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# 1st Annual
# Stem Cells In Regenerative Medicine Symposium
# (SCiRM)

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SCIRM TRAINING PROGRAM

NYSTEM NEW YORK STATE STEM CELL SCIENCE

## NYS Center of Excellence in Bioinformatics & Life Sciences, University at Buffalo

## June 8-9th, 2017

## [Acknowledgements]

The Stem Cells in Regenerative Medicine (SCiRM) was established last fall with funding from the New York Stem Cells Stem Cell Board. SCiRM bring together 19 faculty from the School of Engineering and Applied Sciences, the Jacobs School of Medicine and Biomedical Sciences and Roswell Park Cancer Institute 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** (Professor and Chair, CBE) and co-directed by **Sriram Neelamegham** (Professor, CBE) and **Richard Gronostajski** (Professor, Biochemistry).

***The SCiRM symposium has received generous financial support from our sponsors:***

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# Stem Cell in Regenerative Medicine Faculty

**Stelios Andreadis, Professor and Chair, Chemical Engineering, UB**

**Director, Stem Cells in Regenerative Medicine (SCiRM) Training Program**

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

**John Canty, 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, Research Assistant Professor, Medicine, UB**

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

Research Interests: Cardiology; Critical Care Medicine

**Jian Feng, Professor, Physiology & Biophysics, UB**

*Ph.D., University of Tennessee*

Research Interests: Aimed at finding the cause and a cure for Parkinson's disease.

**David Goodrich, Professor Oncology, Roswell Park Cancer Institute**

*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

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# 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, Chairman, Molecular & Cellular Biology, Roswell Park Cancer Institute**

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

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

**Michael Higgins, Associate Professor, Associate Member (Genetics), Molecular and Cellular Biology, Roswell Park Cancer Institute**

*Ph.D.*

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

**Techung 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

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# Stem Cell in Regenerative Medicine Faculty

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

*Ph.D., Rice University*

Research Interests: Biomedical Engineering, Pathways in Inflammation & Thrombosis and Cell Biomechanics and Adhesion Molecules

**Michael Nemeth, Assistant Member, Medicine & Immunology, Roswell Park Cancer Institute**

*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**

*Ph.D., Rutgers University*

Research Interests: Stem cell biology, engineering, therapy, and multimodality molecular imaging laboratory.

**Steven Pruitt, Professor, Oncology, Molecular and Cellular Biology, Roswell Park Cancer Institute**

*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**

**Daniel Swartz, CEO, Angiograft LLC**

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

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

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# Keynote Speakers

## Ali Brivanlou, *Ph.D.*

Professor, Molecular Embryology Laboratory, Rockefeller University

**Research Interests:** The research focuses on the molecular events and cellular interactions that regulate the emergence of key structures in the early embryo. Most of the work in his laboratory focuses on the molecular basis of cell fate specification and patterning during early embryonic development. The ultimate objective of the work is to understand the molecular circuitry underlying embryonic induction, with a special emphasis on the formation of the nervous system. Toward this aim, he performs comparative studies using both amphibian and mammalian model systems, including human embryonic stem cells (hESCs).

## Steve Goldman, *M.D., Ph.D.*

URMC Distinguished Professor of Neuroscience and Neurology, Chief of the Department's Division of Cell and Gene Therapy, and Co-Director of Rochester's Center for Translational Neuromedicine, University of Rochester

**Research Interests:** In the division of Cell and Gene Therapy, within the Center for Translational Neuromedicine (CTN), the goal is to understand the regulatory control of stem and progenitor cells of the human CNS, and to utilize that knowledge to design new approaches for treating neurological diseases, primarily using cell and gene therapy. There are two principal focuses, one on the glial disorders potentially modifiable through a better understanding of glial progenitor cell biology, and the other on those neuronal disorders potentially treatable through the induction of neuronal addition or replacement from mobilized endogenous stem and progenitor cells.

## Gordana Vunjak-Novakovic, *Ph.D.*

Mikati Foundation Professor of Biomedical Engineering, Medical Sciences, and Director of Laboratory for Stem Cells and Tissue Engineering, Columbia University

**Research Interests:** The focus of the research is on engineering functional human tissues, by an integrated use of stem cells, biomaterial scaffolds and bioreactors, which are culture systems designed to regulate and stimulate tissue development. In her work, she has laid the theoretical and experimental foundation for the development of new biomaterials and scaffold architecture to regenerate tissue.The complex cell biological studies address fundamental problems such as the growth and differentiation of stem cells for functional skeletal and cardiac mechanics, the growth of mammalian cells in microgravity environment, and the role of chondrocytes in cartilage tissue biomechanics.

# Keynote Speakers

## Sean Palecek, *Ph.D.*

Milton J. and A. Maude Shoemaker Professor of Chemical & Biological Engineering and Biomedical, Materials Science & Engineering, University of Wisconsin

**Research Interests:** The focus of the research is on characterizing the nature in which quantitative changes in the flow of cellular signals can control a wide variety of useful processes. With this information Professor Palecek's Lab will design strategies to stimulate or inhibit cellular signaling pathways either at the chemical or physical level, and thereby regulate cell functions. Specifically, focus on human pluripotent stem cells (hPSCs) and high throughput protein arrays. His lab is also investigating microfluidic devices to design a high throughput protein array for cancer diagnosis. They aim to immobilize substrates for breast cancer kinases in order to detect the overactive kinases present in a cell lysate. This will help patient specific therapy by determining which kinase inhibitors are most effective for which patients.

## Robert Sackstein, *M.D., Ph.D.*

Professor, Harvard Medical School
Director, Program of Excellence in Glycosciences
Physician, Brigham & Women's Hospital/Dana Farber Cancer Institute

**Research Interests:** The bench research efforts aim to elucidate biologic processes critical to improving outcomes for patients undergoing HSCT, such as: (1) hematopoiesis (and other cell/tissue regeneration from stem cell-based therapies), (2) tissue-specific lymphocyte migration (including the immunobiology of lymphocyte migration in host defense and in pathologic reactions such as graft-versus-host disease), and (3) pathobiology of tumor cell proliferation and tumor metastasis. The common thread for all these efforts is to manipulate the biology of cellular trafficking, as it pertains to stem cell transplantation and tissue regeneration, to host defense/inflammation, and to cancer growth and metastasis.

## David Schaffer, *Ph.D.*

Professor of Chemical and Biomolecular Engineering, Bioengineering, and Neuroscience & Director of the Berkeley Stem Cell Center, University of California, Berkeley

**Research Interests:** Professor Schaffer's research employs molecular and cellular engineering approaches to investigate biomedical problems. Many of the efforts are dedicated to understanding the biology and exploring the therapeutic potential of stem cells. Stem cells are immature cells that exist in various locations of the body. Throughout our lifetimes, these cells divide and develop into the specialized cells that perform the functions necessary for organismal development and adult tissue function. Schaffer lab has efforts in novel signal discovery, computational and experimental analysis of the biological networks that cells use to interpret and implement these signals, and on the integration of these signals into biomaterial microenvironments for optimal stem cell control.

# Symposium Agenda Day 1:

## THURSDAY, JUNE 8TH

| | | |
|---|---|---|
| 8:00 am | 8:00 am | Continental Breakfast |
| 8:55am | | Welcome |
| 9:00am | 9:45am | **Robert Sackstein, M.D., Ph.D. Harvard University**<br>"GPS for Stem Cells: Creating a Pathway to Cure" |
| 9:45am | 10:15am | **Michael Nemeth, Ph.D. Roswell Park Cancer Institute**<br>"Targeting Quiescence in Myeloid Malignancies" |
| 10:15am | 10:45am | Break |
| 10:45am | 11:15am | **Fraser Sim, Ph.D. University at Buffalo**<br>"Paired Related Homeobox Protein 1 Regulates Quiescence in Human Oligodendrocyte Progenitors" |
| 11:15am | 11:45am | **Stelios Andreadis, Ph.D. University at Buffalo**<br>"Reprogramming of human skin cells to neural crest lineages" |
| 11:45am | 1:30pm | Lunch and poster session |
| 1:30pm | 2:15pm | **Steven Goldman, M.D., Ph.D. University of Rochester**<br>"Stem cell based treatment and modeling of glial disease" |
| 2:15pm | 3:00pm | **Ali H. Brivanlou, Ph.D. Rockefeller University**<br>"Self-Organization of Human Embryonic Stem Cells and Human Embryos" |
| 3:00pm | 3:30pm | Break |
| 3:30pm | 4:00pm | **Richard Gronostajski, Ph.D. University at Buffalo**<br>"NFI transcription factors in Neural Stem Cell Homeostatis" |
| 4:00pm | 4:45pm | **David Schaffer, Ph.D. University of California, Berkeley**<br>"Molecular Elucidation and Engineering of Stem Cell Fate Decision" |

# Symposium Agenda Day 2:

## FRIDAY JUNE 9TH SCHEDULE:

| | | |
|---|---|---|
| 8:00 am | 8:00 am | Continental Breakfast |
| 8:55am | | Welcome |
| 9:00am | 9:45am | **Sean Palecek, Ph.D. University of Wisconsin - Madison**<br>"Biomanufacturing Cardiovascular Cells from Human Pluripotent Stem Cells" |
| 9:45am | 10:15am | **Gen Suzuki, M.D., Ph.D. University at Buffalo**<br>"Cell-based therapy in large animal model with ischemic heart disease" |
| 10:15am | 10:30am | Break |
| 10:30am | 11:00am | **Steven Pruitt, Ph.D. Roswell Park Cancer Institute**<br>"Cell Proliferation and Somatic Stem Cell Aging" |
| 11:00am | 11:45am | **Gordana Vunjak-Novakovic, Ph.D. Columbia University**<br>"Engineering Human Tissues" |
| 11:45am | 1:00pm | Lunch and poster session |
| 1:00pm | 3:00pm | **Advisory Board Meeting, Room B1-306 Center of Excellence** |

**Reduction of Endoplasmic Reticulum Stress Improves Angiogenic Progenitor Cell function in a Mouse Model of Type 1 Diabetes**

Maulasri Bhatta[1, 2], Krishna Chatpar[3], Joshua J. Wang[1, 2], Sarah X. Zhang[1, 2, 4]

1. Department of Ophthalmology and Ross Eye Institute, University at Buffalo, State University of New York, Buffalo, NY, USA; 2. SUNY Eye Institute, State University of New York, NY, USA; 3. Department of Biological Sciences, University at Buffalo, The State University of New York, Buffalo, NY 14260, USA; 4. Department of Biochemistry, University at Buffalo, State University of New York, Buffalo, NY, USA

Persistent vascular injury and degeneration in diabetes are attributed in part to defective reparatory function of angiogenic cells. Our recent work implicates endoplasmic reticulum (ER) stress in high glucose-induced bone marrow (BM) progenitor dysfunction. Herein, we investigated the *in vivo* role of ER stress in angiogenic abnormalities of streptozotocin-induced diabetic mice. Our data demonstrate that ER stress markers and inflammatory gene expression in BM mononuclear cells and hematopoietic progenitor cells increase dynamically with disease progression. Increased CHOP and cleaved caspase3 levels with stunted adaptive unfolded protein response (UPR) were observed in BMderived early outgrowth cells (EOCs) after 3 months of diabetes. Inhibition of ER stress by *ex vivo* or *in vivo* chemical chaperone treatment significantly improved colony formation and migration while reducing apoptosis of diabetic EOCs, increased the number of circulating angiogenic cells in peripheral blood and retina, alleviated BM pathology, and enhanced retinal vascular repair following ischemia/reperfusion in diabetic mice. Mechanistically, knockdown of CHOP alleviated high glucose-induced EOC dysfunction and mitigated apoptosis, suggesting a pivotal role of CHOP in mediating ER stress-associated angiogenic cell injury in diabetes. Together, our study suggests that targeting ER signaling may provide a promising and novel approach to enhancing angiogenic function in diabetes.

**The Role of Intercellular Adhesion in Reprogramming of Epidermal Keratinocytes Toward Neural Crest Stem Cells**

Samaneh M. Boroujeni, Pedro Lei, Surya Selvam, Georgios Tseropoulos, Stelios T. Andreadis
Department of Chemical and Biological Engineering, University at Buffalo, SUNY

Our laboratory has recently shown that epidermal keratinocytes (KC) can be reprogrammed to neural crest stem cell (NC) by treatment with a growth factor cocktail including FGF2 and IGF1. An array of methods including RNA-seq showed that the resulting KC-NC expressed key NC markers including transcription factors such as SOX10, FOXD3 and intermediate filament, NES. Additionally, we demonstrated that KC-NC could be coaxed to differentiate into all NC-specific lineages including neurons, Schwann cells, melanocytes, smooth muscle cells (SMC), osteoblasts, adipocytes and chondrocytes. These results suggest that skin can be a source of multipotent stem cells for the treatment of devastating diseases, including myelinopathies. However, the mechanism of KC reprogramming into NC remains unknown.

The first step in the process of KC-NC reprogramming is the formation of KC colonies, which then give rise to NC by delamination, suggesting that cell-cell contact might play an important role in KC-to-NC reprogramming. To address this hypothesis, we used a combination of knockdown (E-cadherin, alphacatenin), dominant negative (ECAD-DN) and gain-of-function approaches to examine the role of E-cadherin in the process of reprogramming. In addition, the strength of homotypic E-cadherin interactions was modulated by varying concentrations of $Ca^{2+}$, cadherin blocking antibodies and cadherin-decorated surfaces. Our results support a model while E-cadherin is necessary for keratinocyte cluster formation, prolonged expression of cadherin prevented KCto-NC transition. Instead, disruption of adherens junctions was necessary for epithelial-to-mesenchymal transition (EMT), NC conversion and ultimately migration of NC cells out of the KC clusters. This KC-to-NC reprogramming course mimics the developmental process of neurulation, where NC migrate from the area between the dorsal neural tube and overlying ectoderm. Understanding the mechanism of KC-NC transition is expected to facilitate application of these cells in the treatment of neurodegenerative disorders with autologous epidermal stem cells.

**Role of Ars2 in self-renewal and differentiation capabilities of mouse embryonic stem cells**

Elahi S.[1], Olejniczak Scott H.[2],

[1] Department of pathology and anatomical sciences, University at Buffalo [2] Department of Immunology, Roswell Park Cancer Institute

Poorly controlled self-renewal in stem cells can generate either a large population of undifferentiated cells prone to malignant transformation or inadequate mature cells resulting in organ failure, degenerative diseases and aging. Therefore, maintaining a balance between selfrenewal and differentiation requires tight regulation. A great deal is known about how transcription factors regulate gene expression in stem cells in a way that balances selfrenewal with differentiation. However, while it is clear that critical regulation of gene expression happens subsequent to the activity of transcription factors, how co-transcriptional mechanisms contribute to stem cell fate decisions and how these mechanisms are fine-tuned are still unclear. Ars2 is a highly conserved RNA binding protein that mediates co-transcriptional interactions between the nuclear cap-binding complex and various downstream RNA processing machineries. Constitutive knockout of Ars2 in mice led to embryonic lethality around the time of implantation, while inducible knockout of Ars2 in adult mice led to specific reduction of long term hematopoietic stem cells. As these data suggest that Ars2 is playing a critical role during early development, we hypothesized that Ars2 is required for maintaining the balance between stem cell self-renewal and differentiation. To test our hypothesis, we generated tamoxifeninducible Ars2 knockout mouse embryonic stem cells (mESCs) in which both Ars2 alleles can be deleted by addition of 4hydroxytamoxifen (4-OHT) to culture medium. Ars2 knockout

(KO) mESCs had reduced capacity for self-renewal, indicated by significantly reduced proliferation rate and reduced number and frequency of alkaline phosphatase (AP) stained colonies. Additionally, Ars2 KO mESCs displayed decreased glycolytic capacity and mitochondrial oxygen consumption, both of which are associated with differentiation of ES cells. To directly assess the ability of Ars2 KO mESCs to differentiate, embryoid bodies (EBs) were generated. EBs generated from Ars2 KO mESCs were significantly smaller in size and lacked the morphological layering observed in control EBs. Efforts to measure specific effects of Ars2 KO on expression of pluripotency factors and markers of germ layer differentiation in EB cultures are underway and will be presented at the symposium. Future studies will address the molecular details of how ESC and EBs utilize Ars2 to execute gene expression programs that facilitate their self-renewal and/or differentiation.

# Molecular Dissection of the Oncogenic Role of ETS1 and its network in Head and Neck Squamous Carcinoma

Christian Gluck[1] and Satrajit Sinha[1]

[1]Department of Biochemistry, SUNY at Buffalo

Head and Neck Squamous Cell Carcinoma (HNSCC) is a heterogeneous disease of significant mortality and morbidity and with limited treatment options. Genomic analysis of HNSCC tumors has identified four distinct molecular classes, of which the mesenchymal subtype is 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. In accordance, our meta-analysis of TCGA datasets reveal that HNSCC patients with $EMT^{HI}$ index are more likely to suffer from poor disease outcome and high incidence of recurrence. To decipher the molecular drivers of the mesenchymal/$EMT^{HI}$ tumor subtype, we have generated RNA-seq and histone ChIP-seq datasets and analyzed the oncogenic-specific cistrome and the regulatory epigenomic landscape of representative HNSCC cancer cells. Our analysis has led to the identification of ETS1, a prototypic oncogenic transcription factor (TF), as a candidate regulator of the mesenchymal subtype. We show that ETS1 is not only preferentially enriched in the mesenchymal HNSCC, but its high expression is associated with both increased mortality and tumor recurrence. Additionally, interrogation of transcriptomic datasets of many other types of epithelial cancers show that ETS1 is selectively overexpressed in CSC populations, suggesting a much broader and pervasive Ets1-CSC link in tumorigenesis. To better understand the ETS1-driven molecular processes in $EMT^{HI}$ tumors, we have performed global transcriptomic analysis of effects of ETS1 knockdown and identified genome wide transcriptional targets of ETS1 in SCC25, a representative mesenchymal HNSCC cell line. These studies have uncovered an intrinsic core gene signature that implicate ETS1 as a crucial regulator of crucial oncogenic processes,

which includes proliferation and angiogenesis, and importantly mesenchymal subtype specific phenotype, such as drug resistance and stem cell enriched pathways. In sum, our study has revealed an intricate ETS1-dependent regulatory network of CSC and tumors that can be leveraged for targeted therapeutics and broadly highlights the power of genomics approach as a valuable tool for further tumor classification and discovery of cancer-subtype specific pathways.

## Tet1 catalytic domain knocking-out impairs neural differentiation of human embryonic stem cells.

**Hanqin Li[1], Zhixing Hu[2], Houbo Jiang[2], Jiali Pu[3], Jian Feng[2]**

1. SCiRM program, State University of New York at Buffalo, Buffalo, NY 14214, USA

Neuroscience PhD program, State University of New York at Buffalo, Buffalo, NY 14214, USA

2. Department of Physiology and Biophysics, State University of New York at Buffalo, Buffalo, NY 14214, USA

3. Department of Neurology, Second Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China

### Background

DNA methylation is one of the major epigenetic mechanism regulating gene expression. Tet family encode 5-methylcytosine (5mC) demethylases, and play critical roles in a variety of developmental, physiological and pathological processes. Tet1 is the highest expressed tet in both mouse and human embryonic stem cells (ESC). So far, functions of tet1 in maintaining pluripotency of mESC are controversial, and the functions of tet1 in human ESCs haven't been elucidated.

### Results

In this study, we successfully generated tet1 catalytic domain knock-out (tet1-CDKO) hESCs using CRISPR-Cas9 system. Tet1-CDKO hESCs express classical pluripotency markers and are able to differentiate to all three germ layers, even though their 5hmC level decreasing to as low as 20% comparing to wild type hESCs. When differentiated to neuroectoderm via embryoid body (EB), tet1 CDKO expressed 10 folds less PAX6 mRNA than wild type at day 7, and the final percentage of PAX6+ cells were also significantly less than the number observed in wild type. These evidence suggest tet1 catalytic domain knockingout impair *in vitro* neural differentiation of hESC. Our next step is trying to figure out how loss of tet1 catalytic activity causes this developmental defect.

## Novel role of Cadherin-11 in cell signaling via direct interaction with the PDGF receptor

Yayu Liu [a], Sindhu Row [a], Sandeep Agarwal [b], Stelios T. Andreadis [a a]
[a] Chemical and Biological Engineering, University at Buffalo, Buffalo NY [b] Department of Rheumatology, Baylor College of Medicine, Houston TX

**Introduction:** Cellular contact via cadherins has been recognized as an important event in mediating cell's proliferation decisions. Our laboratory recently reported that cadherin 11 (CDH11, type II cadherin) has been intimately involved in the process of MSC (mesenchymal stem cell) differentiation into smooth muscle cells (SMC) via ROCK pathway. Furthermore, homotypic CDH11 engagement significantly promotes ECM synthesis by activating the TGF-beta and ROCK pathways. Here we report that CDH11 plays a critical role in regulating dermal fibroblast proliferation by activating the PDGF receptor.

**Materials and Methods:** Skin tissues were obtained from 8-week old mice (*Cdh11*$^{-/-}$ and WT-wild type) and subjected to immunostaining. Mice dermal fibroblast were isolated from both KO (*Cdh11*$^{-/-}$ knockout) and WT (wild type) skin tissue as well as shRNA targeting CDH11 in human dermal fibroblasts to study the effect of CDH11 deficiency. An engineered surface with and immobilized fusion protein containing the extracellular CDH11 domain fused to the Fc domain (CDH11-Fc) was employed as a tool to study immediate downstream effects and identify the mechanism of intracellular signaling following CDH11 engagement. Immunoprecipitation was used to test the partners of CDH11.

**Results and Discussion:** Our *in vivo* data demonstrate that deficiency in CDH11 results in significant reduction of dermal fibroblasts entering cell cycle. *In vitro,* WT mouse dermal fibroblast proliferation rate is significantly higher than KO (Figure 1). Similarly, by knocking down CDH11 in human fibroblasts reduced the proliferation rate as well as expression of PDGF receptor. Conversely, culturing cells on CDH11-Fc coated plate promoted cell cycle entry, following AKT phosphorylation within the first 30 minutes of

attachment. In addition, pathway inhibition studies identified and coimmunoprecipitation illustrated CDH11 bound to and activated PDGF receptor β, leading to AKT pathway activation and increased proliferation.

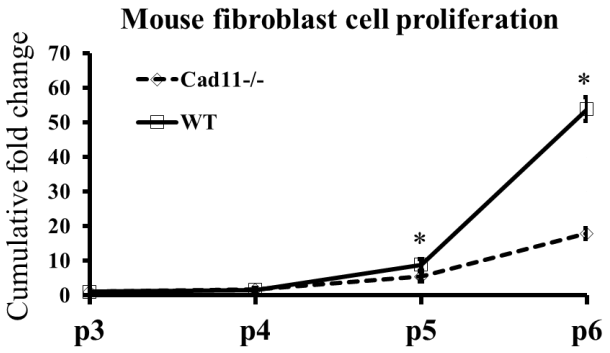


**Figure 1.** Mouse dermal fibroblast were isolated from 8 weeks WT and KO mice (n=3). Proliferation rate were tracked during passage 3 to passage 6. All quantitative results are mean±s.d. *P<0.05 as compared to KO (unpaired two-tailed Student's t-test).

**Conclusions:** Overall, our research indicates a novel function of CDH11 in mediating dermal fibroblasts proliferation via AKT pathway activation, possibly as a result of CDH11 binding and activating the PDGF receptor.

This novel discovery may have implications in wound healing and cancer metastasis.

# Controlling endodermal cell states by understanding and reengineering developmental master regulatory gene circuits (DRGC)

Saber Meamardoost[1], Natesh Parashurama[1,3,4]

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[4] Department of Medicine, Clinical Translational Research Center (CTRC), 875 Ellicott St, Buffalo, NY 14214

Currently, we are unable to produce functional liver cells (hepatocytes) from human pluripotent stem cells (hPSC). Endoderm, which gives rise to hepatic endoderm and eventually hepatocytes, is the most poorly understood germ layer. Endoderm arises during gastrulation, or germ layer formation during early embryogenesis. It is known that relatively large changes in gene expression take place during these stages. Not surprisingly, current approaches directed towards endoderm formation and differentiation from hPSC result in low yields of a particular cell type, mixed populations of cells, heterogeneous gene expression and protein synthesis, and poor maturation. Genetic, in vivo studies demonstrate that developmental master regulatory gene circuits (DRGC), hard-wired into the genome, control endoderm formation. It is believed that the DRGC coordinate these large changes in gene expression by controlling downstream transcription factors (TFs), and cross-regulation of other endodermal TFs. We hypothesize that heterogeneity of TF levels of the DRGC and their downstream effects during hPSC are a root cause of the problems during hPSC-derived endoderm and its progeny. The overall objective

of this work is to characterize the DRGC within hPSC-derived endoderm, to understand the interactions between governing TFs in cell fate determination during early PSC differentiation, and to reengineer ES cells differentiation with a measurable improvement in endoderm. To help us visualize, understand and predict DRGC phenotypes, we have constructed a theoretical gene network of how we believe endodermal TFs are hardwired into the genome based on data in lower organisms. We are performing quantitative RT-PCR (qRTPCR) of endoderm TFs in a model cell line, HepG2 (human hepatoma) cell line. The endoderm TFs include factors like Brachyury, Foxa2, Sox17, and GATA4. Next we will analyze endoderm TFs, by qRT-PCR and western blots, by inducing endoderm from hPSC using traditional techniques (Activin induction in culture). In order to analyze the function and contribution of each TF to endodermal state, RNA interference (RNAi) technique will be used to knock-out target gene(s) at different times. In particular, Foxa2 has extremely unique properties and we believe central to controlling the DRGC and preventing DRGC stochasticity. RNA interference will enable us to understand how each endodermal TF effects other endodermal TFs and downstream differentiation, and to control precise levels of endoderm TFs in hPSCderived endoderm at a specific times. Genome-wide techniques will be used to characterize improved phenotypes. Finally, we will develop new synthetic biology/genetic engineering techniques to improve hPSCderived endoderm quality, differentiation, and uniformity. Taken together, we will have a greater understanding of DRGC dynamics and improved endoderm differentiation towards hepatocytes for biomedical and biotechnology applications.

**DEVELOPMENT OF A TARGETED CARDIOMYOCYTE DELIVERY SYSTEM UTILIZING CARDIOSPHERE-DERIVED CELL EXOSOMES**

Kyle Indiana Mentkowski
Department of Biomedical Engineering
Masters Candidate

Mentor: Dr. Jennifer K. Lang, MD
Department of Medicine
Division of Cardiology

Cardiovascular disease continues to be the leading cause of mortality and morbidity in the United States. Current treatment options are aimed at preventing additional injury and helping the heart work more efficiently, but are limited in their regenerative capacity. Recently, research has shown that treating the heart with various 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. To fully realize the therapeutic potential of efficient delivery to the heart, we engineered CDC-derived exosomes to express a cardiomyocyte-specific peptide fused with Lamp-2b, an exosomal trans-membrane protein. Preliminary experiments showed enhanced exosome uptake by cardiomyocytes *in vitro*, establishing a novel tool for targeted delivery of anti-apoptotic drug and gene therapy.

**CELL SURFACE GLYCOENGINEERING IMPROVES SELECTIN-MEDIATED ADHESION OF MESENCHYMAL STEM CELLS (MSCS) AND CARDIOSPHERE-DERIVED CELLS (CDCS): PILOT VALIDATION IN PORCINE ISCHEMIA-REPERFUSION MODEL**

Momeni A.[1,3], Lo C.Y. [1,3], Weil B.R. [2,3], Palka B.A. [2,3], Canty J.M. Jr. [2,3], Neelamegham S. [1,3]

[1]Chemical and Biological Engineering, [2]Cardiovascular Medicine and [3]Clinical and Translational Research Center, University at Buffalo, State University of New York at Buffalo, Buffalo, NY 14260

Stem cell therapy has been shown to have promising results in cardiovascular applications and there are many ongoing 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. In this paper, we show that glycoengineering of stem cells enables them to mimic neutrophils and address this limitation. Here two modifications have been implemented on CDCs and MSCs to facilitate their interaction with selectin molecules expressed on the vascular endothelium: i. a P-selectin glycoprotein ligand-1 (PSGL-1) mimetic 19Fc[FUT7$^+$] was non-covalently coupled to the surface of the stem cells via lipid-protein G, and ii. α(1,3) fucosyltransferase FUT7 was overexpressed in these cells. Functionalized cells with 19Fc[FUT7$^+$] showed enhanced binding to P-selectin, while cells overexpressing FUT7 had elevated interaction with E-selectin. In an *ex vivo* setting, these surface modifications aided the capture and rolling of mesenchymal and cardiosphere-derived stem cells, under physiological shear, on substrates bearing either IL-1β stimulated endothelial cells or immobilized selectin proteins. A pilot study using a swine ischemia-reperfusion injury model confirmed the safety of the implemented modification technique, and the efficacy of the functionalized cells to target and home to sites of ischemia. Further studies are ongoing, to improve the efficiency of this cell capture process, and to enhance engraftment. Overall, the glycoengineering of stem cells may enable the targeting of stem cells, thus enhancing the success rate of current and future clinical trials.

**Title: Modelling Early Stage Liver Development through the Liver Diverticulum**

Ogechi P. Ogoke, SUNY Buffalo, Co-Author: Natesh Parashurama M.D. PhD.

The prevalence of chronic liver disease (CLD), emanates from the increasing incidence of fibrosis/cirrhosis and consequently end stage liver disease. In developed and developing countries, CLD rates have risen to become a leading cause of death, and CLD predisposes patients to hepatocellular carcinoma, itself a deadly disease. In the US, more than 5 million Americans are live with CLD and by 2020, cirrhosis is projected to be the 12th leading cause of mortality. The field of liver regenerative medicine aims to address CLD therapy, but unfortunately, the only therapeutic option available for CLD is liver transplantation. Liver transplantation, developed in 1960's, has been a tremendous success, with excellent 1-year (85%-88% ) and 5-year (74%) survival rates, and 20,000 orthotopic liver transplants conducted annually worldwide. However, donor livers are scarce, heterogeneous with respect to quality, and allogeneic. Unfortunately, other approaches within liver regenerative
medicine that address liver failure, including bio-artificial liver, hepatocyte cell transplantation, liver decellularization and recellularization, and liver micro-devices, cannot fully support or treat cirrhosis, and new approaches are needed.

Pluripotent stem cells (PSC), including both embryonic stem cells (ESC) and patient specific, induced pluripotent stem cells (iPSC), can self-renew, and can be directed along the liver lineage into hepatocyte-like cells (PSC-Heps). However, PSC-Heps, by definition, can't be used to generate the entire liver. Thus, new methods are needed for generating three dimensional, functional liver tissue from human PSC. Normal liver development entails not only differentiation, but also key processes like liver diverticulum and subsequent liver bud formation, morphogenesis, microarchitecture formation, and massive expansion ((~$10^6$ fold expansion in only 12 days in murine fetus), into the fetal/neonatal liver If we could use human PSC to simultaneously mimic these structures and processes that occur during liver organogenesis, then we can conceive of building large amounts of personalized functional liver tissue, rapidly, efficiently, and inexpensively, from human PSC.

We hypothesize that the geometric, cellular, biophysical, biochemical, and regulatory cues that initiate liver organogenesis can be mimicked by modeling the liver diverticulum, the earliest steps in liver development. Further, we hypothesize that key aspects of liver organogenesis, like liver cell migration and growth, will be enhanced in the tissue models compared to traditional techniques, like monolayer, aggregate or spheroid cultures.

To model the liver diverticulum, we began by using electronic data bases that accurately capture embryogenesis, to estimate liver bud size and geometry. The results of our analysis indicates a $10^6$ fold increase in liver cell density over 6 days in early mouse development . Next, we are using a self-assembly approach to assemble the initial layers of the liver diverticulum. The liver diverticulum constitutes an outpocketing of the foregut endoderm, a layer of hepatic endoderm that is lined by endothelial cells. We are thus using model cell lines to create layered epithelial structures that model the layers within the liver diverticulum. Our microscopy, cell proliferation, and immunostaining data indicates the initial re-organization of dispersed cell into multicellular structures. Our next step is to perform cell migration studies to understand how cell signaling affects early liver development. Ultimately, we aim to develop an accurate representation of the liver diverticulum in-vitro and subsequently transplant it in-vivo to study CLD in living animals.

**NANOG RESTORES MECHANICAL PROPERTIES AND EXTRACELLULAR MATRIX DEPOSITION BY SENESCENT STEM ELLS**

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Extracellular matrix (ECM) is critical in regulating the mechanical properties of many tissues including arteries and skin. It is well established that aging is accompanied by significant loss of tissue strength and elasticity mainly due to loss of elastin fibers, increased collagen crosslinking and decreased collagen synthesis. Therefore, there is a need to develop strategies to restore the impaired deposition of ECM.

Here we report that ectopic expression of NANOG could restore the impaired ECM deposition by human senescent stem cells and mechanical properties of bioengineered tissues prepared from these cells. To this end, we employed two widely established models of aging - replicative senescence of human Mesenchymal Stem Cells (MSC), and myofibroblasts derived from Hutchinson's Guilford Progeria Syndrome (HGPS) patients.

Our results show that senescence reduced synthesis of both collagen 3 and elastin. Consequently, tissues engineered using senescent cells showed inferior mechanical properties. Notably, NANOG restored the capacity of senescent cells to express both ECM molecules and increased the mechanical properties of engineered tissues. NANOG restored the activity of the TGFβ pathway that was impaired in senescent stem cells and is critical for ECM synthesis and crosslinking. Chemical inhibition (SB431542) or shRNA knockdown of SMAD4 (shSMAD4) prevented reversal of the senescent phenotype, suggesting that TGFβ pathway activation mediated the effects of NANOG. Further, knockdown and gain of function approaches indicated that both SMAD2 and SMAD3 were necessary to mediate the effects of NANOG but only SMAD3 was sufficient. In summary, our study provides a novel strategy to restore the impaired production of ECM by senescent stem cells and may have broad applications in stem cell therapies for tissue regeneration as well as anti-aging treatments.

## NANOG Restores the Myogenic Differentiation Potential of Senescent Myoblasts

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Age-related loss in muscle mass, sarcopenia, is a major problem facing the elderly. 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 present data showing that late passage myoblasts exhibited significantly impaired the myogenic differentiation potential that was accompanied by impaired expression of myogenic regulatory factors (Myf5, Myod, Myogenin, and MRF4) and members of myocyte enhancer factor 2 family. Notably, ectopic expression of NANOG for at least two weeks preserved the morphology and restored the myogenic differentiation capacity of senescent myoblasts, possibly by restoring the expression level of these myogenic factors. Muscle regeneration was effective in 2D cultures and in 3D skeletal microtissues mimicking the skeletal muscle niche. Interestingly, myoblasts maintained the rejuvenated capacity for 20 days after NANOG withdrawal, suggesting that NANOG might have imparted epigenetic changes. However, the presence of NANOG during differentiation inhibited myotube formation, suggesting that NANOG might have primed the cells for myotube formation but inhibited the differentiation process itself. 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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**ALLOGENEIC CARDIOSPHERE-DERIVED CELLS IMPROVE INFARCT REMODELING AND LEFT VENTRICULAR FUNCTION WHEN GIVEN VIA GLOBAL INTRACORONARY INFUSION EARLY AFTER REPERFUSION IN SWINE WITH MYOCARDIAL INFARCTION**

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**Background:** Intracoronary administration of cardiosphere-derived cells (CDCs) is a novel strategy to protect the heart from reperfusion injury associated with acute myocardial infarction (MI). However, time-dependent changes in post-MI left ventricular (LV) remodeling following CDC treatment are poorly understood. We therefore utilized computed tomography (CT) to test the hypothesis that allogeneic CDCs favorably influence chronic LV remodeling when administered via global intracoronary infusion early after reperfusion in a porcine model of MI.

**Methods:** Immunosuppressed swine (cyclosporine 100mg/day, n=15) underwent a 60-minute LAD occlusion to produce MI. After 30-minutes of reperfusion, animals were randomized to receive vehicle or $20x10^6$ allogeneic CDCs via global intracoronary infusion in a blinded fashion. Parameters of infarct remodeling and LV function were assessed via CT 2-days and 1-month post-MI. Post-mortem histopathological assessment of myocyte morphometry, proliferation (Ki67), and apoptosis (TUNEL) was also performed.

**Results:** Despite a similar reduction in infarct mass from 2-days to 1month post-MI, a markedly different pattern of infarct remodeling was observed in each group. Vehicle-treated animals exhibited detrimental changes in infarct circumferential extent and transmurality, while CDC treatment tended to reduce both parameters. Histopathological analysis revealed increased myocyte proliferation and reduced myocyte apoptosis in viable myocardium of CDC-treated animals, which resulted in preservation of myocyte

number in this region. This favorable pattern of infarct remodeling with CDC treatment was

associated with enhanced regional wall thickening and a significant improvement in LV ejection fraction.

**Conclusion:** Global intracoronary delivery of allogeneic CDCs early after reperfusion promotes favorable changes in infarct remodeling during the month following MI without affecting total infarct mass. This effect is associated with an increased number of myocytes in viable myocardium and improved LV contractile function, supporting the use of allogeneic CDCs as a therapeutic tool to effectively ameliorate adverse LV remodeling after MI.

# INVESTIGATING THE ROLE OF FGF2 AND DOWNSTREAM PATHWAYS IN REPROGRAMMING OF EPIDERMAL KERTINOCYTES TOWARD NEURAL CREST FATE

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**Introduction:** 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 differentiation diversity these cells have been a focal point of research for disease therapeutics via cell transplantation. However, an easily accessible autologous cells source for therapeutic cell transplantation remains one of the main challenges facing the field. Recently our lab discovered that human epidermal inter-follicular keratinocytes (KC) can turn into neural crest (NC) stem cells without ectopic expression of transcription factors or reprogramming to the pluripotent state. Here we studied the role of FGF2 and its involvement in downstream pathways and transcription factors that enable the KC to NC transition.

**Materials and Methods:** Keratinocytes were isolated from glabrous foreskin of 1-3 day old neonates and exposed to KC-NC induction medium containing FGF2 growth factor. FGF receptor knockdown through lentiviral

transfection was used, as well as chemical inhibitors for various signaling pathways. Clonal density experiments were performed using single keratinocyte cells to create a pure KC population that can give rise to NC. NC were further differentiated into Schwann cell, peripheral neuron, melanocyte and smooth muscle cell lineages. Gene expression was assessed with RNA-Seq and validated through quantitative real time RT-PCR. On protein level immunocytochemistry was used in order to detect markers in NC and differentiated lineages.

**Results and Discussion:** Our results show that FGF2 is necessary for KCNC reprogramming (Fig:1A), as evidenced by use of chemical inhibitors as well as gene knockdown using shRNA. We identified the FGF receptor that mediates the signaling as well as subsequent pathways including Akt and Mek/Erk. Ultimately, FGF signaling leads to expression of the NC-specific transcription factor, *SOX10*, which is necessary for NC reprogramming through *SNAI1* and other transcription factors. The newly produced NC

express NC genes including *SOX10, Nestin, Pax3, FoxD3* (Fig:1B) and could be coaxed to differentiate into peripheral neurons, Schwann cells, melanocytes and smooth muscle cells as shown by molecular as well as functional assays.

C**onclusion:** In conclusion, we provide mechanistic insight into the process of keratinocyte reprogramming to neural crest stem cells. This work represents a paradigm shift in stem cell biology as it demonstrates the unusual plasticity of human adult KC that can turn into many different cells types without genetic modification or reprograming to the pluripotent state. The dearth of cell sources for treatment of neurogenic disorders, combined with the accessibility and growth potential of human epidermal cells suggest that the proposed work could have tremendous implications for the use of cell therapy for treatment of many debilitating diseases.

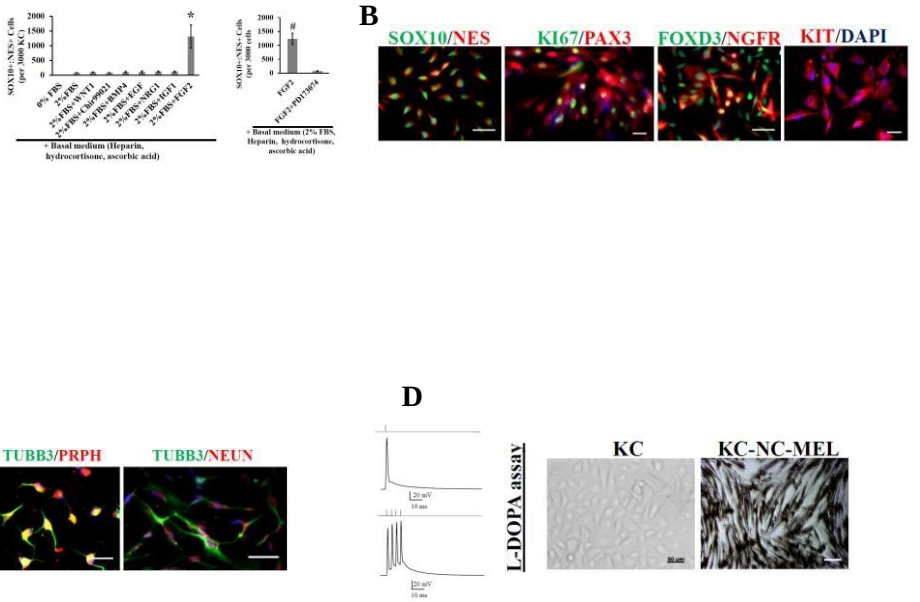


**Fig.1:FGF2 induces KC-NC reprogramming and KC-NC exhibit pluripotency potential.** (A) FGF2 is necessary for KC-NC induction, (B) KC-NC expressing representative markers SOX10, Nestin, FoxD3, Pax3, (C) Peripheral neuron differentiation markers and electrophysiological response (D) L-DOPA melamin assay for melanocyte differentiation (E) Schwann cell expressed markers MPZ, GAFP, S100B, PLP1 . (*):p<0.05 (n=3)

**The function of Nuclear Factor One (NFI) genes in lineage commitment of postnatal neural stem cells**

Karstin Webber, P.I: Dr. Richard Gronostajski

Previous studies have shown that sequential expression of transcription factors is important for the differentiation of postnatal NSCs. NFIs are a family of transcription factors that are known to play an important role in prenatal activation, proliferation and differentiation of neural stem cells (NSCs). However, due to perinatal lethality of germline *Nfia, Nfib,* and *Nfix* deletions in mice, their role in the regulation of adult NSCs remains insufficiently studied. Recently, conditional knockout (CKO) mice for NFIs have been generated which will allow me to determine how NFIs affect lineage specification within the dentate gyrus (DG) region of adult mice. I will also address whether the phenotypes observed in CKO mice are due to the overall level of NFI proteins present (quantitative function), or to unique characteristics of each gene product (qualitative function).

The phenotypes of single NFI knockouts prenatally suggest that individual NFIs play both unique and overlapping roles in NSC lineagespecification. I postulate that the different phenotypes seen are due to

similar or different changes in NFI target gene expression in the knockout mice. To test this hypothesis, I will: characterize NFI CKOs *in vitro* and *in vivo*, assess differential gene expression and chromatin accessibility in NFI CKOs, and perform rescue and overexpression studies in $Nfib^{-/-}$ NSCs. This information will provide valuable information regarding transcription factor regulation of adult NSCs.

## An optimized method to edit genes in short-lived primary human neutrophils differentiated from human hematopoietic stem cells using CRISPR-Cas9

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Leukocyte recruitment and extravasation at sites of inflammation follows a multi-step cascade. It is facilitated by specific binding, interactions between adhesion molecules expressed on the blood leukocytes and vascular endothelium. The first step in this adhesion cascade involves three members of the selectin family (L-, P- and E-selectin), which shared structural and functional homology. These selectins bind carbohydrates or glycans expressed on the leukocyte surface. Controlling selectin binding function can allow the development of novel anti-inflammatory therapy. This, however, requires fundamental understanding of the nature of the carbohydrate ligands of each of the selectins. The overall goal of this project is to define the glycanstructures of the selectin ligands and the glycoprotein ligands of human E-selectin.

This project focuses on developing an optimized method to differentiated CD34+ hHSCs (human hematopoietic stem cells), isolated from cord blood, towards neutrophils. Then it will use a novel CRISPRCas9 based genome editing tool for edit primary human neutrophils differentiated from hHSCs. This technology is used to knockout putative E-selectin ligands in human neutrophils. To test the ability of those modified cells binding E-selectin *ex vivo*, we will perform flow cytometry and microfluidics based cell adhesion assay.


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