell Biology section of Experimental Biology and Medicine.

## Christian Gluck

MD/Ph.D. Candidate, Department of Biochemistry, University at Buffalo

My current work involves the molecular characterization of the network governed by Ets1, an oncogenic transcription factor, in specific subtypes of epithelial cancers that show enriched expression of stem cell and epithelial mesenchymal transition (EMT) markers. Several studies have shown that tumor cells undergoing EMT exhibit Cancer Stem Cell (CSC) and tumor initiating properties and importantly, tumors that show enriched expression of EMT/CSC markers are chemoresistant to standard therapy. Hence, it is important to investigate the molecular mechanisms by which specific drivers of CSC and EMT, such as transcription factors, govern tumor formation, progression, dissemination and ultimately treatment. I am using an integrative approach involving biochemical experiments, bioinformatics analysis and genetic studies to elucidate the gene-regulatory network governed by Ets1 in the stem/EMT enriched tumors and tumor initiating cells of both Head and Neck Squamous Cell Carcinoma and Breast Cancer. We hope to leverage the findings of these studies to discover new therapeutic targets and biomarkers for these epithelial cancers.

## Xiuqian Mu, MD, Ph.D.

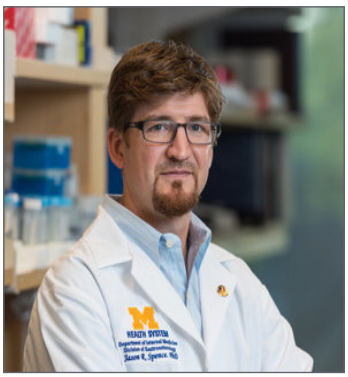
Associate Professor, Department of Ophthalmology, Jacobs School of Medicine & Biomedical Sciences, University at Buffalo

Dr. Mu graduated from Qingdao Medical College with an MD and subsequently from Peking Union Medical College with a PhD in Biochemistry and Molecular Biology. He then conducted research in developmental biology as a postdoctoral fellow at the NIH with Dr. Alan Kimmel. He joined Dr. Bill Klein's group at the MD Anderson Cancer Center in 1999 to study cell differentiation in the developing retina. In 2008, he joined the Department of Ophthalmology at University at Buffalo as a tenure track Assistant Professor, and was promoted to Associate Professor with tenure in 2015. Dr. Mu is interested in how gene regulation regulates the shift of cellular states from progenitors to fully functional mature cell types along individual cell lineages, particularly the ganglion cell lineage, during retinal development. Major efforts in his lab are on identifying key regulators in this process, uncovering their roles in individual lineages, and understanding how they carry out these roles at epigenetic, transcriptional, and post-transcriptional levels. He has made major contributions to our understanding of retinal cell differentiation as demonstrated by his many publications in the field.

## Juliane Nguyen, PharmD., Ph.D.

Assistant Professor, Department of Pharmaceutical Sciences, School of Pharmacy, University at Buffalo

Juliane Nguyen, Ph.D., is an assistant professor of Pharmaceutical Sciences at the University at Buffalo. The Nguyen Lab is developing novel protein-, RNA-, and lipid-based biochemical and delivery platforms for treating myocardial infarction and cancer. One of her research foci is the development of biomaterials equipped with molecular zip codes for the subcellular delivery of macromolecules. Dr. Nguyen's research has received recognition through the Biomedical Breakthrough Award, the UB Exceptional Scholar Young Investigator Award, and the NSF CAREER Award. Dr. Nguyen received her Ph.D. in Pharmaceutical Sciences from the Philipps-University of Marburg (Germany). She then trained at UCSF under Dr. Frank Szoka, where she was a Deutsche Forschungsgemeinschaft Postdoctoral Fellow.



## Jason Spence, Ph.D.

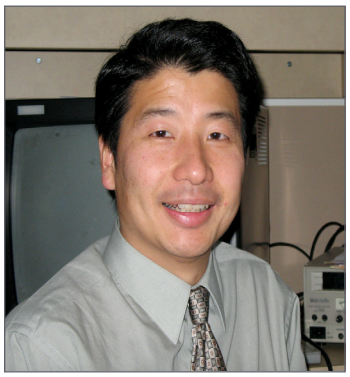
Associate Professor, Internal Medicine, University of Michigan

Dr. Spence was born in Chatham, Ontario Canada and attended elementary and high school in Blenheim, Ontario. Dr. Spence moved to the U.S. for his postsecondary education, and attended Canisius College, a small liberal arts school in Buffalo, NY, graduating in 2001. He attended graduate school at Miami University (Ohio) where he earned his Ph.D. in 2006. His Ph.D. work, conducted in the lab of Katia Del Rio-Tsonis, focused on understanding mechanisms that drive regeneration and tissue repair in unique model organisms that maintain regenerative ability throughout life, including Notophthalmus viridescens (Eastern Newt), Ambystoma mexicanum (Axolotl) and the chick. Following his Ph.D., he performed a research fellowship in the lab of Jim Wells at Cincinnati Children's Hospital, where he turned his focus to understanding mechanisms that regulate embryonic development of endoderm-derived tissue (pancreas, liver, intestine) and utilizing human pluripotent stem cells (hPSCs) to understand human differentiation and development. During this time, he pioneered efforts to generate 3-dimensional intestinal organoids from human pluripotent stem cells using an approach know as directed differentiation. In October 2011, Dr. Spence joined the faculty of the University of Michigan Medical School. The strengths and focus of the Spence lab include using mouse models to study embryonic development of GI and associated organs, and in using 3-dimensional human models to study human development and disease. The lab is currently pursuing projects aiming to understand intestinal development and function and also studies lung development, using mice and a new 3-dimensional human lung organoid model the Spence lab has recently described.

## Lorenz Studer, MD

Director, Center for Stem Cell Biology and Member of the Developmental Biology Program at Memorial Sloan Kettering

Lorenz P. Studer, MD, is the Director of the Center for Stem Cell Biology and a Member of the Developmental Biology Program at Memorial Sloan Kettering. As a native of Switzerland, he received his MD and doctorate degree from the University of Bern where he co-developed the first cell-based therapy for Parkinson's disease in the country. He subsequently trained as postdoctoral fellow with Dr. Ron McKay at the National Institutes of Health pioneering the therapeutic application of neural stem cell-derived neurons in models of neurodegeneration. In his laboratory, he has led the development of techniques that can turn human pluripotent stem cells into many of the specific cell types that comprise the central and the peripheral nervous system. He has also been among the first to realize the potential of patient-specific stem cell in modeling human disease and in drug discovery, and he described methods to manipulate cellular age in pluripotent stem cell-derived lineages. Furthermore, he is currently leading a multidisciplinary consortium to carry out the first clinical application of human stem cell-derived dopamine neurons for the treatment of Parkinson's disease. Dr. Studer's work has been recognized by numerous awards including the Boyer Young Investigator award, the Annemarie Opprecht Award, a MacArthur fellowship and the Ogawa-Yamanaka Stem Cell Prize.



## Sean Wu, MD, Ph.D., FACC

Endowed Faculty Scholar, Lucile Packard Foundation for Children; Associate Director, Cardiovascular Institute, Associate Professor of Medicine (Cardiovascular Medicine) and, by courtesy, of Pediatrics, Stanford University

Sean Wu graduated from Stanford University in 1992 where he completed majors in Biological Sciences and in Mechanical Engineering with Honors and Distinction. He subsequently completed an MD-PhD training at Duke University School of Medicine and an internal medicine resident at the Duke University Hospital. He then completed a clinical and research fellowship in cardiovascular medicine at the Massachusetts General Hospital/Harvard Medical School (HMS) where worked with Stuart Orkin at Boston Children's Hospital to identify a bi-potent cardiac progenitor cell from developing mouse embryos and embryonic stem cells (Wu et al, Cell 2006). He was an Assistant Professor of Medicine at HMS from 2009 until 2012 when he returned to Stanford where he is now a tenured Associate Professor of Medicine and, by courtesy, Pediatrics, an Associate Director at the Stanford Cardiovascular Institute, and an Endowed Faculty Scholar at the Lucile Packard Foundation for Children, Stanford University School of Medicine. His research is dedicated to the identification of molecular mechanisms regulating cardiac lineage commitment during embryonic development and the biology of cardiac progenitor cells in development and disease. His research work has led to the identification Yin Yang 1 as a master regulator of mesodermal commitment into cardiac lineage, the discovery of a robust compensatory response in the early fetal heart towards cell loss, and the role of IGF signaling to expand the number of cardiac progenitor cells, among others. More recently, his lab has explored the transcriptional signature of single cardiac cells from the mouse embryos and embryonic stem cells using single cell RNA sequencing and the use of 3D bioprinting and bioacoustics patterning to generate bioengineered cardiac tissue. His research is funded by awards from the NIH/NHLBI, NIH Director's New Innovator Awards, NIH Director Pioneer Award, American Heart Association Established Investigator Award, California Institutes for Regenerative Medicine, the Endowed Faculty Scholar Award from the Lucile Packard Foundation for Children/Child Health Research Institute at Stanford, among others.

# SPEAKER ABSTRACTS

## Engineering of Biomimetic Skeletal Muscle

Nenad Bursac, Ph.D.
Duke University

Engineering three-dimensional skeletal muscle tissues is motivated by the need for improved physiological systems that can serve for modelling and studying of muscle diseases, pre-clinical drug development, and potential muscle regenerative therapies. In this talk, I will describe first-time engineering of contractile human engineered muscle tissues made of primary myogenic cells isolated from muscle biopsies and myogenic progenitors derived from induced pluripotent stem cells by transient overexpression of satellite cell marker Pax7. Resulting engineered microtissues ("myobundles") exhibit aligned architecture, multinucleated and striated myofibers, and a Pax7+ cell pool. They contract spontaneously and respond to electrical stimuli with robust calcium transients, twitch and tetanic contractions. During culture, myobundles maintain functional acetylcholine receptors and structurally and functionally mature, as evidenced by increased myofiber diameter, improved calcium handling and contractile strength, and enhanced expression of muscle-specific maturation genes. When treated with diversely acting drugs, myobundles undergo dose-dependent physiological responses or toxic myopathy, similar to clinical outcomes. In response to exercise-mimetic electrical stimulation, myobundles show increased force generation and metabolic flux and undergo gross tissue hypertrophy induced by both the growth of existing and the formation of new myofibers. When derived using cells from patients with congenital skeletal muscle diseases, myobundles exhibit expected pathological phenotypes. Implanted myobundles progressively vascularize and maintain functionality for at least 3 weeks in vivo. Tissue-engineered myobundles provide an enabling platform for predictive drug and toxicology screening and development of novel therapeutics for degenerative muscle disorders.

## New Neurons in the Posnatal Hippocampus: What are they good for? And what is good for them?

Amelia J. Eisch, Ph.D.
University of Pennsylvania

More than 50 years ago, it was discovered that new neurons are made throughout life in the mammalian hippocampus, a brain structure central to memory and mood regulation. As the hippocampus is a target of many neurologic and psychiatric disorders – including Alzheimer's disease, epilepsy, and major depressive disorder – the discovery of lifelong hippocampal neurogenesis raises the enticing possibility that we may be able to harness new neurons and their stem cell precursors to develop novel therapeutic intervention for these disorders. For this 2nd Annual Stem Cells in Regenerative Medicine Symposium, I will highlight recent findings the literature and unpublished research from my laboratory suggesting adult hippocampal neurogenesis is important for our understanding, treatment, and perhaps prevention of psychiatric disorders, like depression and addiction. I will also discuss the emergence of new molecular, cellular, and network level approaches to manipulate neurogenesis and hippocampal function in laboratory animals, and comment on the future of such approaches for translational application, particularly outside the scope of early life and into maturity. Finally, in emphasizing work from our laboratory and others on the heterogeneity of the hippocampal neural stem cell population in vivo, this presentation will provide a current view not only of "what new neurons are good for" and "what is good for them", but also how neural stem cell manipulations influence neurogenesis and hippocampal function.

## Decoding Parkinson's Disease with Patient-Specific Neurons

Jian Feng, Ph.D.
The State University of New York at Buffalo

Human nigral dopaminergic (DA) neurons have massive axon arborization and utilize an oxidation-prone neurotransmitter to subserve the computational requirements of basal ganglia circuits in motor control. These unique vulnerabilities of human nigral DA neurons are exposed when parkin is mutated. Through the generation and analysis of midbrain DA neurons from patient-specific induced pluripotent stem cells (iPSCs), we have found that parkin mutations disrupt the precision of dopaminergic transmission by increasing spontaneous DA release and reducing DA reuptake. Dysfunctional dopaminergic transmission leads to oscillatory neuronal activities in iPSC-derived midbrain neurons from parkin patients, but not from normal subjects. Similar oscillatory neuronal activities in PD patient brains are believed to cause motor deficits by reducing the information content of dopaminergic transmission. Mutations of parkin elevate dopamine-induced oxidative stress by increasing the transcription of monoamine oxidases, mitochondrial enzymes responsible for the oxidative catabolism of cytosolic dopamine, a reaction that produces large amounts of reactive oxygen species. Furthermore, parkin mutations reduce the complexity and length of neuronal processes by destabilizing microtubules. The microtubule-stabilizing agent taxol restores the morphology of iPSC-derived midbrain DA neurons from parkin patients, while treating normal neurons with the microtubule-depolymerizing drug colchicine induces similar morphological changes found in parkin-deficient neurons. Our studies illustrate the usefulness of patient-specific neurons in deciphering the biological functions of parkin in human nigral DA neurons. The rapid development of stem cell technologies affords more exciting opportunities to reconstruct human nigral DA neurons for the development of a disease-modifying therapy of PD.

## Exploring The Link Between ETS1 and Tumor Heterogeneity: Identifying a Novel Regulator in EMT Enriched Tumor Subtypes

Christian Gluck, MD/Ph.D., Candidate and Satrajit Sinha, Ph.D.,
The State University of New York at Buffalo

Large-scale, massively parallel sequencing of human cancer samples has revealed tremendous heterogeneity across tumors of the same anatomical location. These tumor-to-tumor variations in genetic mutations, epigenetic aberrations and gene expression are recognized in almost every type of cancer. However, current therapies treat cancer as a homogenous disease. Understanding the specific driving forces behind different intrinsic subtypes of tumors will facilitate a better understanding of the nature of cancer and will provide insight into the development of more effective cancer therapies.

Transcriptional dysregulation is a hallmark of cancer. These dysregulated programs, which define the expression of genes that define tumor phenotypes, is driven by the function of transcription factors. As the function of specific Master transcription factors can dictate cell identify, it can be assumed that key transcription factors are driving the gene expression patterns that cause intertumoral heterogeneity. Hence, understanding the dependencies of tumors on these specific regulators of gene expression will highlight the critically important genes and pathways to which malignant cells are addicted as well as identify novel therapeutic interventions in cancer.

We have characterized the role that ETS1, an oncogenic transcription factor, plays in the dysregulated transcriptional networks found within specific subtypes of both Breast Cancer (BRCA) and Head and Neck Squamous Cell Carcinoma (HNSCC). We discovered that ETS1 is preferentially expressed in the Basal and Claudin-Low subtypes of BRCA as well as the Mesenchymal subtype of HNSCC, which are all aggressive forms of cancer that currently lack targeted therapy. These subtypes are associated with the overexpression of key drivers of the Epithelial to Mesenchymal Transition (EMT). Recent evidence has shown that cancer cells undergoing EMT are endowed with stem-cell like self-renewal properties and that the Cancer Stem Cells (CSC) likely confer resistance of tumors to chemotherapy treatments.

Using an integrative analysis that identified the direct transcriptional targets of ETS1 within cell-line models of the aforementioned cancer subtypes, we uncovered core gene-signatures that highlight ETS1 as a crucial regulator of oncogeneic processes that are intrinsically linked to the phenotypes that define specific subtypes of BRCA and HNSCC, such as EMT and cellular invasion. Thus, our studies portray ETS1 a critically important regulator of transcriptional networks that drive cancer subtype specific phenotypes and maintain intertumoral heterogeneity, which can be leveraged for targeted therapeutics. Additionally, our work broadly highlights the power of genomics approach as a valuable tool for further tumor classification and discovery of cancer-subtype specific pathways

## Multi-levels of gene regulation control retinal cell differentiation

Xiuqian Mu, MD, Ph.D.
The State University of New York at Buffalo

Glaucoma is a major neurodegenerative disease affecting the vision of millions of people. It is characterized by progressive loss of retinal ganglion cells in the retina. Stem cell-based cell replacement therapy holds the promise of restoring the lost vision in glaucoma patients. Understanding how retinal ganglion cells form during development will provide essential knowledge for developing efficient stem-cell based therapy for glaucoma. Our recent studies have revealed the gene regulatory pathway essential for the fate-commitment and differentiation of retinal ganglion cells. Our current study focuses on the shift of the epigenetic landscape along the different stages of retinal ganglion cell differentiation. In addition, we discovered that regulation of mRNA decay also plays a critical role in regulating the balance between proliferation and differentiation in the developing retina, indicating multi-levels of gene regulation control retinal cell differentiation.

## Utilizing miRNA-enriched MSC Exosomes for Cardiac Regeneration: An Activity Composition Analysis

Juliane Nguyen, PharmdD., Ph.D.
The State University of New York at Buffalo

Mesenchymal stem cell (MSC)-derived exosomes mediate tissue regeneration in a variety of diseases including ischemic heart injury. Despite an increasing number of studies reporting the regenerative effects of MSC exosomes, the underlying molecular mechanisms and their miRNA complement are poorly characterized. Thus, we microRNA (miRNA)-profiled MSC exosomes and conducted a network analysis to identify the dominant biological processes and pathways modulated by exosomal miRNAs. At a system level, miRNA-targeted genes were enriched for (cardio)vascular and angiogenesis processes in line with observed cardiovascular regenerative effects. Targeted pathways were related to Wnt signaling, pro-fibrotic signaling via TGF-β and PDGF, proliferation, and apoptosis. When tested, MSC exosomes reduced collagen production by cardiac fibroblasts, protected cardiomyocytes from apoptosis, and increased angiogenesis in HUVECs. The intrinsic beneficial effects were further improved by virus-free enrichment of MSC exosomes with network-informed regenerative miRNAs. In a mouse model of myocardial infarction MSC exosomes enriched with miR-101 significantly decreased infarct size and improved cardiac function.

## Human pluripotent stem cells to model and treat neurological disease

Lorenz Studer, MD
Memorial Sloan Kettering Cancer Center

Human pluripotent stem cells (hPSCs) present a powerful new avenue for studying human disease and for developing new cell-based therapies in regenerative medicine. Our group has developed strategies to coax human PSCs into specific neurons on demand and at scale. The work demonstrates that insights from developmental biology can guide the rationale design of human stem cell differentiation. Here, I will discuss new strategies to use human PSCs for neural disease modeling including next generation differentiation technologies that enable the study of many genetic variants in parallel. Such approaches may be particularly powerful in the study of psychiatric disorders such as autism or Schizophrenia, disorders that involve many genes in their pathogenesis. The new technology should accelerate hPSC-based studies in the field towards unraveling the underlying genetic architecture of those still poorly understood human disorders. Furthermore, I will present an update on our work geared towards the translation of hPSC technology, in particular for Parkinson's disease (PD). I will discuss the progress of developing a cell based therapy that is at the verge of clinical translation with a first in human clinical trial scheduled to start later this year. Those studies involve a multidisciplinary effort involving neurologist, neurosurgeons, cell manufacturing specialists, rodents and non-human primate experts and experts in regulatory affairs and working closely with the FDA towards human applications. In addition to our planned trial in New York, we have close interactions with teams in Kyoto, Japan and in Cambridge UK / Lund Sweden as members of the G-Force PD, a global alliance of investigators focused on brining hPSC-derived dopamine neurons to the clinic in a safe and thoughtful manner. After nearly two decades of human PSC research the field is at an exciting stage where human testing is imminent with PD being one of the main therapeutic targets.

## Stem Cell-based Approaches to Cardiac Development and Regeneration

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Heart diseases such as ischemic heart failure and complex congenital heart defects have been studied intensively in the past four decades using animal models in vitro and in vivo. However, with rare exceptions, the mechanistic etiology of most of these diseases remain largely unknown and the methods to treat them remain quite primitive. Some of the challenges are due to the inaccessibility of human cardiac tissue for detailed investigation and the heterogeneity of the disease phenotype and genotype. Recently, human stem cell from autologous sources have been proposed as a cell source for cardiac repair and regeneration. However, results from adult cell transplantation studies have demonstrated minimal benefit. To gain greater insights into the biology of cardiac development and differentiation, we have employed pluripotent stem cells (e.g. embryonic stem cells and induced pluripotent stem cells) to gain critical insights into the logic of cardiac cell differentiation and to identity various cardiac cell types for potential therapy. My lab uses iPSCs to model adult and congenital heart diseases and to apply high throughput screening methods to identify new drugs that can ameliorate disease phenotype in vitro. We also incorporating tissue engineering approaches with the goal of generating thick, perfusable, contractile, cardiac tissues for better modeling of complex multi-cellular disease processes and for cardiac regenerative therapies. The successes and challenges of various approaches to use stem cells-derived cardiac cells for discovering new therapeutic modalities for heart disease applications will be presented and discussed.

## Defining the Role of BK Channels in Human Oligodendrocyte Progenitor Cells

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Multiple sclerosis is a neurodegenerative disorder that is characterized by chronic destruction of myelin, leading to an attenuation in saltatory conduction and ultimately axonal dystrophy. Recently, inhibitory muscarinic receptor signaling acting via M1R and/or M3R has been shown to impair spontaneous myelin repair or remyelination that is mediated by adult neural stem/progenitor cells known as oligodendrocyte progenitor cells (OPCs). Previously, we found that treatment of human OPCs (hOPCs) with a non- selective muscarinic agonist Oxotremorine (Oxo-M) induced intracellular calcium release and store-operated calcium entry (SOCE). In this study, we hypothesized that muscarinic agonist-induced SOCE could contribute to differentiation block via the activation of calcium-activated potassium BK channels (Kca1.1). BK channels are known to play a functional role in various physiological processes including membrane hyperpolarization during action potential, proliferation, and transcriptional regulation. However, the functional role of BK channels in hOPCs remains unexplored, and may mediate the inhibitory effects of Gq-coupled receptors such as M1/3R. To test this hypothesis, we treated primary PDGFαR+ hOPCs with pharmacological inhibitors of BK channels, Iberiotoxin (IBTx) and Paxilline (Pax) in the presence or absence of muscarinic agonist for 3 days. As previously shown, activation of muscarinic receptors significantly impaired primary hOPC differentiation to O4+ oligodendrocytes (n=3 individual human samples). Interestingly, we found that inhibition of the BK channel rescued the anti-differentiative effects of muscarinic agonist treatment leading to an increase in oligodendrocyte differentiation. These results provide novel insight into the downstream signaling mechanisms of muscarinic receptors and identify BK channels and their downstream signaling pathways as possible candidates to improve OPC differentiation in demyelinating diseases.

## Tet1 Deficiency Impairs the Differentiation of Human Embryonic Stem Cells to Neuroectoderm

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Tet family of 5-methylcytosine (5mC) dioxygenases play critical roles in development by modifying DNA methylation. While the expression of Tet1 is the highest in human embryonic stem cells (hESCs), its function in hESCs is unclear. Using CRISPR, we mutated both alleles of Tet1 gene in H9 hESCs by introducing stop codons in its catalytic domain. H9 cells with mutated Tet1 exhibited normal morphology, expressed pluripotency markers and were able to differentiate to cells of all three germ layers in serum-containing medium, even though the level of hydroxymethylcytosine (5hmC) decreased to 20% of that in parental H9 cells. Neural differentiation induced by double SMAD inhibitors was not significantly affected by loss of Tet1 activity. However, in morphogen-free condition, Tet1 deficiency significantly reduced the generation of SOX1+PAX6+Nestin+ neuroectoderm cells from 70% in parent H9 cells to 20% in the mutant H9 cells. This was accompanied by a 20 fold reduction in the expression level of PAX6 and a significant decrease in the amount of 5hmC on PAX6 promoter. Our results suggest that loss of tet1 catalytic function impairs the intrinsic ability of hESCs to differentiate to neuroectoderm, possibly by decreasing the expression of PAX6, a key transcription factor in the development of human neuroectoderm.

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## Cadheirn 11 as a Novel Regulator of Cell Growth in Cooperation Platelet Derived Growth Factor Beta

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Cellular adhesion mediated by homotypic cadherin interaction is recognized as a critical event during cell development including morphology, differentiation, and migration as well as maintain tissue integrity and homeostasis. Cadherin 11 (CDH11) is a typical type II cadherin that is mainly expressed in mesenchymal cells. Recently, our lab reported that cadherin 11 is necessary for differentiation of human mesenchymal stem cells (hMSC) into smooth muscle cells. In addition, we identified cadherin 11 as a mediator of extra cellular matrix (ECM) synthesis by in fibroblasts and MSC via TGFβ and ROCK pathways. Here we report our recent discovery that in cooperation with platelet derived growth factor receptor beta (PDGFRβ), CDH11 regulates cell growth through the PDGFRβ-PI3K-AKT signaling cascade. Binding of cells to CDH11 elevates their sensitivity of PDGFRβ to its ligands by 10-100 times, ultimately enhancing the effect of PDGF-BB on cell proliferation. Conversely, loss of CDH11 compromises AKT signaling and cell growth. In agreement, we observed significant reduction in the thickness of the dermal layer of cdh11-/- mice model, as compared to wild type controls. Interestingly, losing of CDH11 reduces PDGFRβ expression, as evidenced by shCDH11 knock down in human fibroblast and cdh11-/- mouse cells. Cutaneous wound healing experiment further revealed the cdh11-/- mice failed to heal full thickness dermal wounds even at two weeks while post-injury, while wild type control mice were completely healed by that time. In summary, we discovered that a cadherin, CDH11 acts in synergy with a growth factor receptor, PDGFR , to promote cell survival and proliferation even under conditions of nutrient deprivation. This finding may have significant implications in various biological processes including epithelial-to-mesenchymal transition, wound healing and tumor metastasis.

## Induction of Definitive Endoderm from Human Pluripotent Stem (hPS) Cells

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Currently, we are unable to produce functional hepatocytes, and the precursors to functional liver cells remain poorly understood. Endoderm, which gives rise to liver, pancreas, and lung, is the most poorly understood germ layer, and has never been isolated from humans. Also, human pluripotent stem cell (hPSC)-derived endoderm, is heterogeneous, and further investigation into the factors that regulate its differentiation are essential. Genetic in vivo studies demonstrate that developmental master regulatory gene circuits (DRGC) control endoderm formation, and since relatively large changes in gene expression occur during germ layer formation, the DRGC coordinate large changes in gene expression. In order to achieve a high yield of hepatocytes, it is necessary to obtain pure endoderm lineage from embryonic stem (ES) cells. Since DRGC control downstream transcription factors (TFs) and each other levels, it is critical to define and understand DRGC to better understand endoderm formation, and eventually hepatocyte formation; then, the produced pure cells may be used for different research and clinical applications. Hence, it is of highly importance to understand how different soluble factors and signaling pathways activate or inhibit particular transcription factors and how that affects the expression of downstream genes to govern endoderm formation during early stages of development.

The primary objective of our research is to understand how Foxa1 and Foxa2 (Foxa1/2), regulate DRGC composed of master regulatory transcription factors and can eventually be used to control hPSC-derived endoderm induction, maintenance, and differentiation. Foxa1/2 together with other endodermal transcription factors, compose the poorly understood DRGC. Foxa2 was identified as the first known pioneer TF, which binds to silent differentiation genes, like Albumin, to prime them for activation. The binding of Foxa2 appears to open normally inaccessible heterochromatin and provides access for other TF of DRGC, like Hnf1, HNF4a, and HNF6 to bind. This enables thousands of genes to be activated, within endoderm derivatives, in a relatively short amount of time during development. Consistent with this, mouse genetic studies of Foxa1/2 double knockdown (Foxa1/2-/-) within hepatic endoderm, demonstrates a complete absence of the liver bud and a failure to initiate the hepatic differentiation (Lee et al., Nature 2005). Similarly, Foxa1/2-/- phenotype in pancreatic endoderm, severely blocks pancreatic growth and differentiation. The developmental role of Foxa1/2 is in contrast to its role in mature liver tissue, in which Foxa1/2 knockdown in adult hepatocytes has no effect on transcription factors or liver- specific genes.

In order to better understand the relationship between major signaling pathways, soluble factors and main transcription factors that govern endoderm formation, we apply multiple differentiation protocols for endoderm induction. We use UCSF4 human embryonic stem cell line, various growth factors and small molecules to activate or inhibit specific signaling pathways such as Nodal, Wit, BMP and FGF during endoderm differentiation. Our optimized protocol requires less cytokines than previously reported methods and we use serum and feeder free system in low oxygen which resembles in vivo endoderm development condition. Interestingly, we demonstrate that how small changes during hPS cells differentiation towards definitive endoderm results in a different configuration of the DRGC and various expression of pluripotency, mesendoderm and endodermal markers in produced endoderm.

We show that activating Nodal pathway is essential but not sufficient for proper endoderm induction because this results in formation of a mixture of various cell types, including undifferentiated cells, mesendoderm and definitive endoderm. Activating Wnt signaling pathway facilitates the differentiation of pluripotent population. After mesendoderm formation, however, Wnt signaling favors mesoderm formation and thus its presence reduces the purity of endoderm. To overcome this contradictory roles of Wnt pathway before and after mesendoderm formation, we stop Wnt activation which reduces mesoderm formation significantly. Further, in order to completely inhibit Wnt pathway we use small molecules that target endogenous Wnt to direct differentiation towards definitive endoderm. In addition, BMP pathway plays a similar role as Wnt and inhibition of both Wnt and BMP pathways results in higher expression of endodermal markers and lower expression of mesendoderm and mesodermal markers. Although, it is yet unknown what configuration of the DRGC is ideal for endoderm but further in vivo analysis can reveal the effect of the transcription factors network on the quality of endoderm and how it undergoes patterning in the next developmental stages.

Furthermore, it has been shown that keratinocyte growth factor (KGF or FGF7), is capable of opening chromatin regions at developmental enhancers so that either Foxa1/2 or PDX1 can bind and in the presence of proper developmental cues they can regulate hepatic or pancreatic differentiation, respectively (Wang et al., Cell Stem Cell 2015). Therefore, definitive endoderm requires epigenetic priming of developmental enhancers, pioneer transcription factors and proper developmental signals in order to differentiate further towards its downstream derivatives. However, it is still unclear how endodermal state and fate change in the absence of any of these factors. We seek to differentiate PSCs towards definitive endoderm, and then by manipulating the DRGC we maintain endoderm and lock the cells in that state. This provides a unique system that results in a better understanding of transcriptional network involved in endoderm formation, maintenance and differentiation and also enables us to reduce heterogeneity of endoderm which is vital for clinical and translational applications. Consistent with in vivo process, our data indicates that endoderm is a transient lineage and its state cannot be maintained unless endoderm DRGC is manipulated. We show that, how genetic manipulation of endoderm can enhance its maintenance and differentiation towards endoderm derivatives.

## CDC-Derived Exosomes Polarize Macrophages to a Distinct Arg1 High Phenotype

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Introduction: Cardiosphere-derived cells (CDCs) have been shown to prevent abnormal heart remodeling, tissue fibrosis, and improve systolic function post myocardial infarction (MI). These effects appear to be largely mediated by paracrine signaling, of which exosomes have been shown to play a role. CDCs have also been shown to mediate cardioprotection via modulation of the macrophage response post MI. The role of CDC-derived exosomes on macrophage polarization, however, is less clear as is the contribution of anti-inflammatory macrophages to a cardioprotective CDC-exosome effect. Identifying the mechanisms of action of CDC-derived exosomes will enable identification of novel therapeutic approaches to augment cardiac repair.

Hypothesis: CDC-derived exosomes polarize cardiac macrophages to a distinct phenotype responsible for mediating cardioprotection and limiting myocardial injury through suppression of inflammation and fibrosis.

Methods: Mouse CD68+ macrophages were isolated by peritoneal lavage, plated at 4 mil cells/ml in RPMI media, and treated with CDC-exosomes or media control. Following a 6 hour incubation, RNA was extracted from macrophages and qPCR performed for a number of M1 and M2 genes. Additionally, to characterize what phenotype was dependent on exosome release, murine peritoneal macrophages were cocultured with either nSMase2 knockdown human CDCs unable to secrete exosomes or a scrambled CDC control. Following 24hrs, macrophage RNA was again extracted and qPCR performed.

Results: Purified CDC-exosomes polarized murine macrophages to a unique phenotype characterized by a downregulation of M1 genes (TNFα, Nos2, Il1b, Il6, and Fpr2) and a dose-dependent upregulation of the M2 gene, Arg1+. Coculture of CDCs with macrophages induced an Arg1High phenotype which was dependent on exosome release, as coculture with nSMase2 KD CDCs was unable to induce Arg1 expression in macrophages.

Conclusions: CDC exosomes polarize macrophages towards an anti-inflammatory phenotype with downregulation of M1 genes and a dose dependent upregulation of Arg1. CDC-exosomes are both necessary and sufficient for macrophage Arg1 expression, a gene previously implicated in suppressing Th2-dependent inflammation and fibrosis.

## Development of a Cardiomyocyte Targeting Delivery System Utilizing Cardiosphere-Derived Cell Exosomes

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Research has shown that treating the heart with stem cell populations, including cardiosphere-derived cells (CDCs), post myocardial infarction stimulates regeneration, angiogenesis, and functional improvement. While this treatment has shown promise in early stage clinical trials, there remains a gap in the ability to efficiently deliver tissue-specific agents directly to the heart while avoiding nonspecific delivery to other organs. Improving exosomal uptake by cardiomyocytes following myocardial infarction would allow for improved delivery of beneficial drug and gene therapies and may result in an increase in viable myocardium and improved functional capacity of the heart. Therefore, our aim was to develop an efficient delivery system that can target cardiomyocytes and transport drugs, siRNA, and/or miRNA to aid in the treatment of cardiovascular diseases. To achieve this aim, Lamp-2b was cloned with cDNA from C2C12 cells and ligated downstream of the CMV promoter in a lentiviral transfer plasmid. Primers designed to encode a cardiomyocyte targeting peptide were used to introduce the targeting ligand between XhoI and BspEI at the N-terminus of Lamp-2b forming the final vector (LV-CMP). We subsequently generated LV-CMP lentivirus by triple transfection of LV-CMP with helper plasmids pLP/VSVG and psPAX2 into 293T cells. CDCs were transduced at 1 MOI in the presence of polybrene. Exosomes were isolated from infected and non-infected CDCs and labelled with a nucleic acid dye. Cultured mouse cardiomyocytes were incubated with 10 ug of fluorescent labeled CDC-exosomes or LV-CMP CDC-exosomes and imaged at 2 and 24 hours. Primary cardiomyocytes showed a 17-fold increase in uptake of targeted CMP CDC-exosomes in vitro when compared with their non-engineered CDC control at 24 hours, establishing the efficacy of our system and laying the groundwork for further experiments to assess the efficiency of cardiac localization and cardiomyocyte uptake in vivo.

## Neural Crest Stem Cells from Human Epidermis Skin Tissue

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Neural crest Stem Cells (NCs) are transient cells generated during early vertebrate development. These multipotent cells show extensive migratory and proliferative capacity as well as the potential to differentiate into multiple lineages from craniofacial skeletal tissues to cells of the peripheral nervous system. However, clinical application of these cells is hindered by the limited availability of cell sources. Previously we showed that NC can be derived from neonatal keratinocytes (KC) with a differentiation protocol that did not involve genetic modification. Although a very promising result, it was not clear whether NC could also be derived from the skin of adult donors, who are most likely in need of cellular therapies. Here we showed that NCs can be obtained from epidermal KC of human skin tissues of adult donors ranging from 78 to 97 years of age. Adult NCs derived from KC expressed key NC markers including lineage-specific transcription factors such as SOX10, FOXD3 and intermediate filament, NES. Notably, adult NC did not exhibit the hallmarks of cellular senescence (aging) as evidenced by examination of multiple markers of cellular senescence including p16, p21, p53, oxidative DNA damage or SA- -galactosidase expression. Using protein expression and functional assays, we demonstrated that KC-derived NC could be coaxed to differentiate into all NC-specific lineages including neurons, Schwann cells, melanocytes, and smooth muscle cells using appropriate differentiation strategies. Finally, implantation of adult NC in chick embryos showed that these cells contributed to the full repertoire of NC derivatives, from neural and glial cells to mesenchymal and pigment cells. Our results suggest that multipotent NC stem cells can be derived from adult skin, without genetic modification or reprogramming to the pluripotent state. These findings may have significant implications for treatment of neurodegenerative diseases for which cell sourcing remains a major roadblock to development of cellular therapies.

## Developing Cell Adhesion Engineering (CAE) Technology to Improve Stem Cell Delivery Following Myocardial Ischemia-Reperfusion Injury

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The blockage of the coronary artery results in myocardial infarction, tissue damage and loss of heart function. This can happen at the time of vessel-reperfusion following an ischemic attack (i.e. ischemia-reperfusion injury). Stem cell therapy has been shown to have promising results in reversing heart remodeling and improving heart function after an ischemia reperfusion injury in animal models with a number of attempted clinical trials. One of the shortcomings of such clinical trials is the poor homing and engraftment ability of the natural stem cells as they lack the ability to target the site(s) of injury.
As a result of an ischemia reperfusion injury, the inflammatory nature of the injured/inflamed myocardial tissue encourages excessive neutrophil recruitment via the selectin-dependent neutrophil adhesion cascade. We hypothesize that by modifying stem cells with selectin ligands, they can both home at the inflamed site of injury independently, and form aggregates with neutrophils and being carried out to the targeted site of therapy more efficiently. The current work summarizes the establishment of cell surface engineered stem cells and preliminary results on neutrophil stem cells aggregate formation in-vitro. More work is in progress to reinforce the efficiency of these modified stem cells in binding to neutrophils and their capture by stimulated endothelium and the validation of these techniques for improving stem cell delivery in animal models. Overall, the cell surface engineering of stem cells may enable the targeting of stem cells, thus enhancing the success rate of current and future clinical trials.

## 6-O Sulfation Modulates Cell-Fate Signaling in Oligodendrocyte Progenitor Cells

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Following pathological loss of myelin, oligodendrocyte precursor cells (OPCs), an adult stem cell population, infiltrate regions of demyelination and differentiate into myelin-producing oligodendrocytes (OLs). In Multiple Sclerosis (MS), the inhibitory tissue environment in chronically demyelinated lesions acts to prevent efficient myelin repair and remyelination. Therefore, the identification of mechanisms which promote OPC differentiation is critically important for the development of new and effective treatments. In this study, we sought to identify candidate drug targets that would be interfere with several signaling cascades acting en masse to impair differentiation. Transcriptional network analysis of primary human OPCs (hOPCs) revealed that SULF2, which encodes an extracellular 6-O endosulfatase, was highly expressed by OPCs and via whole genome network analysis associated with maintenance of the progenitor state. Pharmacological inhibition of sulfatases via PI-88 treatment or specific lentiviral knockdown of SULF2 switched hOPCs from a basal state of low heparin sulfate 6-O sulfation to a highly sulfated state and in so doing abrogated their transcriptional responsiveness to both inhibitory BMP and WNT signaling. Inhibition of SULF2 attenuated the negative effects of BMP treatment on oligodendrocyte differentiation suggesting that sulfatase inhibition may be sufficient to promote differentiation in an inhibitory lesion environment. Importantly, we found that inhibition of sulfatases via OPC-specific conditional deletion of Sulf1/2 or pharmacological treatment with PI-88 led to accelerated OPC differentiation and myelin repair following lysolecithin-induced demyelination in the spinal cord. Taken together, these results suggest that sulfatases represent a novel and effective therapeutic target for the acceleration of OPC differentiation in the treatment of demyelinating disease.

## Application of Genome-Wide CRISPR Library in Understanding How Nanog Reinstates Myogenic Potential in Senescent Hair Follicle Derived-Mesenchymal Stem Cells

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Hair follicle derived-mesenchymal stem cells (HF-MSC) have shown therapeutic potential in smooth muscle regeneration in animal models with peripheral vascular diseases. Despite these encouraging results, organismal ageing and culture senescence in HF-MSC reduces myogenic capacity, which remains a major hindrance to further preclinical studies. Previously, our laboratory has demonstrated that the pluripotency factor, Nanog, reverses the effect of ageing in HF-MSC and restores myogenic contractility. Herein, we proposed to employ a combination of a LentiViral Dual promoter (LVDP) and a CRISPR/Cas9 guide RNA library to uncover genes responsible for these events. The LVDP contains a myogenic specific marker controlling the expression of ZsGreen, allowing monitoring of myogenic differentiation as well as a constitutive promoter expressing DsRed to assess transduction efficiency and for data normalization. To seek an appropriate myogenic specific marker, we generated LVDP vectors containing the smooth muscle alpha-2 actin promoter, or the transcriptional response elements, Smad2/3-RE and CArG-RE, all of which have been shown to be activated during smooth muscle cell differentiation. Upon induction to differentiation in TGF-β1 containing medium (DM), Smad2/3-RE, CArG-RE, and ACTA2-P activity were ~7-, ~ 5-, and ~27-fold, respectively, greater than cells in growth medium lacking TGF-β1 (GM). These results suggested that all the 3 Pr/RE tested could be used to monitor myogenic differentiation. We also compared the efficiency of gene knockout using a 2-vector versus a 1-vector CRISP/Cas9 guide RNA system. The 2-vector system required a lower virus titer to achieve the same level of gene knockout as the 1-vector system. Hence the 2-vector system was chosen to avoid adverse effect of high virus titer on cell viability.Currently, we have engineered HF-MSC to express Nanog and the LVDP-ACTA2 promoter. We have also transduced the engineered HF-MSCs with a CRISPR/Cas9 pooled library containing 120,000 single guide RNA targeting ~19,000 genes. Cells that spontaneously differentiate under GM conditions and cells that fail to differentiate under DM conditions will be sorted and genomic DNA will be isolated and sequenced using high throughput Next Generation Sequencing to identify potential myogenic inhibitors and promoters, respectively. Our study may identify novel genes essential for myogenic differentiation and may shed light on the design of small molecules to promote smooth muscle cell regeneration.

## NANOG Restores Collagen Type III Production in Aged Stem Cells

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Collagen type III (Col3) is one of the three major collagens in the body and loss of expression or mutations in the Col3 gene have been associated with the onset vascular diseases such the Ehlers-Danlos syndrome. Previous work reported significant reduction Col3 in tissues such as skin and vessels with aging. In agreement, we found that Col3 was significantly reduced in senescent (aged) stem cells. Most notably, we discovered that that ectopic expression of the embryonic transcription factor, NANOG, restored collagen expression and the mechanical properties of Col3 containing tissues.

To study the effect of NANOG on aged cells, we employed two widely established models of aging i.e. replicative senescence of human Mesenchymal Stem Cells (MSC), and myofibroblasts derived from patients suffering from Hutchinson's Guilford Progeria Syndrome (HGPS), a premature aging syndrome. MSC were induced to senescence by serially passaging. To express NANOG, cells were transduced with a tetracycline-regulatable lentivirus, which enabled expression of NANOG by addition of Doxycycline (Dox) after cells reached senescence. Production of ECM was evaluated in NANOG expressing senescent cells and young and control culture senescent and HGPS cells.

Our results showed that the level of Col3 diminished in senescent cells but was completely restored by ectopic expression of NANOG. To further explore the underlying molecular mechanism, we performed RNA sequencing and Gene Ontology and Ingenuity Pathway Analysis, which showed that the TGF-βsignaling pathway was activated by NANOG. Genes associated with the activation of TGF-β pathway such as SMAD2/3, TGF  2, TGF  R1 and LTBP1 were upregulated, while negative regulators of the pathway Smad6, Smad7 and Smurf2 were downregulated upon NANOG expression. These results were verified with experiments that employed chemical inhibitors and shRNA knockdown. NANOG increased the expression and nuclear accumulation of Smad2/3 leading to increased Col3 transcription. ChiP-Seq experiments revealed that NANOG also bound to the SMAD2 and SMAD3 promoters, which was also verified by immunoprecipitation with antibodies against Smad2/3. In addition, loss and gain of function experiments showed that SMAD3 was mostly responsible for mediating the effects of NANOG in increasing Col3 synthesis. In conclusion, we report that NANOG restored production of Col3, which was impaired by cellular aging, and suggests novel strategies to restore the impaired ECM production and biomechanical function of aged tissues including skin and arteries, with broad applications in tissue regeneration and potentially anti-aging treatments.

## Store-operated Calcium Signaling Regulates Human Oligodendrocyte Progenitor Cell fate

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In multiple sclerosis (MS), destruction of myelin sheaths and insufficient repair can lead to axonal degeneration, and severe neurological dysfunction. Endogenous remyelination is contingent upon the differentiation of adult CNS stem/progenitors known as oligodendrocyte progenitor cells (OPCs). As such, the development of effective pharmacological interventions for MS requires an essential understanding of the key molecular mechanisms that regulate OPC homeostasis.

Previous research in our lab identified that muscarinic M3 receptor (M3R) negatively regulates hOPC differentiation, and genetic ablation of M3R in hOPC promotes remyelination in vivo. The primary goals of this study were to investigate M3R signaling in human OPCs (hOPC) and identify intrinsic mechanisms regulating hOPC fate. We hypothesized that the muscarinic agonist oxotremorine (Oxo-M) causes an ER-dependent rise in intracellular calcium ([Ca2+]i) in hOPCs, and that M3R directly contributes to this signaling.

To test our hypothesis, we developed an EF1  -driven lentiviral GCaMP6s calcium reporter to directly study [Ca2+]i levels in hOPCs. We demonstrate that Oxo-M dose dependently induces [Ca2+]i release in hOPCs, and that M3R knockdown attenuates the extent and magnitude of this signaling.

Suggesting a common mechanism for blockade of OPC differentiation, we also identified [Ca2+]i signaling following activation of G  q metabotropic glutamate subtype-5 receptors (mGluR5), and found that selective mGluR5 agonist treatment significantly attenuated hOPC differentiation.

Importantly, using pharmacological inhibitors revealed store operated calcium entry (SOCE) to be an essential component of Oxo-M induced M3R signaling. To determine whether SOCE is sufficient to block hOPC differentiation, we used an optogenetic STIM1 lentivirus to independently modulate SOCE in hOPCs and found that optogenetic-induced SOCE attenuates hOPC differentiation.

These findings afford valuable insights into the regulatory mechanisms guiding hOPC fate, and provide new alternative strategies for developing regenerative therapies for the treatment of MS.

## NANOG Restores the Myogenic Differentiation Potential of Senescent Myoblasts

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Skeletal muscle loss due to aging, sarcopenia, is a major medical problem facing elderlies. Adult skeletal muscle regeneration relies on the activity of resident satellite cells in skeletal muscle niche. However, systemic and intrinsic factors decrease the myogenic differentiation potential of senescent satellite cells. Here we show that expression of embryonic transcription factor NANOG in late passage myoblasts restored their myogenic differentiation potential which was impaired due to cellular senescence. This reversal in the myogenic differentiation was proven both at the functional level, by formation of myotubes in 2D and 3D, and at the molecular level by restoring the expression level of myogenic regulatory factors (Myf5, Myod, Myogenin, and MRF4) and members of myocyte enhancer factor 2 family.
The anti-aging effects of NANOG were accompanied by pluripotency effects of NANOG on myoblasts, in which expression of NANOG in differentiation phase could inhibit myotube formation, therefore removal of this factor was required before the start of differentiation. Interestingly, the rejuvenating effects of NANOG on senescent myoblasts were stable for 2 weeks after removal of this factor, suggesting that NANOG might have imparted epigenetic changes. This rescue of myogenic differentiation was accompanied by reversal of cellular morphology and amelioration of aging hallmarks (i.e. trimethylation of H3K9, reduction of reactive oxygen species, and decrease in the DNA damage marker gH2AX).
In conclusion, these results shed light on the potential of NANOG to restore the myogenic differentiation potential of senescent myoblasts and to reverse the loss of muscle regeneration due to aging.

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## Monocytes are Circulating Stem Cells for Vascular Tissue Regeneration

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Introduction: Recently our group demonstrated that immobilized VEGF can capture circulating progenitor cells from the blood in-vitro. Furthermore, we have demonstrated proof of concept by implanting a-cellular tissue engineered vessels (A- TEVs) comprised of SIS immobilized with heparin and vascular endothelial growth factor (VEGF) into the arterial system of sheep which remained patent (92%, n=12) for 3mo (Fig.1). Upon analysis, the lumen of these grafts was comprised of a fully functional endothelium as early as 1mo post implantation. This study sought to identify the type of cells that are captured by VEGF on the lumen of A-TEVs in-vivo and understand how these cells turn into an endothelial (EC) monolayer that is capable of maintaining patency in-vivo.

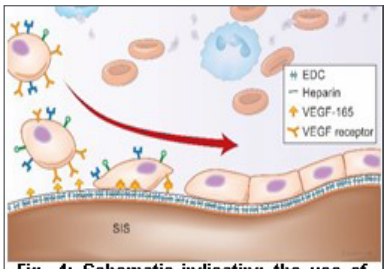

Fig. 1: Schematic indicating the use of VEGF to capture blood borne, VEGFR-expressing cells and repopulate the vascular graft lumen.

Materials and Methods: A-TEV implantations were performed as previously published. In-vivo Analysis: Fixed explants of 1wk, 1mo, 3mo, and 6mo VEGF functionalized A-TEVs are assessed via IHC for MC and EC markers. Blood borne mononuclear cells that are captured on surface immobilized VEGF are coaxed to differentiate into EC with a combination of soluble and biophysical signals.

Results and Discussion: A-TEVs were implanted as interpositional grafts into the arterial circulation of an ovine animal model. As early as 1mo post-implantation, the graft lumen was fully endothelialized as shown by IHC for EC markers, CD144 and eNOS. At the same time, luminal cells co-expressed leukocyte markers CD14 and CD163 (Fig. 2). To understand these results, we performed cell capture experiments under flow using microfluidic devices. Interestingly, blood mononuclear cells expressing high levels of VEGF receptors were captured on CHV surfaces with high specificity under a range of shear stresses. Initially, these cells expressed high levels of CD14 and CD16. Under the right conditions they were coaxed to differentiate into an EC phenotype as shown by expression of CD144, VEGFR2, and eNOS (Fig. 3-4), additional IC analysis, qRT-PCR, and flow cytometry also confirmed this observation. We will also discuss the role of soluble signals and biophysical forces in transdifferentiation of blood cells into EC that maintain graft patency.

Conclusions: We demonstrate the ability of VEGF functionalized
surfaces to capture progenitor cells directly from the blood in-vitro and in vivo. In the presence of the right biochemical and biophysical signals these stem-like cells differentiate into EC like cells that maintain graft patency and vascular function. Our results shed light into the process of vascular tissue regeneration in situ using the body's regenerative capacity via circulating monocytes. Furthermore, this study suggests monocytes as a possible stem cell population for vascular regeneration.

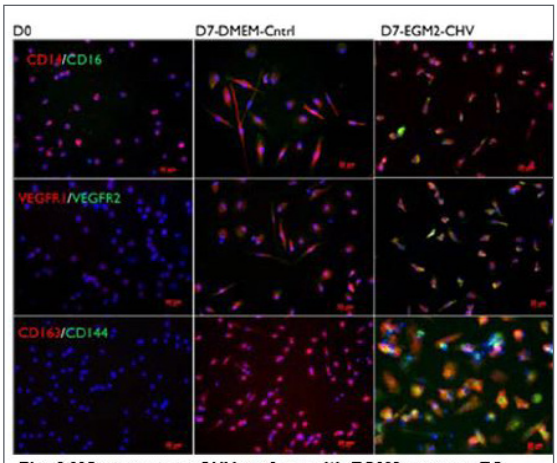


Fig. 3 MCs grown on CHV surface with EGM2 express EC markers after 7d, control surface of FN with DMEM media do not express EC markers. Blue=DAPI.

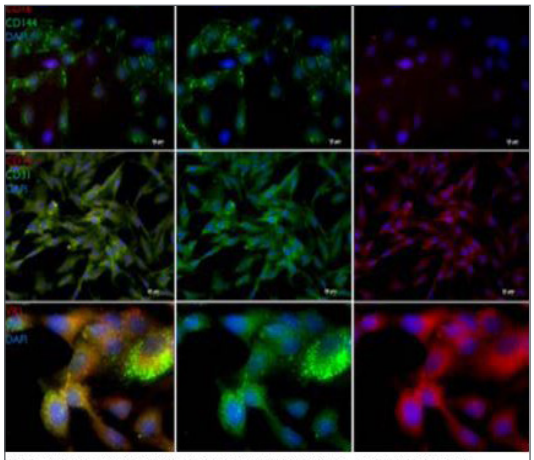

Fig. 4 MCs differentiated in the presence of platelet rich plasma on FN into more mature MC-EC. Blue=Dapi

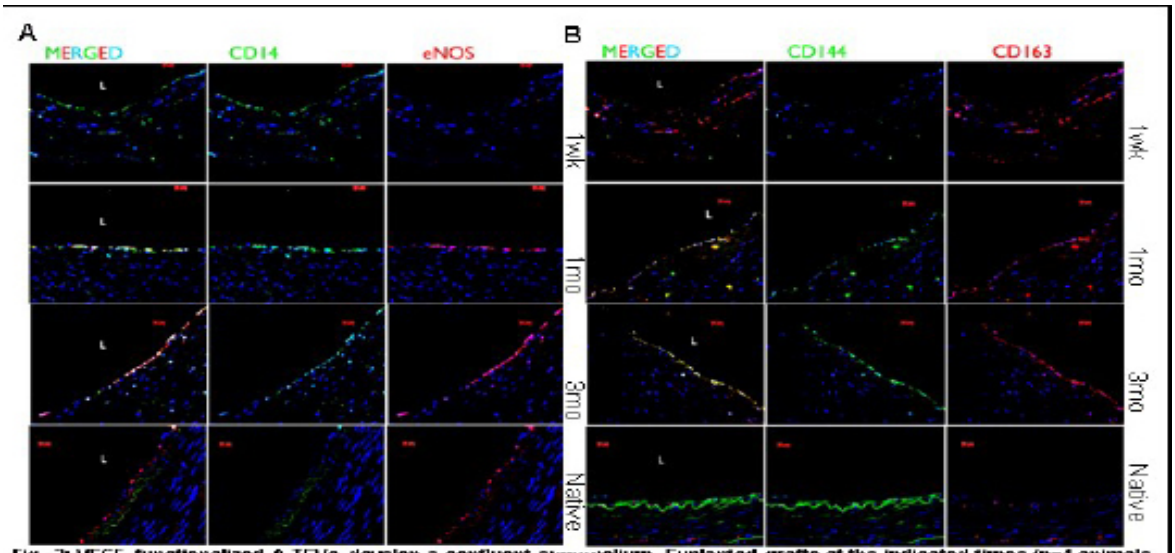


Fig. 2: VEGF functionalized A-TEVs develop a confluent endothelium. Explanted grafts at the indicated times (n=4 animals at each time point) were stained for EC markers eNOS (A) and CD144 (B) and MC/MΦ markers CD14 (A) and CD163 (B). Blue stain=DAPI. Note that the basal lamina of native arteries is highly auto-fluorescent (wavy structure under the EC monolayer).

## Enhancing Cardioprotection with Allogeneic Cardiosphere-Derived Cells Using Cyclosporine in Swine with Myocardial Infarction

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Introduction: Allogeneic cardiosphere-derived cells (CDCs) have been shown to be cardioprotective in the setting of acute myocardial infarction and reperfusion in several animal models. The cells reduce inflammation and scarring while enhancing cardiac regeneration and contractility. Unlike other stem cell therapies which rely on direct differentiation, these cells mediate their protective effects via release of paracrine factors. Indeed, the effect of these factors persists even after most of the delivered cells have disappeared from the heart. It is plausible then, that the clearance of these allogeneic cells may be delayed with immunosuppression using cyclosporine, thus prolonging and enhancing their efficacy.

Methods: Swine underwent a 90-minute balloon occlusion of the LAD coronary artery to produce MI. Ten minutes prior to reperfusion, animals were given either IV cyclosporine or saline. Thirty minutes after reperfusion, animals were again given either CDCs or saline as three 10 minute coronary infusions, one into each of the three coronary arteries. The cyclosporine treated animals then received oral cyclosporine daily for the next month until termination. This produced three groups of animals that were randomized to treatment, either saline/saline, saline/cells, or cyclosporine/cells. After 1 month, infarct size, cardiac function, and cardiac remodeling were assessed.

Anticipated Results: We expect that CDCs alone will reduce infarct size and improve cardiac function measured at 1 month. We anticipate that cyclosporine treatment will enhance the cardioprotective effects of the CDCs thus reducing infarct size and improving cardiac function and remodeling beyond that of cells alone.

## From Skin to Nervous System : Investigating Signaling in Neural Crest Stem Cells from Interfollicular Human Epidermis

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Neural Crest cells (NC) play a central role in forming the peripheral nervous system, the craniofacial skeleton and pigmentation of the skin during development due to their broad multilineage differentiation potential into neurons, Schwann cells, melanocytes, and mesenchymal stem cells. Recently, we identified an easily accessible source of pluripotent neural crest stem cells from human inter-follicular keratinocyte (KC) cultures. Here, we investigate the importance of two growth factor, FGF2 and IGF1 in the induction of KC to NC, with respect, to the expression of potent NC markers Sox10 and FoxD3 and proliferation potential. Our approach contains both experimental and high-throughput genomic sequencing tools in order to shed light to the gene regulatory networks and pathways that govern our system. Using chemical inhibition and shRNA knockdown strategies, we uncovered that the downstream regulatory pathways AKT/PI3K, MEK/ERK and cJun are critical in Sox10 and FoxD3 regulation in our system. In addition, 10X single cell RNA-sequencing is employed to analyze the different stages of the KC to NC induction over time. After identifying clusters with discrete genetic profiles, such as KC expressing epidermal markers or pluripotent NC (towards the later days of induction), we compare our findings with genetic profiles from human embryonic stem cell or induced pluripotent stem cell-derived NC. This high-throughput RNA-seq analysis provides insights into the genes and pathways that play a critical role in our KC-NC system. In summary, our study provides a better understanding of the role of FGF2 and IGF1 on the induction of NC from KC cultures and sheds light on the pathways through which these growth factors regulate key NC transcription factors Sox10 and FoxD3, which can prove pivotal in sustaining the pluripotent phenotype of NC, rendering them a potent stem cell source for applications in cell therapies.

## Distinct and Synergistic Roles of NFIA, NFIB and NFIX in Postnatal Mouse Neural Stem Cell Self-Renewal and Lineage Specification

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Understanding postnatal neural stem/progenitor cell (pNSPC) self-renewal and lineage specification is key to future stem cell therapies. Here we assess the effects of loss of single or multiple Nfi genes on murine pNSPC self-renewal and differentiation in vitro. Germline loss of Nfia or Nfib reduces astrogenesis in cortex and spinal cord and results in prenatal dysgenesis of the corpus callosum. Conversely, germline loss of Nfix has minor effects on astrogenesis but promotes oligodendrogenesis. We generated floxed alleles of Nfia, Nfib and Nfix. Mice homozygous for these alleles and carrying R26CreERT2 are viable and pNSPCs were cultured from the subventricular zone (SVZ) of such mice.

pNSPCs of P10-20 mice were cultured in the presence of EGF and bFGF (proliferation (prolif.) conditions) then placed into medium lacking these growth factors (differentiation (differen.) conditions). Transcript levels of markers of self-renewal and differentiation were assessed by qPCR from RNA of cells cultured without (WT) or with 4HT (NFI-deleted) during both prolif. and differen. conditions. Treatment with 4HT during prolif. efficiently deleted all floxed alleles with >99% loss of transcripts within 3 days.

Deletion of Nfix resulted in no changes in prolif. or neuronal or astrocytic differen., but a bias towards the oligodendrocyte lineage, consistent with our previous studies on germline loss of Nfix. Deletion of Nfib also resulted in no obvious changes in self-renewal or neuronal differentiation but reduced the expression of astrocyte markers, consistent with our previous studies on loss of Nfib in vivo. Surprisingly, simultaneous deletion of Nfia & Nfib resulted in a major reduction in self-renewal as seen by reduced PCNA and Nestin expression and the loss of colony forming ability. Upon differentiation there were increases in the neuroblast marker DCX and reduced expression of astrocyte and oligodendrocyte markers. This loss of self-renewal appears specific for the combined loss of Nfia & Nfib as the combined loss of Nfib & Nfix does not result in this phenotype.

We are currently assessing the molecular mechanisms that influence these changes in self-renewal and lineage-specification by RNA-seq analysis of prolif. WT and NFI-deleted pNSPCs and quantification of the cell types formed upon differentiation.

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- Epidermal Stem Cells
- Induced Pluripotent Stem Cells & Reprogramming (iPSC and hESC)
- Mesenchymal Stem Cells
- Neural Stem Cells

## Learn more at:

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