Maryland TEDCO & MSCR Commission

Calendar Year

2009

Annual Report

MSCRF Awarded Research

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2009 FY Awarded Research Abstracts

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Sabrina Barksdale, MSCRF Administrative Coordinator, TEDCO

MSCR Commission Members

Maryland Stem Cell Research Commission: This independent Commission functions within TEDCO and consists of the following members:

The Attorney General or Designee

- **Ira Schwartz**, Senior Assistant Attorney General and Counsel to the Maryland Technology Development Corporation.

Three Patient Advocates

- **Bowen P. Weisheit, Jr.**, Board member of the Maryland Chapter of Cystic Fibrosis Foundation and lawyer with the Law Office of Bowen Weisheit, Jr. (appointed by the Governor)

- **Brenda Crabbs (Vice Chair)**, previous chairwoman of the Maryland chapter of the Arthritis Foundation and member of the organization's Medical and Scientific Council (appointed by the President of the Senate)

- **John Kellermann**, a Parkinson's sufferer who has advocated for the passage of the Stem Cell Research Act (appointed by the Speaker of the House of Delegates)

Three Individuals with Experience in Biotechnology

- **Margaret Conn Himelfarb**, Health Advisory Board and Institutional Review Board, Johns Hopkins Bloomberg School of Public Health; Embryonic Stem Cell Research Oversight Committee, Johns Hopkins School of Medicine (appointed by the Governor)

- **Linda Powers**, managing director of Toucan Capital, an early and active supporter of biotech companies (appointed by the President of the Senate)

- **Dr. Curtis P. Van Tassell**, Research Geneticist, USDA-ARS, Beltsville, MD (appointed by the Speaker of the House of Delegates)

Two Individuals Who Work as Scientists for the University System of Maryland and Do Not Engage in Stem Cell Research (Appointed by USM)

- **Dr. Suzanne Ostrand-Rosenberg**, professor of biological sciences and the Robert and Jane Meyerhoff Chair of Biochemistry at UMBC's Department of Biological Sciences

- **Dr. Steven Salzberg**, director of the Center for Bioinformatics and Computational Biology, and the Horvitz Professor at the University of Maryland, College Park's Department of Computer Science

Two Individuals Who Work as Scientists for the Johns Hopkins University (JHU) and Do Not Engage in Stem Cell Research (Appointed by JHU)

- **Dr. Diane Griffin**, professor and chairman of Molecular Microbiology and Immunology at Johns Hopkins Bloomberg School of Public Health

- **Dr. Murray Sachs**, professor and director of the Biomedical Engineering Department at the Johns Hopkins University School of Medicine

Two Bioethicists, One Appointed by USM and One by JHU

- **Karen Rothenberg (Chair)**, Marjorie Cook Professor of Law, founding Director of the Law & Health Care Program, and served as Dean of the University of Maryland School of Law from 1999-2009. (appointed by USM)

- **Dr. Jeremy Sugarman**, Harvey M. Meyerhoff Professor of Bioethics and Medicine, Berman Institute of BioethicsÂ and Department of Medicine Johns Hopkins University (appointed by JHU)

Two Individuals with Expertise in the Field of Biomedical Ethics As It Relates to Religion, Appointed by the Governor

- **Rabbi Joel Zaiman**, Rabbi Emeritus of Chizuk Amuno Congregation, and Jewish scholar at the Institute for Christian and Jewish Studies

- **Vacant**, (to be appointed)

OVERVIEW

This is the third annual report from the Maryland Stem Cell Research Fund (MSCRF). The report identifies each grantee that received funding, as well as the amount of funding from the MSCRF. These grants were made from the State's FY2007, FY2008 and FY2009 appropriation. This report also provides an abstract of the research activity as provided by the respective Principal Investigator to the MSCRF.

Funding Programs

In the past three years, the MSCRF has committed $56 million to 140 different research projects. The MSCRF has funded three types of research Programs: Investigator-Initiated, Exploratory and Post-Doctoral Research Grants. It has funded a variety of human stem cells: adult, embryonic, cancer stem cells, iPS or combinations of these. It has also funded both basic and translational research projects on diseases and conditions such as neural, Alzheimer's, Lou Gehrig's, tumors, spinal cord, Parkinson's, MS, heart and vascular, kidney, diabetes, muscle repair, bone repair, lung disease and Gaucher's. Most importantly, the MSCRF has funded research from a number of institutions in the State, some connecting private sector collaborators with academic institutions. All awardees presented their research results at the Maryland Stem Cell Research Symposium on September 21, 2009, and all detailed research descriptions may be found in this report. The three types of research Programs are:

Investigator-Initiated research grants which are designed for investigators with preliminary data supporting the grant application, for up to five years (extended from the first two years at the request of Investigators and with the approval of the Maryland Stem Cell Research Commission {Commission}).

Exploratory research grants which are designed for investigators who are new to the stem cell field and for exploratory projects without preliminary data, for up to two **years.**.

Post-Doctoral research grants from exceptional pre-doctoral students and post-doctoral fellows who wish to conduct post-doctoral research on human stem cells in the State of Maryland, for up to two years.

Fiscal Year 2007

The MSCRF has continued supporting awards from FY2007. Principal Investigators from the 24 projects submitted their progress reports and funding has either continued or no-cost extensions have been granted.

Fiscal Year 2008

For FY2008, the Commission recommended and the TEDCO Board approved 58 new awards which are now into their second year of funding. These awards include 11 Investigator-Initiated research grants, 32 Exploratory research grants and 15 Post-Doctoral Fellowship research grants.

Fiscal Year 2009

For FY2009, TEDCO received 147 applications for funding. From this pool of proposals, the Commission recommended and the TEDCO Board approved 58 new awards. These awards include 6 Investigator-Initiated research grants, 31 Exploratory research grants and 21 Post-Doctoral Fellowship research grants. These grant agreements have been executed and the research has begun.

Fiscal Year 2010

For FY2010, TEDCO received a record number of 193 Letters of Intent in response to its three official Requests for Applications (RFAs). The applications will be reviewed by an independent Scientific Peer Review Committee prior to full review by the Commission in April 2010. The final research grants will be in place no later than June 30, 2010.

Maryland Stem Cell Research Commission Meetings

In calendar year 2009, the Commission had four scheduled meetings, three of which were open to the general public and one full-day meeting (April) which was a closed session. All Commission meetings complied with the Maryland Open Meetings Act. In addition, the Commission established sub-committees to focus on ethics issues and application criteria and process. One of the major issues the Commission focused on this year was encouraging collaboration between for-profit companies and academic institutes. The sub-committees reported their activities to the full Commission in the open meetings and meeting minutes can be found the MSCRF website.

2009 World Stem Cell Summit and the 2nd Annual Maryland Stem Cell Research Symposium

The 2009 World Stem Cell Summit, held in Baltimore, was the largest stem cell meeting in the United States this year. The Summit was hosted by the Genetics Policy Institute (GPI), Johns Hopkins University, the University System of Maryland, the University of Maryland, Baltimore, MSCRF, TEDCO and DBED. This Summit attracted 1,200 participants from 40 states and 27 countries, and included scientists and researchers; representatives of industry; stem cell research funding organizations and economic development officials; members of Institutional Review Boards and Stem Cell Research Oversight Committees; patient advocacy organizations, patients, and representatives of related not-for-profit organizations; government agencies, such as NIH, FDA, NIST; and professors and students of medicine, physiology, biology, other relevant sciences, ethics and law. Many of the Commission members took an active role in the Summit; the Commission Chair was a co-chair of the Summit, MSCRF Director organized and presented with the Commission chair in a session that focused on the State's commitment to stem cell research, and other commission members presented in talks and discussions.

An integral part of the Summit was the 2nd Annual Maryland Stem Cell Research Symposium. Over 100 Maryland stem cell scientists presented scientific posters that summarized the work funded by the MSCRF. The Symposium was free and open to the public and many of the Commission members participated and were available for discussions by the scientists and the general public.

2009 World Stem Cell Report

The World Stem Cell Report (WSCR) is the most comprehensive publication on the state of stem cell research and over 3,000 copies of the report will be distributed in the upcoming year. The 2009 WSCR was sponsored by the State of Maryland and features Maryland's Research Institutes, highlights the Governor Bio 2020 initiative, MSCRF, the State incubator program and the Bio Parks and research conducted by Maryland researches.

Maryland-California Collaboration Agreement

TEDCO and the California Institute for Regenerative Medicine (CIRM) announced a collaboration on stem cell research during the 2009 Summit. The agreement is first step for joint Maryland-California research to advance stem cell therapies. The goal of the agreement is to stimulate California and Maryland researchers to collaborate. Maryland and California stem cell scientists will meet during 2010 to discuss the most effective way to proceed with the collaboration.

MSCRF Website Updates

The Commission and TEDCO continue to update the MSCRF website on a daily basis. The informative website, www.mscrf.org, provides information to researchers and the general public. The website contains information on the Maryland Stem Cell Research Act of 2006; funding opportunities; information on past awardees; information on Commission members, meetings, press releases and regulations; events; and general information on stem cells.

2010 3rd Annual Maryland Stem Cell Research Symposium

The Commission will host The Third Annual Maryland Stem Cell Research Symposium on September 22nd, 2010. The Symposium will be held in collaboration with the National Institute of Standards and Technology (NIST), the National Institute of Health (NIH), Johns Hopkins University and the University of Maryland, Baltimore. This collaboration would not have been available without the strong investment the State has made in stem cell research. As in prior years, the Symposium will be open to the scientific community and the general public to view the research supported by our State.

FY 2007

Investigator Initiated

Awarded

Research Abstracts

Name of Principle Investigator: **Angelo H. All** Project Budget: **$ 1,081,128.00**

Grantee: **Johns Hopkins University**

Title: **MicroRNA Expression Profiling during Human Embryonic Stem Cell Differentiation into the Oligodendritic Lineage**

Abstract of stem cell research (as submitted by Principle Investigator):

MicroRNA Expression Profiling during Human Embryonic Stem Cell Differentiation into the Oligodendritic Lineage

Problem: Oligodendrocytes (OLs) are glial cells of the central nervous system that synthesize myelin, the multilamellar membrane which insulates axons. Myelin is responsible for the functional role of OLs by enabling saltatory conduction of neuronal action potentials. As such OLs play a significant role in the pathogenesis of many neurological afflictions including spinal cord trauma, multiple sclerosis, and cerebral palsy. Human embryonic stem cells (hESCs) provide novel prospects for cellular replacement strategies due to their ability to provide seemingly unlimited stem cell numbers in vitro, their amenability to genetic engineering, and their broad developmental capacity toward neural cell-types. To determine the utility of hESCs in generating oligodendrocytes with therapeutic utility, it will be essential to understand the mechanisms which regulate their differentiation into myelin producing oligodendrocytes. MicroRNAs (miRNA) are small noncoding RNAs which regulate gene expression at the posttranscriptional level by binding and silencing the translation of mRNA into proteins. Hypothesis: The generation of myelin-producing oligodendrocytes from hESCs is regulated in part by miRNAs specific to their development. These miRNA can be distinguished by their changes in expression during hESC-OL differentiation in vitro. Research: To investigate miRNAs which regulate the development of OLs from hESCs, we used global miRNA arrays to follow expression patterns during multiple stages of hESC-OL differentiation. These time points consisted of defined culture conditions which promote undifferentiated ESCs, neural embryoid body induction, late neural progenitors, glial progenitors as well as early, mid and late OL progenitors (OPCs). Individual miRNAs which appear to gradually change during this process were selected for further studies with a focus on miRNAs which have targets for various aspects of OL development and myelin production. For this purpose, qRT-PCR was used to confirm their expression patterns. Observations: Immunofluorescence revealed that hESC-derived OPCs expressed oligodendrocyte markers, including CNPase, NG2, O1, O4, and Olig1 while earlier neural progenitors expressed A2B5, Nestin, and Sox10 expression. Results revealed several miRNAs that correlated with changes in differentiation. Changes in microRNA expression fell within three distributions linear, bimodal and normal over the course of differentiation. Several miRNAs with putative binding sites to genes associated with OL function and development such CLDN11, PMP22, and MYT1 demonstrated correlative changes within these distributions. Conclusions: Differentiation of OLs from hESCs express changes in their miRNA profiles which have biological relevance to pathways involved in myelin production. Future studies will be required to confirm their roles in this process and to utilize this information for enhancing myelin-producing OLs for transplant therapy.

Name of Principle Investigator: **Jeffrey W.M. Bulte** Project Budget: $ **1,190,276.00**

Grantee: **Johns Hopkins University**

Title: **Immunomodulation and Magnetic Resonance Tracking of Transplanted Human Embryonic Stem Cell-Derived Oligodendroglial Progenitors in a Mouse Model of Multiple Sclerosis**

Abstract of stem cell research (as submitted by Principle Investigator):

Problem Multiple sclerosis (MS) is an autoimmune disease that attacks the central nervous system, which has limited ability to regenerate damaged cells or repair lesions. Stem cell therapy is an attractive application by which to repair or ameliorate MS, but whether transplanted stem cells migrate toward lesions, and how well these cells can repair the lesions and interact with inflammatory cells is unknown. Background Experimental autoimmune encephalomyelitis (EAE) is an animal model of MS. Human embryonic stem cells (hESCs) are pluripotent stem cells, and can differentiate to oligodendrocytes. Magnetic resonance imaging (MRI) is a clinically applicable technology that can be used to track transplanted cells using contrast agent. Hypothesis Transplanted hESC-derived oligodendroglial progenitors (OPs) can repair lesions, modulate the immune system in the EAE model, and these cells can be tracked in the brain using MRI. Research C57Bl/6 mice were immunized with MOG35-55 peptide in Freund's adjuvant containing H3RA, and were observed daily for clinical signs of EAE. At 14 days post-immunization, mice were injected into the right cerebral ventricle with 5x105 hESC-derived OPs that were magnetically labeled with contrast agent (Molday ion), while control mice received only PBS. After transplantation, mice were monitored to determine the primary migration route of transplanted cells in the brain using MRI, and spleens were obtained from both groups to confirm the immunomoduation effect of transplanted hESC-derived OPs on the antigen-specific T cell proliferation. Observations The severity and duration of EAE paralysis in the hESC-derived OPs-transplantated groups was significantly suppressed and reduced, respectively. Serial in vivo and ex vivo MRI of EAE mice that were transplanted with hESC-derived OPs showed that hypointense MRI signals were observed mainly in the ventricle and subventricular zones, and in the white matter tracts. hESC-derived OPs-treated mice showed a significant decrease in antigen-specific T cell proliferation in response to MOG, compared to control mice. Conclusions We postulate that cell migration mainly occurs in the peri-ventricular white matter, and intracerebrolateral ventricle administration of hESC-dervied OPs inhibits the progression of EAE through a systemic immunosuppressive effect. Our findings suggest the signals generated from transplanted hESC-derived OPs in the ventricle modulate the systemic immune response.

Name of Principle Investigator: **Curt I. Civin** Project Budget: **$ 1,725,000.00**

Grantee: **University of Maryland, Baltimore**

Title: **MicroRNA Regulation of Hematopoiesis and Leukemias**

Abstract of stem cell research (as submitted by Principle Investigator):

Informatic analysis of our microRNA and mRNA expression profiles of purified subsets of human and mouse hematopoietic stem-progenitor cells (HSPCs) suggested that certain HSPC-expressed microRNAs can down-regulate key hematopoietic molecules and thereby regulate hematopoiesis. For example, we found that miR-155 was expressed in human CD34+ HSPCs and in mouse Kit+Sca1+Lin- early HSPCs. MiR-155 is known to be over-expressed in several types of cancer, including many leukemias and lymphomas where it acts as an oncogene by uncharacterized molecular mechanisms. To study the functional role and mechanisms of action of miR-155 in hematopoiesis and leukemias, we developed molecular tools to efficiently up- and down-regulate miR-155 in hematopoietic cells. Enforced miR-155 expression (via transfection or lentiviral transduction) increased hematopoietic cell proliferation and increased the percentage of cells in S phase of the cell cycle, in addition to inhibiting hematopoietic differentiation. For loss-of-function experiments, we designed an antisense locked nucleic acid (LNA)-containing antimiR-155 that potently bound to the complementary miR-155. Upon transfection into hematopoietic cells, this LNA antimiR-155 blocked miR-155-mediated inhibition of target mRNA translation. Since modulation of miR-155 and the pathways it regulates may be useful both in ex vivo expansion of HSPCs and in leukemia treatment, we are intensively studying the targets of miR-155 that may explain its effects on HSPC proliferation/differentiation. Targets of miR155 over-expression were studied in the K562 human leukemia cell line using microarray screening and qRT-PCR confirmation. Down-regulated targets included cell cycle-related molecules including MS4A3, transcription factors including JARID2, chemokine receptors including CCR7, and the SDC-2 co-receptor for GM-CSF. MiR-27a appeared to have functional effects opposite to those of miR-155. In general, miR-27a was expressed at lower (or absent) levels in human leukemias, as compared to normal human and mouse HSPCs. Lipofection of synthetic miR-27a or lentiviral expression of miR-27a decreased human leukemia cell proliferation. Drug-resistant human leukemia cell lines exhibited increased spontaneous apoptosis and became more susceptible to drug- and growth factor withdrawal-induced apoptosis upon enforced expression of miR-27a. Using luciferase assays, we showed that the anti-apoptotic molecules YWHAQ and PLK2 and the drug-resistance pump ABCC4 were targets of miR-27a. Leukemia cells with enforced miR-27a expression had reduced proliferation and decreased percentages of cells in the G1 cell cycle phase. Thus, based on its expression, functional effects, and targets, miR-27a may function as a tumor suppressor miR -- lack of miR-27a expression in leukemias may contribute to development and/or progression of these cancers. Conversely, inhibition of miR-27a in normal HSPCs might stimulate their ex vivo expansion.

Name of Principle Investigator: **William J. Lederer** Project Budget: **$ 1,724,988.00**

Grantee: **University of Maryland Biotechnology Institute**

Title: **Mesenchymal Stem Cells Stimulate Protective Genetic Reprogramming of Injured Cardiac Ventricular Myocytes.**

Abstract of stem cell research (as submitted by Principle Investigator):

Despite the growing enthusiasm for an intramyocardial injection stem cell approach, the understanding of how human mesenchymal stem cells (hMSCs) evoke cardiac benefit is ever more controversial. We have new evidence that hMSCs provide protection of cardiac myocytes from damage or enable cellular repair through a provocative paracrine stem cell signaling mechanism. This study explores the hypothesis that these beneficial effects are manifest in the genetic reprogramming of cardiac myocytes themselves. Cultures of neonatal mouse ventricular myocytes (nMCMs) were subjected to two distinct stress stimuli shown to cause profound cardiac injury, either the endotoxin, lipopolysaccharide (LPS) 1g/ml or toxic cytokine, IL-1 (100 ng/ml) for 3 hr. Previous studies reveal that these stressors evoke abnormal, chaotic Ca2+ signaling in nMCMs that is prevented by hMSCs when grown in co-culture. In search of a molecular explanation, we have found that LPS provokes marked increases in cardiac cell proinflammatory cytokine release from the nMCMs themselves, including mouse TNFa, IL-10 and IL-6. These increases are markedly inhibited when hMSCs are present. This abrogation of the LPS response is not seen in control nMCM-HUVEC cell co-cultures. Intruigingly,this reprogramming of cytokine production is accompanied by attenuation of specific intracellular signaling cascades in nMCMs which have been linked to stress responses and hypertrophy in heart. In particular, flow cytometry analyses of signaling in nMCMs from control and hMSC co-cultures revealed that the LPS- and IL-1-dependent activation of cardiac transcription factor, NF-kB, quantified as increases in phospho-p65, are blocked hMSCs are present. hMSCs may evoke such protective reprogramming through a paracrine mechanism. This notion was directly tested in transwell cultures where nMCMs and hMSCs were separated by a permeable membrane, preventing stem cell-heart cell contact. In this configuration hMSCs still blocked the increases in phospho-p65 signaling evoked by LPS or IL-1. The specificity of hMSC signaling was examined in control human fibroblast transwell cultures where LPS-evoked increases in phospho-p65 were still observed. Taken together, these results reveal new evidence that hMSCs elicit a protective effect on cardiac tissue through molecular reprogramming of the cardiac myocytes themselves. Further this cellular system provides a compelling model to identify specific hMSC signaling molecules that underlie this paracrine signaling cascade. Thus these studies provide novel new insight into the cellular and molecular mechanisms that underlie the therapeutic benefit of hMSCs in the setting of heart failure.

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Name of Principle Investigator: **Lloyd Mitchell** Project Budget: **$ 890,362.00**

Grantee: **Retro Therapy, LLC.**

Title: **Preservation of Potentiality in Genetically Altered Stem Cells**

Abstract of stem cell research (as submitted by Principle Investigator):

Preservation of Potentiality in Genetically Altered Stem Cells

The long-term objective of this project is to enable genetically enhanced embryonic or adult stem cells to retain their capacity to function as stem cells. The ability of stem cells to persist while repopulating tissues is the property that drives the field of stem cell research. Many therapeutic strategies hope to achieve long-term persistence and appropriate differentiation of cultured or transplanted stem cells. For those applications requiring genetic modification of stem cells, premature expression of the transgene may disrupt the status of the stem cell by inhibiting the cell's ability to divide or differentiate appropriately. Our project intends to develop a technology to overcome genetic defects in patient derived (autologous) stem cells and minimize ectopic expression of the transgene (making protein at the wrong time). Autologous cells should be most compatible and may avoid rejection following transplantation. The treatment of genetic diseases using patient derived stem cells generally requires that they are modified to express one or more therapeutic proteins. However, stem cells do not normally express most of these proteins until they begin to differentiate. Therefore, it may be necessary to prevent or limit expression of the therapeutic transgene until the appropriate stage of differentiation is reached. Proper regulation of gene expression may be critical to the maintenance of stem cells and the ultimate success of stem cell therapy. One way to accomplish this goal is to tie expression of the therapeutic gene to a promoter that is expressed at the appropriate stage of development. Cystic fibrosis is one of the most prevalent genetic diseases. It is caused by mutation within a single gene that encodes the cystic fibrosis transmembrane conductance regulator protein. Cystic fibrosis affects the lungs and digestive system of about 30,000 children and adults in the United States and an estimated 70,000 worldwide. In the US, the predicted median age of survival is 37 years. Over the past 15 years, a number of clinical trials have been performed in an attempt to treat cystic fibrosis with gene therapy using poorly regulated vectors. However, there is evidence to suggest that premature expression of the therapeutic gene interferes with the division and differentiation of pulmonary stem cells, which are the primary target for gene therapy. Our project intends to develop a genetic construct that can provide the missing protein under the natural regulation of the endogenous gene. Patient derived stem cell models will be used in the later stages of our project. We hope to demonstrate that therapeutic levels of protein and function can be achieved while preserving the capacity of pulmonary stem cells to divide and differentiate in both in vitro and in vivo model systems. If development is successful, we anticipate that this technology could be adapted to regulate the expression of other therapeutic genes in stem cells.

Name of Principle Investigator: **Hongjun Song** Project Budget: **$ 1,431,750.00**

Grantee: **Johns Hopkins University**

Title: **Characterization of neuronal potentials of human embryonic stem cells and adult neural stem cells**

Abstract of stem cell research (as submitted by Principle Investigator):

Stem cells are special cell types with the capacity of unlimited self-renewal and differentiation into fully functional cell type(s). Recent successful derivation of stem cells from humans, including both pluripotent human embryonic stem cells (hESCs), induced pluripotent stem cells (iPSCs) as well as multipotent human adult neural stem cells (hANSCs), has raised exciting possibilities to model human diseases and to develop cell-replacement therapy for many human diseases and injuries. Before these potentials can be realized, we need to characterize the capacity of these stem cells, in particular, functional properties of progeny from these stem cells. We have derived multipotent neural stem cells from surgical sample of adult human patients and shown their ability to give rise to neurons and glia cells. We have been able to in vitro differentiate hESCs into neural stem cells, neurons and glia, and then further into neuronal subtypes, such as dopaminergic neurons and motor neurons. We have also generated iPSCs from normal human skin fibroblasts and patients with genetic defects. We showed that these stem cells express all known markers as hESCs, and can differentiate into cells types in three germ layers in vitro and form teratoma after transplantation in SCID mice. Using similar approaches for hESC differentiation, we have differentiated human iPSCs into neural progenitors, immature neurons and neuronal subtypes. Electrophysiological analysis showed functional maturation of neuronal excitability and synapse formation by neurons derived from human iPSCs. In parallel, we have been able to transplant iPSCs into the embryonic developing rodent brains and shown successful neuronal development and integration of human neurons into the adult brain. Such approach allows us to examine the neuronal potential of human stem cells in vivo and could serve as a preclinical model when we use human stem cells with genetic defects. Our study will provide fundamental information on functional potential of different types of human stem cells for future application of human stem cells in cell replacement therapy.

Name of Principle Investigator: **Elias T. Zambidis** Project Budget: $ **1,110,826.00**

Grantee: **Johns Hopkins University**

Title: **Human ESC and iPSC-derived erythroid progenitors can be expanded in mass quantities for hemoglobinopathy and malaria studies**

Abstract of stem cell research (as submitted by Principle Investigator):

Despite improvements in the treatment of beta-hemoglobinopathies such as sickle cell disease (SCD) or beta-thalassemia, that include preventive care, fetal hemoglobin (HbF) induction with hydroxyurea, improved iron chelation during transfusions, and hematopoietic stem cell (HSC) transplantation, life expectancy for such patients remains diminished. Transplantation of genetically-corrected erythroid cells from patient-specific induced pluripotent stem cells (iPSC) would avoid the risk of allogeneic transplantation. Since increased HbF expression ameliorates the clinical symptoms of both beta-hemoglobinopathies and also malaria, we propose that transfusion of long-lived, fetal globin expressing erythroid cells from iPSC may serve as a treatment for these individuals. However, although iPSC possess characteristics of human embryonic stem cells (hESC), their full capacity for differentiation into specific cell types remains untested. In these studies, we quantitatively compared the ability of hESC and iPSC from reprogrammed fibroblasts to differentiate to the hematopoietic lineage. Human iPSC gave rise to various lineages of hematopoietic cells, and produced E-CFU, M-CFU, G-CFU and GEMM-CFU in colony forming assays. Moreover, we demonstrated, that human ESC/iPSC-derived erythroid progenitors can be mass expanded under serum-free conditions in liquid culture for 60-80 days. Primitive iPSC-derived erythroblasts expressed CD71, CD36, and CD235a (Glycophorin A), and also embryonic and fetal globins at expression levels similar to hESC-derived erythroid cells. Next, we showed that hESC/iPSC erythoid cells could be efficiently infected with the malaria parasite Plasmodium falciparum. However, despite efficient infection rates, we observed that malaria parasite development in these infected hESC/iPSC-derived erythroid cells was significantly delayed in comparison with normal adult red blood cells (RBC). These data demonstrate the feasibility of generating erythroid progenitors for hemoglobinopathy and malaria studies and treatment. Further optimization of our methods for mass expansion of hESC and iPSC-derived fetal type erythroid progenitors using bioreactor systems should provide broad applications in molecular and developmental biology, microbiology, gene therapy, pharmacology, and disease modeling.

FY 2007

Exploratory

Awarded

Research Abstracts

Name of Principle Investigator: **Shyam Biswal** Project Budget: $ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **Mesenchymal Stem Cell Therapy for Intervention of H1N1 Influenza Virus-induced Acute Lung Injury and Mortality**

Abstract of stem cell research (as submitted by Principle Investigator):

Problem: Influenza virus poses a serious recurrent annual public health problem and with ever growing threat of highly virulent influenza pandemic outbreaks. Due to continuous antigenic changes, developing effective vaccination has been a great challenge. Besides, high mutation rate of the virus has lead to the development of resistance to antiviral medications. Background: Excessive and/or dysregulated lung inflammation after influenza virus infection has been suggested as the underlying mechanism for influenza related mortality. Therefore, therapies that can mitigate host inflammatory response may have the benefits to limit morbidity and mortality following virus infection. Recent studies have demonstrated the therapeutic potential of bone marrow derived mesenchymal stem cells (BM-MSC) for treatment of inflammatory disorders. Besides pluripotency, BM-MSC have shown immunomodulatory effects without immunological rejections. Hypothesis: We hypothesize that human BM-MSC can attenuate influenza virus (H1N1)-induced acute lung injury and improve survival. Research: Mice were inoculated with influenza A/WSN/33 H1N1 mouse adapted strain of virus. Six hours later, mice were treated with human BM-MSC (hMSC; 106 cells/mouse) through intratracheal delivery. Body weight, lung injury, inflammation, viral titer and mortality were assessed. Observations: Administration of hMSC significantly decreased morbidity, lung injury, inflammation, viral titer, and improved survival in H1N1 virus infected mouse model. Conclusions: Our results demonstrate the therapeutic efficacy of hMSC to inhibit H1N1 virus induced lung injury and improve survival. Further studies will be carried out to study how hMSC modulate antiviral activity as well as virus-induced inflammation. Our data may provide a basis for the development of an innovative approach for the treatment of acute lung injury in influenza patients.

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Name of Principle Investigator: **Srinivasan Chandrasegaran** Project Budget: $ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **Targeted Manipulation of Mammalian Genomes using Designed Zinc Finger Nucleases**

Abstract of stem cell research (as submitted by Principle Investigator):

ZFN-mediated gene targeting is emerging as a powerful tool to site-specifically and permanently modify plant and mammalian genomes, including the human genome. While homology-directed repair of a targeted genomic DSB in presence of investigator-provided donor DNA results in gene correction in cells, repair of the DSB by non-homologous end joining (NHEJ) in absence of the donor DNA results in targeted mutagenesis. The high efficiency and accuracy of ZFN-mediated gene targeting combined with the ability to design ZFNs that target almost any DNA sequence makes ZFN technology a powerful research tool for targeted engineering of a variety of cells and cell types. Here, using a pair of designer 3-finger ZFNs, we have shown that ZFN-mediated gene correction successfully repaired the chromosomal tyrosinase gene mutation in albino mouse melanocytes. We have also shown that efficient ZFN-mediated gene correction occurs at a transduced, transcriptionally active, mutant GFP locus encoded with CCR5 target sites, by homology-directed repair; and that efficient mutagenesis by non-homologous end joining (NHEJ) occurs at the endogenous, transcriptionally silent, CCR5 locus in HEK293 Flp-In cells, using designed 3- and 4-finger ZFNs. However, the 3-finger ZFNs appear to be toxic to cells. To address the issue of ZFN cytotoxicity, we re-engineered ZFNs with obligate heterodimer FokI nuclease domain variants, which appear to improve the efficiency and efficacy of ZFN-mediated gene targeting in human cells by completely eliminating or greatly reducing toxicity to cells. Thus, ZFN-mediated gene targeting has great potential for various biological and biomedical applications, which include targeted genome engineering of human primary cells and human stem cells (which at present cannot be done effectively by routine technologies) for use in laboratory research and for use in human therapeutics (as a form of cell-based therapy using genetically-modified stem cells) in the future.

Name of Principle Investigator: **Nancy Craig** Project Budget: $ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **Genome Engineering of Human Stem Cells for Gene Therapy**

Abstract of stem cell research (as submitted by Principle Investigator):

The ability to specifically modify the genomes of stem cells would be of great benefit in the treatment of human disease. In diseases that result from the lack of a particular gene product because of a defective gene, addition of an intact copy of the gene to stem cells could lead to the alleviation of disease upon reintroduction. Alternatively, it may be useful to supplement stem cells with a gene product from a heterologous gene such that the modified cells would produce an agent that would kill other cells, for example, an anti-tumor agent, when introduced into a host. We are exploring the use of DNA cut & paste transposons as vehicles for the modification of stem cell genomes. One aspect of our work has been to isolate and characterize new DNA cut & paste transposons that have activity in stem cells. To avoid making random mutations, i.e. having selective insertion of an element, is highly desirable. We have been working to generate "target site-specific transposons" by making chimeric transposases in which a highly target site-specific DNA binding domain is fused to the transposase. iPS cells can be generated by the introduction of several transcription factors and others have established that the cut & paste transposon piggyBac is a useful vehicle for the introduction of the gene encoding these transcription factors. In other work, we (Liqin Zhou1, Kosuke Yusa2, Xianghong Li1, Allan Bradley2 & NLC) have isolated hyperactive piggyBac mutants that are about 15x more active than wild-type piggyBac that will facilitate the generation of iPS cells. 1 = Howard Hughes Medical Institute, Department of Molecular Biology & Genetics, Johns Hopkins University School of Medicine, 2 = Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom

Name of Principle Investigator: **Shengyun Fang** Project Budget: **$ 219,420.00**

Grantee: **University of Maryland Biotechnology Institute**

Title: **DPF2/REQUIEM is a ubiquitin ligase for Oct4**

Abstract of stem cell research (as submitted by Principle Investigator):

Topic investigated Oct-4 is a transcription factor required to maintain the pluripotency and self-renewal of embryonic stem cells (ESCs). The amount and activity of Oct-4 protein is strictly controlled. Any up- or downregulation induces divergent cell fates. Oct-4 also plays a critical role in the genesis of human testicular germ cell tumors. Therefore, understanding the regulation of Oct-4 activity will provide means to control ESC identity and differentiation, and may have therapeutic value for cancer.Background Ubiquitin ligase (E3) regulates protein function via catalyzing attachment of ubiquitin to the protein, called ubiquitination. E3 interacts with substrate protein and thus determines substrate specificity of ubiquitination. Three E3s, WWP2, RNF2 and DPF2, have been shown to interact directly or indirectly with Oct-4, but ubiquitination-mediated regulation of Oct-4 remains elusive. Hypothesis We hypothesize that DPF2/REQUIEM regulates Oct-4 function through ubiquitination of Oct-4. To test the hypothesis, we propose two Specific Aims: (1) To determine the role of the Oct-4-interacting E3s in the regulation Oct-4 protein stability, ESC self-renewal and differentiation, and (2) To elucidate the mechanisms by which E3s regulate the stabilities and functions of Oct-4 protein. Researches and Results To follow the fate of Oct4 protein during ESC differentiation, we used retinoic acid (RA) to induce H9 cell differentiation for up to 29 days. We confirmed the previous reports that Oct4 is downregulated during H9 cell differentiation. To the contrary, the expression of DPF2 exhibits an initial downregulation followed by upregulation. Notably, Oct4 protein expression almost disappeared after 5-day's RA induction, while the DPF2 protein expression reaches to its peak level at the same time point. These results suggest that DPF2 may regulate the differentiation of H9 cells by targeting Oct4 for degradation. To test this possibility, we demonstrated that DPF2 interacts with Oct4 by in vitro binding and co-immunoprecipitation assays. When Oct4 was co-expressed with GFP-DPF2 in 293 cells, both were localized mainly in the nuclei. In HeLa cells ectopically expressed Oct4 was evenly distributed in the nuclei but not in nucleoli. When co-expressed with DPF2, Oct4 was colocalized with DPF2 along with the nuclear DNA in a fine network-like pattern. Interestingly, a RING finger mutant DPF2 co-aggregates with Oct4, but not nuclear DNA in the nuclei. The nuclear Colocalization of Oct4 and DPF2 are also seen in H9 cells. Moreover, Oct4 immunoprecipitated from proteasome inhibitor-treated H9 cells is polyubiquitinated. To determine if DPF2 is involved in Oct4 ubiquitination, we demonstrated that wild type DPF2 but not its ring finger mutant increased Oct4 ubiquitination in 293 cells. We are also studying the regulatory role of DPF2 for Oct4 in neuronal differentiation of H9 cells. Conclusions These results support a role of DPF2 as a candidate E3 for Oct4 and a potential regulator for Oct4 activity involved in stem cell differentiation.

Name of Principle Investigator: **Ricardo Feldman** Project Budget: $ **230,000.00**

Grantee: **University of Maryland Biotechnology Institute**

Title: **Generation of glucocerebrosidase-specific human embryonic stem cells for modeling and treating Gaucher disease**

Abstract of stem cell research (as submitted by Principle Investigator):

Gaucher disease is an autosomal recessive lipid-storage disease that is caused by mutations in the glucocerebrosidase (GC) gene. This results in the accumulation of glucosylceramide in cells of the reticuloendothelial system (type 1 disease), and to a lesser extent in the nervous system (types 2 and 3). At the present time the most effective therapy for type 1 disease is enzyme replacement, using recombinant GC that has been modified to enter macrophages through mannose receptors. However, this therapy involves bi-weekly intravenous infusions for life, the treatment is very expensive, and it does not provide a cure. We are modeling Gaucher disease using two different approaches. In the first approach, we have simulated the loss of endogenous GC enzyme in hES cells using shRNA specific for GC to knock down GC expression. To determine whether endogenous GC can be knocked down, we tested a panel of lentivirus vectors encoding different GC-specific shRNAs (GC-shRNA). To measure GC activity we used an intact cell assay in which hydrolysis of glucocerebroside by the enzyme releases a FITC-labeled product that can be measured by flow cytometry. One of the shRNA vectors we tested was found to inhibit endogenous glucocerebrosidase activity in shRNA-infected hES cells by 80%. To assess whether GC activity in cells in which GC was knocked down could be restored, we constructed a therapeutic hGC lentivirus vector in which the nucleotide sequence of GC had a codon modification in the shRNA target region so that it could not be silenced by GC-shRNA introduced in the first round of infection. In order to be able to discriminate infected from uninfected cells we also engineered a new IRES lentivirus vector in which a truncated CD8 surface molecule (CD8t) was cloned downstream of IRES. Superinfection with the therapeutic vector allowed us to simultaneously measure GC activity in cells where GC was knocked down (CD8t-) and in cells where GC activity was restored (CD8t+). Our results showed that the therapeutic GC vector was able to overcome the GC-shRNA knockdown and restore high GC activity in GC-shRNA expressing cells. Since mutant GC in some Gaucher patients may not fold properly and may clog the endoplasmic reticulum en route to the lysosome, treatment of these patients may require the simultaneous knockdown of the mutant enzyme as well as introduction of a wild type copy of the gene. In our second approach, we are generating iPS cells derived from Gaucher-affected individuals to determine if iPS-derived monocyte-macrophages display the subcellular and functional abnormalities of Gaucher macrophages. Our latest results will be presented.

Name of Principle Investigator: **John P. Fisher** Project Budget: **$ 229,792.00**

Grantee: **University of Maryland, College Park**

Title: **Human Mesenchymal Stem Cells in Macroporous Cyclic Acetal Hydrogels for Orbital Floor Regeneration**

Abstract of stem cell research (as submitted by Principle Investigator):

Orbital floor injuries are a devastating form of craniofacial trauma. Current clinical treatments, including implantation of plastics or metals, are often inadequate due to loss of function as well as poor aesthetics. These concerns have led us to investigate tissue engineering approaches for the treatment of orbital bone defects. Cyclic acetal biomaterials may be preferred for tissue engineering applications as they hydrolytically degrade to form diol and carbonyl primary degradation products, which should not affect the local acidity of the implant or phenotypic function of a delivered cell population. To this end, EH-PEG hydrogels composed of the cyclic acetal monomer 5-ethyl-5-(hydroxymethyl)-β,β-dimethyl-1,3-dioxane-2-ethanol diacrylate (EHD) and hydrophilic poly(ethylene glycol) diacrylate (PEGDA) were investigated. Macroporous EH-PEG biomaterials were created using a porogen leaching technique. Macroporosity within hydrogels facilitates both molecular diffusion and cell migration, and thus should promote osteogenic signaling among human mesenchymal stem cells (hMSCs) within EH-PEG hydrogels. Human MSCs were loaded in porous EH-PEG hydrogels with a pore size and porosity of 100μm / 65%, 100μm / 70%, 250μm / 70%, and 250μm / 75%. In addition, the influence of fibronectin on osteogenic cell signaling and differentiation of hMSCs in porous EH-PEG hydrogels was studied. Human MSCs were loaded into 100μm / 65% EH-PEG hydrogels with fibronectin concentrations of 0, 0.5, 2.5, and 10 μg/mL gel. At days 1, 4, 8, and 12 total RNA was isolated and reverse transcribed. Results demonstrate that pore size and porosity does impact differentiation, with increased alkaline phosphatase levels observed for hMSCs in 100μm EH-PEG hydrogels. Osteogenic signaling analysis showed elevated levels of BMP-2, BMPR1A, and BMPR2 expression for all groups compared to the control, demonstrating the EH-PEG hydrogels enhance osteogenic signaling of hMSCs. Fibronectin inclusion increased cell attachment and spreading in a dose dependent manner throughout the study. However, the inclusion of macropores within a hydrogel does impact the strength of the biomaterial. As the purpose of the orbital floor is to maintain the orbital contents, the proposed scaffolds must provide the necessary mechanical support. To improve the strength of the construct, a thin layer of crosslinked EHD was bound in the center of the construct creating a three-layer composite. Results demonstrate improved strength with the addition of the EHD layer as well as describe the overall effects of scaffold fabrication parameters on resulting mechanical properties. Overall, this work describes the utility of hMSCs for orbital bone regeneration as well as develops the biomolecular and mechanical properties of a novel EH-PEG hydrogel for orbital bone regeneration.

Name of Principle Investigator: **Paul S. Fishman** Project Budget: $ **230,000.00**

Grantee: **University of Maryland**

Title: **Transcription Factor Directed Differentiation of Neural Progenitor Cells**

Abstract of stem cell research (as submitted by Principle Investigator):

The goal of this project is to enhance the neuronal differentiation of human neural progenitor cells (hNPCs) through the introduction of transcription factors known to be vital for normal development of the nervous system. The two factors chosen for initial evaluation were the BHLH type factor NeuroD2 (ND2) and the homeodomain factor Pitx3 which has been associated with differentiation of dopaminergic neurons of the substantia nigra. As an initial goal adeno-associated viral (AAV) vectors were created that over expressed each of the genes of choice. Commercially obtained hNPCs were maintained as aggregate cultures known as neurospheres, then dissociated into single cells and allowed to adhere and differentiate into neuronal or glia type cells. These cells were transfected with each of the viral vectors and evaluated for both expression of the delivered factors and changes in neuronal or glial differentiation. Transfection with either of the vectors led to sustained expression of either of the factors in up to 40-50% of cells. Immuno-histochemical evaluation showed that both transcription factors were localized to cellular nuclei, an observation that is consistent with a functional nuclear importation domain on the recombinant proteins. Over expression of either protein alone resulted in a significant increase in the number of hNPCs expressing the neuron specific cytoskeletal protein beta III tubulin as well as an increase in the number of cells showing neuronal morphologies such as neurite extension. An alternative strategy to viral vector delivery of the appropriate gene to enhance intracellular concentrations of a transcription factor is direct delivery of the purified protein. A plasmid construct was created to drive over expression in mammalian cells of ND2 which had been linked to the protein transduction peptide Tat. ND2-Tat was purified from transient transfection of the HEK293 cell line. This protein shows both nuclear localization in vivo and the capacity to bind DNA in vitro consistent with a functional transcription factor. Incubation of hNPCs with 1ug/ml of Tat-ND2 produced significant increases in expression of beta tubulin in a manner similar to transfection with the gene for ND2. Increases in expression of the synaptic protein synaptotagmin were also seen, but this effect appeared to vary with culture conditions. The major obstacle of this strategy is the strong preference of hNPCs to differentiate along a glial (astrocytic) lineage. Although transfection or transduction of hNPCs with ND2 enhanced expression of neuronal proteins, a significant fraction of these cell continued to express the astocytic marker glial fibrillary acidic protein (GFAP). Modification of hNPCs with factors that both enhance neuronal differentiation and suppress astrocytic differentiation may be needed to obtain a phenotype suitable for transplantation as replacement neurons.

Name of Principle Investigator: **Gary Fiskum** Project Budget: $ **230,000.00**

Grantee: **University of Maryland**

Title: **Protection Against Oxidative Stress and Death of Neural Cells through Genomic Pre- and Post-Conditioning with Sulforaphane**

Abstract of stem cell research (as submitted by Principle Investigator):

Oxidative stress is an important molecular mechanism responsible for the death of neurons and astrocytes following exposure to hypoxia, ischemia, inflammation, and toxic molecules, e.g., heme present in extravasated blood. Exposure of exogenous stem cells to all these conditions following implantation in the brain after traumatic head injury likely contributes to their >90% death rate, thus limiting their effectiveness at neuroregeneration and neuroprotection. One therapeutic strategy for detoxifying the many different reactive oxygen and nitrogen species that are produced under these conditions is induction of the Phase II gene response by the use of chemicals or conditions that promote the translocation of the transcriptional activating factor NRF2 from the cytosol to the nucleus, where it binds to genomic antioxidant response elements. This study tested the hypothesis that pre- or post-treatment of immature and mature hippocampal neurons or cortical astrocytes with sulforaphane (SFP), an alkylating agent known to activate the NRF2 pathway of gene expression protects against death of these cells caused by transient exposure to O2 and glucose deprivation (OGD) or to heme in the form of hemin. Neurons and astrocytes were exposed to between 0.5 and 5 μM SFP either prior to, or following OGD or exposure to hemin. Pre- or post-treatment significantly reduced cell death and reduced 8-hydroxy-2-deoxyguanosine immunostaining, a marker of DNA/RNA oxidation. Sulforaphane exposure was followed by an increase in cellular and nuclear NRF2 immunoreactivity. RT-PCR analysis indicated that NRF2-inducible cytoprotective genes, including NAD(P)H quinone oxidoreductase (NQO1) and heme oxygenase 1, were upregulated following SFP treatment both in control cells and in cells exposed to OGD and hemin. NQO1 immunoreactivity and enzyme activity were also elevated by SFP. We conclude that SFP stimulates the NRF2 pathway of antioxidant gene expression in neurons and astrocytes and protects them from cell death using in vitro models of hypoxia and heme toxicity. These results provide the rationale for testing the ability of SFP to pre- or post-condition stem cell-derived neural progenitor cells against death caused by OGD and hemin and ultimately determine whether such preconditioning increases neural progenitor cell survival following implantation after traumatic brain injury.

Name of Principle Investigator: **Samir Jafri** Project Budget: $ **230,000.00**

Grantee: **University of Maryland**

Title: **A Delivery System for Stem Cells**

Abstract of stem cell research (as submitted by Principle Investigator):

A critical but underappreciated hurdle in developing effective cell based therapy in humans with neurological disorders is the method by which the cells are delivered to the brain. We propose to address this challenge 1) by developing methodology to monitor in real time the delivery of stem cells or other therapeutic agents to visually confirmed targets in the brain and 2) by combining the emerging real-time optical imaging technology, optical coherence tomography (OCT), with a frameless MRI targeting system. The strengths of catheter-based OCT include its real-time feedback capability and its unparalleled spatial resolution. Combining the two would allow the surgeon instantaneous feedback on the precise position of the needle tip and the movement of the cells as they are being delivered. One challenge of this project was to develop a methodology that allowed the visualization of injected material in real time. To achieve this, we have adapted microbubbles, a contrast agent clinically approved for ultrasound, as a novel contrast agent for OCT. Stem cells expressing green fluorescent protein (GFP) were injected with microbubbles into subregions of rat hippocampus (subiculum, dentate gyrus and CA1). These subregions were identified using OCT images. The microbubbles allowed the progress of the injection to be monitored in real time on OCT. After 5 days, histological evaluation of the tissue showed GFP-labeled cells in the hippocampus. The location of these cells was consistent with the sites visualized on OCT prior to delivery. We have recently published many of these targeting experiments (Jafri et al., J Neurosci Methods, in press). The ability to get real-time visual confirmation of target specificity and of the progress of delivery provides a breakthrough in our ability to accurately and effectively deliver therapeutics to the brain. The parameters established in these experiments pave the way for successful and efficient stem cell delivery in non-human primates (NHPs). The other major innovation outlined in this proposal was to integrate OCT imaging with the Brainsight neuronavigation system for NHPs. We have begun this process by successfully using OCT in conjunction with Brainsight neuronavigation to target the globus pallidus interna (GPi) in two NHPs. The landmarks along the surgical tracks on OCT have correlated with the Brainsight predictions. Histological examination of these brains confirmed these landmarks and targeting of the GPi. We found the systems to be highly compatible and complimentary. Further improvement of the integration of these systems is planned to make the interface more seamless. Once again the real-time OCT visual confirmation of locations deep in the brain provides a new level of confidence that the desired target has been obtained.Our goals in the future include quantitative analysis of the survival of stem cells with and without the microbubble contrast agent as well as determination of whether there is any inflammatory response to the contrast agent. Following that, we plan to deliver stem cells to targets in the NHP brain using the OCT/Brainsight guidance system. Ultimately, if the hybrid system works as planned in NHPs, we will request rapid translation to human use.

Name of Principle Investigator: **Hai-Quan Mao** Project Budget: $ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **Differentiation of Neural Stem Cells on Electrospun Biomimetic Substrates**

Abstract of stem cell research (as submitted by Principle Investigator):

Neural stem cell (NSC) therapy carries great potential for the treatment of neurodegenerative diseases, with functional recovery and tissue integration observed in animal disease models following cell transplantation. Despite these exciting advances, much remains to be understood regarding how NSCs sense and react to cues within their microenvironment, and how these cues can influence cellular behavior. Conventional approaches towards differentiation of neural progenitors call for induction via treatment with small molecules such as retinoic acid, sonic hedgehog, FGF-8 and so on; however, little is understood about the impact of cell-substrate interactions in directing neural stem cell fate. Using neural stem cells as an experimental model, we evaluated the effect of a highly organized substrate with nanoscale features and substrate-presented biochemical signals on stem cell differentiation. Biomimetic fibrous substrates of poly(ε-caprolactone) (PCL) were prepared by electrospinning the polymer solution; by changing the collector from a stationary to rotating aluminum disc, both non-aligned and aligned fiber meshes could be produced. Biopolymers were selected because of the ease for secondary modification to introduce surface chemical cues. The growth factors (GFs) FGF-2, NGF, and BDNF were covalently tethered to the surface of the fibers via acrylic acid grafting and NHS/carboxydiimide chemistry. GF-dependent cell lines were then used to verify the bioactivity of surface-tethered signaling molecules. The impact of the combination of topological and chemical cues on differentiation of NSCs into various subtypes was then evaluated. Preliminary results indicated that NSCs cultured on aligned substrates adopted an elongated morphology and showed preferred differentiation towards a neuronal lineage over oligodendrocyte and astrocyte lineages. This preferential fate choice was not observed in NSCs cultured on the polymer film or non-aligned substrates, as quantified by cellular expression of the early neuronal marker Tuj1. A similar result was observed when cells were cultured at high density, indicating that the substrate cue persisted in spite of heightened paracrine signaling, which is believed to contribute to maintenance of stemness. We further hypothesize that the signaling to NSCs via surface-tethered GFs that are implicated in neurogenesis will potentiate the neuronal induction effect of the aligned topography. Further studies are under way to elucidate the possible mechanisms for substrate-mediated neuronal differentiation, such as cytoskeletal pathways involved in translation of changes in cell morphology into changes in cell phenotype. In summary, we hope to demonstrate that this surface-functionalized scaffold platform is a novel and potentially powerful tool for studying the manipulation of NSCs for therapeutic applications.

Name of Principle Investigator: **Candace Kerr** Project Budget: $ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **Control and Regulation of Pluripotent Stem Cells**

Abstract of stem cell research (as submitted by Principle Investigator):

Recent developments defining new sources for pluripotent stem cells signifies the importance of elucidating the mechanisms involved in regulating stem cell self-renewal and differentiating potential. In general, most pluripotent stem cells have been either derived from the blastocyst or from germ cells. Embryonic stem cells (ESCs) and epiblast stem cells have been derived from blastocysts while germ cells have been the source of embryonic germ cells (EGCs), embryonal carcinoma cells, multipotent germline stem cells, and parthenogenetic stem cells. Yet little is known regarding the mechanisms involved in regulating their pluripotency. The significance of which is highlighted by the successful attempts to convert adult cells into pluripotent-like stem cells, called induced pluripotent stem cells by genetic engineering. While these studies are promising, low conversion rates along with high rates of transformation ascertain the need for a better understanding of pathways regulating the developmental potential of stem cells. This will be critical for the regeneration of clinically relevant cell types while minimizing those associated with carcinogenesis. To date, molecular mechanisms regulating pluripotency include a handful of genes or pathways implicated in this process.This study compares the gene expression of ESCs and EGCs to determine similarities in their expression to select candidate factors involved in regulating pluripotency. Comparisons were also made with the unipotent progenitors of EGCs, primordial germ cells (PGCs). Although PGCs exhibit limited developmental potential and self-renewal, they express common markers of pluripotency such as Oct4, Nanog, cKIT and alkaline phosphatase. This study showed that of the three known regulators of pluripotency, Oct4, Nanog and Sox2, all three cell types expressed Oct4 and Nanog while Sox2 expression was not detected in either EGCs or PGCs. Other differences between human ESCs compared to PGCs and EGCs is that the latter express very little or negligible amounts of Gdf3, Lin28, Rex1, Utf1, and Foxd3. Furthermore, PGCs do not express TRA-1-60 and TRA-1-81 antigens expressed by ESCs, iPSCs, EGCs and ECCs suggesting a role for these markers in pluripotentiality. Growth factors involved with self-renewal of stem cells such as Fgf2, Lif, Bmp4, Gdnf, and Activin as well as their respective pathways including MAPK-ERK, TGFB/activin/nodal, Wnt/b-catenin and Akt/PkB pathways were also studied. Expression analyses showed differences in these pathways distinguishable between human EGCs and ESCs including increased expression of members of the LIF pathway, including LIFR, STAT3, JAK1, and GP130 in EGCs compared to ESCs and PGCs consistent with the requirement of LIF for the derivation of human EGCs. In contrast, expression of key members of the Wnt/b-catenin, BMP4, and MAPK-ERK pathways were very similar in EGCs and ESCs as well in PGCs which may be indicative of a common lineage. Interestingly, members of the TGFβ/Activin/Nodal pathway including TGFβR1 (alk5), ACVR1C (alk7), SMAD2 and SMAD4 are increased in EGCs and ESCs versus PGCs which is consistent with mechanisms involved in determining the potency among these cells. Therefore comparisons between PGCs which exhibit limited developmental potential to that of its pluripotent derivative, EGCs and ESCs provides critical information for defining factors involved in stem cell pluripotency and self-renewal.

Name of Principle Investigator: **Andrew McCallion** Project Budget: **$ 228,373.81**

Grantee: **Johns Hopkins University**

Title: **An Efficient Array-Based Methodology Identifies Novel Genes Associated with Cardiogenesis from Differentiated Human Pluripotent Stem Cells**

Abstract of stem cell research (as submitted by Principle Investigator):

Cardiovascular disease is the leading cause of death in the industrialized world. Cardiomyocyte generation from human pluripotent stem cells offers unique opportunities for cellular therapies that replace damaged heart muscle. However, this strategy requires a greater understanding of the genetic programs that control early human cardiac development, which has been impeded in part due to the inaccessibility of the post-implantation human embryo. To better clarify the genetic programs associated with human cardiogenesis, we used microarray technology to identify genes that are specifically expressed during the development of human embryonic stem cells (hESC) to the cardiac lineage. We utilized a novel and highly efficient hESC cardiomyocyte differentiation system that has been demonstrated to closely mimic in vivo cardiovascular developmental ontogeny. Using this optimized human embryoid body (hEB) differentiation protocol that employs chemically defined and animal product-free media, we produced beating hEBs after 9 days of differentiation with greater than 90% efficiency. We initially established the developmental kinetics for expression of pluripotency markers, cardiac mesoderm, and committed cardiac progenitors using real-time quantitative PCR methods. We then performed microarrays to compare the transcriptomic profiles for each of these populations. These data allowed identification of novel transcripts that likely direct the mesodermal differentiation of human cardiogenesis from a pluripotent state. A cross-comparison to similar data from mouse embryonic stem cell cardiac differentiation, as well as whole mouse embryos will allow the confirmation of in vivo biological relevance to correlative cardiac structures during murine development. These data also demonstrate the power of integrating genomic approaches with efficient methods for human pluripotent stem cell differentiation.

Name of Principle Investigator: **Guo-Li Ming** Project Budget: $ **230,000.00**

Grantee: **Johns Hopkins University**

Title: Mechanisms Regulating Self-renewal of Human Embryonic Stem Cells

Abstract of stem cell research (as submitted by Principle Investigator):

Human embryonic stem cells (hESCs) are pluripotent cells with the potential to generate all cell types found in the human body. Recent advances in our knowledge of derivation, expansion and controlled differentiation of hESCs offer a unique opportunity to investigate human cell biology, to model diseases and screen therapeutic drugs using human cells, and to develop cell replacement therapy for various injuries and degenerative diseases. One of the hallmarks of stem cells is self-renewal, that is to maintain the identity as stem cells when proliferate. Our current understanding of the cellular and molecular mechanisms regulating the self-renewal of hESCs, however, is very limited. Neurotransmitter -aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in mature central nervous system, yet accumulating evidence suggesting that GABA plays a trophic role during early neural development. Interestingly, medium containing GABA appears to be effective in promoting hESC cultures. We examined the expression of different types of GABA receptors in hESCs and found that hESCs express significant level of GABA-A, but not GABA-B receptors under proliferation conditions. Electrophysiological recordings from these cells revealed an inward current when stimulated with GABA, suggesting the expression of functional GABA-A receptor in hESCs. Application of GABA led to an increase of the colony size and this effect was blocked by GABA-A receptor antagonist. Calcium imaging studies showed that GABA induced calcium influx through GABA-A receptors and this calcium elevation is important for the proliferation of hESCs. Given GABA has also been suggested to play a role in the proliferation of neural stem cells during development and in adult brain, these studies suggest there may exist a conserved signaling pathway in GABA-dependent regulation of self-renewal of stem cells.

Name of Principle Investigator: **Akhilesh Pandey** Project Budget: $ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **IDENTIFYING TARGETS OF MIR-302 IN HUMAN EMBRYONIC STEM CELLS USING A QUANTITATIVE PROTEOMIC APPROACH**

Abstract of stem cell research (as submitted by Principle Investigator):

Although the miR-302 family represents an important stem cell-specific class of miRNAs, only one target of miR-302s was experimentally identified. BACKGROUND: Several lines of evidence have revealed crucial roles of miRNAs in self-renewal and differentiation of embryonic stem (ES) cells. For example, the miR-302 family is essential for maintenance of ES cell renewal and pluripotency. Thus far, only one target of miR-302s (Cyclin D1) has been experimentally verified. Based on the fact that miRNAs mainly regulate their targets at the translational level in animals, we propose to employ a quantitative proteomic strategy to systematically identify candidate targets of miR-302s in hESCs. HYPOTHESIS: We hypothesize that modulation of miR-302s levels in hESCs will lead to a significant alteration in the expression level of miR-302 target proteins. Further, this alteration in abundance of target proteins can be identified and quantitated using state-of-the-art high resolution mass spectrometry-based quantitative proteomic approaches. RESEARCH: We propose to identify targets of stem cell specific miRNA miR-302a using an iTRAQ based quantitative proteomic approach. A temporal proteome profile of hESCs transfected with miR-302a antagomir for various time points will be obtained. Relative changes in protein abundance between miR-302a antagomir and control oligonucleotides transfected cells will be calculated based on the intensity of reporter ions generated during tandem mass spectrometry analysis. Candidate miR-302a targets discovered by proteomic analysis will be validated by standard Western blot, immunocytochemical analysis and luciferase assay. OBSERVATIONS: We have successfully inhibited expression of miR-302a in the human embryonic stem cell line H1 (WA-01) cells by a sequence specific miRNA hairpin inhibitor (miR-302a antagomir). The expression of miR-302a in H1 cells was significantly decreased by miR-302a antagomir at 24h, 48h and 72h post-transfection. CONCLUSIONS: miR-302a is highly expressed in hESCs and can be significantly suppressed by miR-302a antagomir. We will carry out a quantitative proteomic approach to identify candidate targets of miR-302a.

Name of Principle Investigator: **Adam Pouche** Project Budget: $ **230,000.00**

Grantee: **University of Maryland**

Title: **Role of Metalloproteinases in the Migration of Transplanted Human Stem Cells**

Abstract of stem cell research (as submitted by Principle Investigator):

The transplantation of stem cells into the central nervous system has the potential for delivering exogenous gene products or a cell replacement therapy. In many cases when stem cells are transplanted into the brain they migrate extensively from the site of engraftment. Regulating the intrinsic capability of stem cells to migrate within the brain is an essential step for targeting stem cell transplantation therapy for maximum effectiveness. In order for cells to penetrate the complex microenvironment of a mature brain, the extracellular matrix must be modulated and matrix metalloproteinases (MMPs) are thought to play key roles in extracellular matrix remodeling. We are exploring the role of MMPs in regulating migration of transplanted stem cells. Our preliminary data show that ES stem cells can be efficiently transfected with MMP expression constructs (MMP2, MMP9, MT5-MMP and TIMP2). Transfected ES cells in vitro display 100-fold increased expression/secretion of MMP9 protein compared to non-transfected control, with commitment increases of enzymatic activity. Transfection with the membrane inserted MT5-MMP construct increased expression in transfected cells by ~4x compared to control ES stem cells. We next transplanted those transfected ES stem cells into mature brain and found that those transplanted and transfected stem cells survive and migrate in adult brain. Preliminary observations suggest that MMP9 transfected ES stem cells were capable of migrating out of the injection sites at great distances, even traversing into the other hemisphere following the corpus callosum pathway. MMP9 transfected cells were co-transplanted with control cells and interestingly the lead/most distant cells were the ones expressing MMP9 expression construct. These preliminary transplants suggest transfected cells may migrate with higher efficiency than non-transfected. Understanding and potentially regulating the intrinsic capability of stem cells to migrate within the brain is an essential step for targeting stem cell transplantation therapy. MMPs represent a novel target to address the fundamental problem of uncontrolled migration that is currently inherent in any transplanted stem cell applications.

Name of Principle Investigator: **Hamid Rabb** Project Budget: $ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **Isolation, expansion and characterization of adult human kidney derived stem Cells**

Abstract of stem cell research (as submitted by Principle Investigator):

The therapeutic potential of stem cells has opened the opportunity to repair and regenerate damaged kidney. Based on the identification of kidney progenitor cells in mouse kidney, we tested the hypothesis that stem cells could also be isolated and expanded from normal adult human kidney and used to reverse kidney damage, initially in a mouse model. We used a kidney progenitor cell isolation technique previously described in heart (Circulation 2007) and mouse kidney (J Am Soc Nephrol 2009), and applied this technique to isolate human adult kidney stem cells. Human kidney biopsy samples were taken from kidney transplant donors immediately prior to implanting the kidney in the recipient (both live and deceased donor samples). The presence of kidney stem cells were demonstrated by flow cytometry measuring expression of stem cell marker (CD133) as well embryonic kidney marker (CD24), and lack of expression of hematopoetic stem cell markers (CD45 and CD34). Approximately 20% of the cell population expressed both CD133+CD24+, indicating renal progenitor cell status. These adult human kidney stem cells as well as control bone marrow derived mesenchymal stem cells were injected in mice with ischemia reperfusion injury. Initial studies focused on safety and trafficking of these cells in vivo, setting the stage for functional studies. Thus, techniques developed in murine models to isolate and expand adult kidney stem cells are feasible in humans. Further expansion and in depth in vitro and in vivo characterization of these adult human kidney derived stem cells will set the stage for therapeutic trials in patients, including those with acute kidney injury, chronic kidney diseases, acute and chronic transplant rejection.

Name of Principle Investigator: **Karen Zeller** Project Budget: $ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **MYC Directly Regulates a Stem Cell Signature in Human ES Cells and Transformed Lymphocytes**

Abstract of stem cell research (as submitted by Principle Investigator):

The MYC proto-oncogenic transcription factor was discovered decades ago as the cellular homolog of the retroviral v-myc gene that is sufficient to initiate a variety of chicken tumors. MYC has proven to be an important human cancer gene, but recently c-myc was shown to be vital for the conversion of murine and human differentiated cells to a primitive induced pluripotent stem (iPS) cell. In addition c-myc is known to play a role in adult skin and blood stem cells. MYC gene amplification found in existing human embryonic stem cell (hESC) lines raises the concerns regarding the stability and safety of existing embryonic stem cell lines for in vivo uses. Because of MYC's role in stem cell biology as well as in human cancer, we seek to understand the role of MYC in hESCs with specific reference to self-renewal and potential tumorogenicity. To the end, we have begun to decipher the direct MYC transcriptome by utilizing chromatin IP followed by hybridization to human promoter arrays in both H9, human ES cells, and P493 cells, which are a model for Burkitt Lymphoma. More than 900 genes are bound by MYC in both cell models including many previously identified direct MYC targets such as NPM1, APEX1 and PAICS. Genes involved in protein synthesis, nucleolar function, RNA and nucleic acid metabolism are overrepresented in this common list as compared to all human annotated genes. In addition, we determined which genes are responsive to Myc in our P493 model, in which MYC is tet repressible, and in a well defined model of trophoblast differentiation. Upon trophoblast differentiation, MYC RNA and protein levels decrease several fold and the expression of more than 350 genes, including LIN28 and SOX2, decrease by at least 2-fold. Comparison of these genes with those that respond to MYC in P493 cells, reveals a set of core MYC targets that are independent of cell type. Of these core human targets, 57 are also bound by Myc in murine embryonic stem cells. Gene set enrichment analysis of these core targets revealed that the most significant overlaps are with gene sets that are overexpressed in embryonic and neural stem suggesting that MYC regulates a "stem cell signature". These data combined represent the first comprehensive study of a direct c-MYC transcriptome in 2 cell models and reveal that MYC plays a crucial role in the regulation of genes involved in nucleolar function, ribosomal biogenesis and RNA metobolism and that these direct targets represent a stem cell signature.

FY 2008

Investigator Initiated

Awarded

Research Descriptions

Name of Principle Investigator: **Laure Aurelian** Project Budget: $ **827,171.00**

Grantee: **University of Maryland, Baltimore**

Title: **GVHD IS ASSOCIATED WITH HSV REACTIVATION IN STEM CELL TRANSPLANT RECIPIENTS**

Description of stem cell research (as submitted by Principle Investigator):

Graft versus host disease (GVHD) is a major cause of late morbidity and mortality in stem cell transplant (SCT) recipients. It shares clinical properties with herpes simplex virus (HSV)-associated erythema multiforme, an inflammatory skin presentation in which infected CD34+ cells are stimulated to differentiate into Langerhans precusors (CD1a+/CD14-) that repopulate the epidermis and function in antigen presentation. The HSV gene Pol is expressed in the infected cells, including lesional skin. Our studies were designed to examine whether HSV reactivation is associated with GVHD development in SCT recipients. STUDY DESIGN. Thirteen recipients of allogeneic SCT were enrolled. They had immunosuppressive therapy and oral acyclovir initiated on day 3 after transplant (D+3). Peripheral blood mononuclear cells (PBMC) and skin biopsies were obtained from normal skin at the time of enrollment (baseline) and from lesional skin. Double immunofluorescence and flow cytometry were used to examine expression of Pol, E-cadherin (associated with virus-induced differentiation), CD34, CD1a and CD14. RESULTS. Patients fell into 3 groups. Group I consists of 4 patients with skin rash diagnosed as GVHD that was positive for Pol antigen. One of them had a skin rash diagnosed as GVHD grade 1 that appeared on D+10, shortly after the onset of genital HSV-2 lesions, which did not respond to acyclovir treatment (D+5). CD34+/Pol+ and CD34+/E-cadherin+ cells increased with time and the skin lesion was Pol+. A new patchy rash diagnosed as GVHD grade 3 developed on D+33 and revealed 41% Pol+ of epidermal and dermal cells. Two other patients also had evidence of HSV infection by Pol staining associated with lesion severity. CD1a+/Pol+ cells were seen in the bloodstream and the skin biopsy when the rash flared and decreased with the resolution of the cutaneous symptoms. Group II consists of 3 patients that had a rash, which did not fit the GHVD criteria by histopathology one of whom had Pol staining associated with lesion severity. Group III consists of 5 patients that did not develop any cutaneous eruptions, although one of them had elevated levels of circulating CD34+/Pol+ cells. In all patients, the baseline samples were negative for Pol staining. CONCLUSION. Skin lesions that follow allogeneic SCT can result from reactivation of latent HSV and infection of circulating CD34+ cells that are stimulated to differentiate along the Langerhans cell pathway. These lesions are generally diagnosed as GVHD, but HSV-associated lesions that are distinct from GVHD were also identified. Additional studies may help to modify existing clinical practices to include improved (parenteral) acyclovir treatment. These studies were supported by the Maryland Stem Cell Research Fund. Current affiliation: Department of Pathology, NYU Langone Medical Center.

Name of Principle Investigator: **Stephen Baylin** Project Budget: $ **1,725,000.00**

Grantee: **University of Maryland, Baltimore**

Title: **Pharmacological Unmasking of a Cancer Related Epigenome that is Created During the Incomplete Reprogramming of iPS Cells**

Description of stem cell research (as submitted by Principle Investigator):

Much excitement has accompanied recent observations that insertion of gene panels can induce mouse and human mature cells to become pluripotent stem cells (iPS) resembling embryonic stem cells (ES). While we expect that successful reprogramming of somatic cells may yield important therapies for regenerative medicine, some of the genes used to produce ES-like properties may also induce tumorigenesis, in vitro and in vivo. It has been documented that iPS formed from reprogramming produces clonal populations with some variability in their resemblance and gene expression compared to ES cells. We now present xenografts derived from these clones which demonstrate foci of clinically relevant pathology common to human tumors. Using global gene expression and methylation analysis, we have also identified key epigenetic abnormalities that arise during incomplete reprogramming of fibroblast cells into ES-like induced pluripotent cells. Using pharmacological methods, we have unmasked a significant number of genes that are abnormally silenced in both multipotent and nullipotent clones. The expression signature we observed for these genes is that of induced re-expression in the iPS clones, but not in blastocyst derived ES cells, by the DNA demthylating agent, 5-aza-2' deoxycytidine (DAC, 5uM, 72 hr). A significant percentage of these same genes are also abnormally epigenetically silenced in cancer. This research not only has significant implications for the use of engineered iPS cells in a therapeutic setting, but also underscores the intriguing possibility that one mechanism for evolution of human cancers could involve cellular reprogramming of mature cells towards more stem/progenitor-like cells. These findings may also help explain recent studies showing that human cancer cells can manifest an embryonic stem-cell like signature, and strengthen the argument that differentiation (or in this case de-differentiation) may create a vulnerable window for epigenetic changes to occur, from which a tumor cell may arise.

Name of Principle Investigator: **Ted M. Dawson** Project Budget: $ **1,725,000.00**

Grantee: **Johns Hopkins University**

Title: **Derivation and Characterization of Parkinson's Disease Inducible Human Pluripotent Stem Cells**

Description of stem cell research (as submitted by Principle Investigator):

Parkinson's disease (PD) is the most common movement disorder that is due, in part, to the preferential loss of dopamine (DA) neurons. Recent developments in the reprogramming of human somatic cells to pluripotency with defined factors has the potential to revolutionize the study of the underlying pathogenesis of a variety of human disorders. Using standard protocols and technology, the co-investigators of this grant have begun to establish inducible Pluripotent Stem (iPS) cells from patients with sporadic PD and familial PD. The ultimate goal is to produce DA neurons from these iPS cells for in vitro and in vivo investigations. To accomplish these goals we are reprograming dermal fibroblasts isolated from skin biopsies. Ultimately these human patient-specific iPS cells will be differentiated into DA neurons and characterized using standard protocols. These DA neurons will be subjected to a variety of assessments to determine whether they form functional DA neurons and whether they are effective in neuronal replacement in an animal model of PD. Human patient-specific DA neurons derived from iPS cells will be genomically and biochemically characterized to potentially identify molecular markers that are common to PD as well unique biochemical or molecular pathways that are involved in the degeneration of DA neurons in sporadic and/or genetic PD. In summary, we have begun an innovative set of studies to explore the potential of human iPS cells as tools to study the pathogenesis of PD and as potential sources of cells to restore motor deficits in PD. These set of investigations and aims have the potential to transform the study and treatment of PD by providing new molecular insights into the pathogenesis of PD and the potential discovery of biochemical and/or molecular markers that could be ultimately used as biomarkers to monitor the progression of PD.

Name of Principle Investigator: **Valina L. Dawson** Project Budget: $ **1,725,000.00**

Grantee: **Johns Hopkins University**

Title: **The novel protein Botch promotes neurogenesis through regulation of the Notch pathway**

Description of stem cell research (as submitted by Principle Investigator):

Neurological diseases, stroke and cranial trauma are the leading cause of death, disability and loss of quality of life worldwide according to the World Health Organization. In US, 4.7 million cases are reported each year and stroke is the third leading cause of death and disability. According to the National Institutes of Health, there are 4.5 million people in the United States suffering from Alzheimer's disease and 1 million people suffering from Parkinson's disease. Unfortunately, there are few effective therapies available for treating these diseases. From many years of combined basic and clinical research, it is becoming clear that to treat patients with neurological problems multiple approaches must be taken. The cause of cellular demise must be uncovered and slowed or stopped. Cell survival and regenerative processes must be activated to spare as much tissue as possible. Finally, since neurological symptoms manifest due to loss of neural cells, cell replacement therapy must be developed. Replacement therapy through the generation of the needed cell population derived from differentiation of stem cells into neurons or oligodendrocytes is an attractive possibility. However, under current protocols for differentiation and maturation into neural cells the bulk of the cells are astrocytes, not neurons or oligodendrocytes. This is not a very efficient or effective approach to providing the more interesting cells for research and ultimately for clinical use. Recently we have found that manipulating a novel protein Botch in rodent neural precursor cells reverses this ratio so that more neural precursor cells differentiate into neurons. We performed both gain-of-function and loss-of-function experiments in mouse embryonic neurogenesis models. Botch overexpression in E14.5 neuroprecursors decreased neurosphere frequency and size. It also promotes neuroprecursors to differentiate towards neuronal fates over glial ones. Loss-of-function of Botch drives more neural stem cells to differentiate into glia. To determine whether Botch plays a significant role in vivo, intraventricular injections and in utero electroporation at E13.5 of Botch-shRNA was used to knock-down endogeneous Botch. Botch-shRNA greatly increases the number of cells in the VZ/SVZ while significantly decreasing the number of cells in the CP/IZ two days after electroporation. Overexpression of Botch has the opposite effect. Taken together, these data indicate that Botch promotes neurogenesis both in vitro and in vivo. To further understand the molecular mechanism of Botch biology, we performed co-immunoprecipitation experiments and found out that Botch interacts with Notch1 in vitro and in vivo. In vitro binding assays using mammalian expressed Botch-AP and Notch1 protein indicate the binding Km is around 10 nM. By using the CBF-1-luciferse assay, we found that Botch blocks CBF-1-dependent Notch signal pathway. In addition, we used mouse muscle cell line C2C12 as a biological readout for Notch signaling. In C2C12 differentiation assay, Botch promotes C2C12 differentiation into myotubes by inhibiting Notch signaling. In summery, we identified a novel protein Botch promotes embryonic neurogenesis by inhibiting Notch signaling pathway. Our goal is to now explore this signaling in human stem cells.

Name of Principle Investigator: **Richard L. Eckert** Project Budget: $ **941,315.00**

Grantee: **University of Maryland, Baltimore**

Title: **Human and Mouse Epidermis-Derived Multipotent Cells for Cell Therapy**

Description of stem cell research (as submitted by Principle Investigator):

Embryonic stem (ES) cells are pluripotent and able to differentiate into virtually every cell type in the body. Because of these properties these cells are considered an important source of cells for cell-based therapy. However, the use of ES cells is controversial and may not be justified or necessary, since appropriately reprogrammed adult somatic cells may provide a source of cells for cell-based therapy. Converting somatic cells can, in principle, be achieved by manipulating expression of key stem cell maintenance proteins. The embryonic transcription factor Oct-4 is often referred to as the master regulator of the undifferentiated state. Although its role in maintaining embryonic stem (ES) cell pluripotency is well established, its ability to directly reprogram committed somatic cells is not well defined. Using transient transfection, we tested the ability of Oct4 to revert mouse epidermal basal keratinocytes to a more ES cell-like state. We found that the Oct-4-transfected keratinocytes expressed Oct-4 target genes including Rex-1, Sox-2, Nanog and undifferentiated transcription factor 1 (Utf1). We also note increased developmental potential in that Oct-4 transfected cells could differentiate into neuronal cells when exposed to neuroectodermal differentiation medium. Empty vector-transfected keratinocytes did not respond to this treatment and remained as keratinocytes. These findings suggest that Oct-4 expression can cause epidermis-derived somatic cells to revert to a more developmentally potent state and this does not require other stem cell maintenance factors. This finding has important therapeutic implications provided it can be applied in human epidermal cells. Additional studies reveal that transfection of human epidermal keratinocytes with Oct-4 plasmid causes human keratinocytes to behave in a similar manner to express neuronal markers. This is an important finding as it suggests that transfection of epidermal keratinocytes with Oct-4 may provide an important cell source of multipotent cells for cell-based therapy.

Name of Principle Investigator: **John T. Iaascs** Project Budget: $ **910,522.00**

Grantee: **University of Maryland, Baltimore**

Title: **Isolating Prostate Cancer Stem Cells Without Contaminating Normal Prostate Cells From Radical Prostatectomy Specimens**

Description of stem cell research (as submitted by Principle Investigator):

A panel of human Prostate Cancer Stem Cell (PCSC) lines derived from a spectrum of differing histological grades of prostate cancer is urgently needed as a platform for rational drug development. Thus, methods are urgently needed for identifying, isolating, and propagate PCSCs from a series of prostate cancers patients.Over the first year of support from the Maryland Stem Cell Support, a series of human prostate cancer xenografts have been used to develop and validate protocols for isolating and propagating PCSCs. These protocols have been modified to establish PCSC cultures from surgical specimens of primary prostate cancer directly from patients. Modification is required because within surgical specimens of localized prostate cancer, there is always an admixture of both malignant and normal prostate epithelial and stromal cells and these normal cells commonly have a higher ability to grow in culture for multiple passages before senescing that the PCSCs. Thus, unless separated initially, these cultures eventually become overgrown with normal not malignant cells. To overcome this problem surgical tissue is enzymatic dissociated into single cells and then the cells are initially cultured for 1-2 weeks in a serum free high calcium (>1mM) growth factor defined media (i.e. termed suspension culture media) using standard tissue culture flasks. This suspension culture media allows formation and growth of PCSC containing spheroids in suspension while simultaneously allowing the attachment of normal prostate epithelial and stromal stem and progenitor cells to the tissue culture dish. After this initial culturing, the PCSC spheroids in suspension are removed, dissociated into single cells and replated into the suspension culture media and the attached normal prostate epithelial and stromal stem and progenitor cells are detached by trypinization and half the cells replated in serum free low calcium (i.e., <100uM) growth factor defined media to propagate the normal adult prostate epithelial stem cells and half of the cells replated in 10% fetal bovine serum containing moderate calcium (i.e., ~400uM) media to propagate the normal adult stromal stem cells. The PCSC spheroids from the second passage are dissociated into single cells and replated in growth factor and attachment protein containing moderate calcium (i.e., ~400uM) media which allows the PCSCs to attach and grow uncontaminated by normal prostate cells. Using this method, we have been able to culture PCSCs separately from patient matched normal adult prostate epithelial and stromal cells.

Name of Principle Investigator: **Dara L. Kraitchman** Project Budget: **$ 1,378,731.00**

Grantee: **Johns Hopkins University**

Title: **Estimation of Cell Viability in X-Ray-Trackable Encapsulated Mesenchymal Stem Cells Transplants for Rabbit Model of Peripheral Arterial Disease**

Description of stem cell research (as submitted by Principle Investigator):

Stem cells have the potential to enhance arteriogenesis to treat ischemic tissue in peripheral arterial disease (PAD). Yet, poor cell survival and difficulties with cell tracking are major issues with all cell transplantation therapies. Background: Using the alginate-poly-L-lysine-alginate (APA) microencapsulation method, (1) cells can be protected from early destruction and thereby avoid immunosuppressive regimes. The addition of perflurooctylbromine (PFOB) to APA microcapsules enables cell tracking by X-ray imaging. However, cell survival within the microcapsule must be inferred. Using cells expressing reporter gene (2) in vivo longitudinal monitoring of cell survival with multimodality imaging has also been performed. Hypothesis: Transfection of mesenchymal stem cells (MSCs) with triple fusion reporter (TF) gene (TF-MSCs) followed by microencapsulation in PFOB-impregnated APA capsules enables cell tracking and survival monitoring in vivo. Systemic injection of a reporter gene probe, i.e., luciferin, would require large systemic doses and may be poorly delivered to ischemic beds. Thus, we sought to test whether C-arm CT for localized administration of the reporter probe could be accomplished. Research: MSCs were isolated from bone marrow of male New Zealand White rabbits, expanded for 3 passages and transfected using Lipofectamine 2000 (Invitrogen) with the TF gene containing red fluorescent protein, truncated thymidine kinase (SPECT/PET reporter) and firefly luciferase (BLI reporter). The addition of 12% PFOB to the APA capsules was used to enable X-ray visualization. Prior to the transplantation in a PAD rabbit model, the microcapsules were incubated with D-luciferin (150 μg/ml, Caliper). Rabbits (n=8) were randomized to received two to six intramuscular injections of PFOB-labeled or unlabeled capsules containing TF-MSCs in the medial thigh. BLI (Xenogen IVIS 200) was performed to assess cell viability immediately after transplantation as well as 1 and 2 days post injection. C-arm CT (Siemens Axiom Artis dFA) was performed using the 8sDR preset (DynaCT®, Siemens Medical Solutions, Forchheim, Germany) with an acquisition time of 8 s, a total 240° projection angle, 0.5° projection increment, 1k-matrix, and a 0.4x0.4x0.4 mm resolution on day 1 and 2 to target luciferin (15 mg per injection site) to the PFOB capsules using a custom needle targeting software (X-Loc). APA capsules were injected blindly. Observations: In vitro bioluminescence of encapsulated MSCs was not blocked by encapsulation itself or the addition of PFOB. Successful targeting to the PFOB microcapsules using C-arm CT was performed at all injection sites. BLI revealed viable MSCs encapsulated in PFOB microcapsules 1 and 2 days post transplantation, except 1 rabbit, where the all the cells were dead just after encapsulation. Blind luciferin injections to unlabeled APA injections resulted in successful BLI signal detection in only ~15% of the injection sites. Conclusions: X-ray-visible microcapsules enable the tracking of stem cell injections using clinically available X-ray imaging systems. Accurate injections of the BLI probe directly into the transplantation sites allow monitoring of cell viability within the microcapsules in a serial manner using non-invasive imaging. References: 1. F. Lim et al., Science 210, 908 (Nov 21, 1980). 2. R. Mayerhofer et al., J Biolumin Chemilumin 10, 271 (Sep-Oct, 1995).

Name of Principle Investigator: **John Laterra** Project Budget: **$ 827,886.55**

Grantee: **Hugo W. Moser Research Institute at Kennedy Krieger**

Title: **DNER, an Epigenetically Modulated Gene, Regulates Glioblastoma-Derived Neurosphere Cell Differentiation and Tumor Propagation**

Description of stem cell research (as submitted by Principle Investigator):

Neurospheres derived from glioblastoma (GBM) and other solid malignancies contain neoplastic stem-like cells (GBM-SCs) that efficiently propagate tumor growth and resist cytotoxic therapeutics. The primary objectives of this study were (1) to determine the effects of histone modifying agents on the GBM-SC phenotype and (2) to use histone-modifying agents to elucidate mechanisms by which the phenotype and tumor-promoting capacity of GBM-derived neoplastic stem-like cells are regulated. Research and observations:Using established GBM-derived neurosphere lines and low passage primary GBM-derived neurospheres, we show that histone deacetylase (HDAC) inhibitors inhibit growth, induce differentiation, induce apoptosis, and diminish the tumor initiating capacity of neoplastic neurosphere cells. A specific gene product induced by HDAC inhibition, Delta/Notch-like epidermal growth factor-related receptor (DNER), inhibited the growth of GBM-derived neurospheres, induced their differentiation in vivo and in vitro, and inhibited their engraftment and growth as tumor xenografts. Conclusions:The differentiating and tumor suppressive effects of DNER, a noncanonical Notch ligand, contrast the previously established tumor-promoting effects of canonical Notch signaling in brain cancer stem-like cells. Our findings are the first to implicate noncanonical Notch signaling in the regulation of neoplastic stem-like cells and suggest novel neoplastic stem cell targeting treatment strategies for GBM and potentially other solid malignancies.

Name of Principle Investigator: **Nicholas J. Maragakis** Project Budget: $ **976,243.00**

Grantee: **Johns Hopkins University**

Title: **Targeting respiratory dysfunction in ALS: Astrocyte replacement in SOD1-G93A rat cervical spinal cord via human Glial-Restricted Precursor (GRP) transplantation**

Description of stem cell research (as submitted by Principle Investigator):

Studies in models of amyotrophic lateral sclerosis (ALS) have suggested that cellular abnormalities are not limited to motor neurons. Given the numerous observations of astrocyte dysfunction in both humans with ALS and in SOD1-G93A rodents, a widely studied ALS model, the present study targeted replacement of non-neuronal cell types for therapeutic benefits via transplantation of human Glial-Restricted Precursors (GRPs), lineage-restricted astrocyte precursors derived from human fetal neural tissue. Our previous findings demonstrated that transplantation of rat-derived GRPs into cervical spinal cord ventral gray matter (in order to target therapy to diaphragmatic function) resulted in therapeutic efficacy in the SOD1-G93A rat. Benefits using rat GRPs included extended survival and disease duration, delayed forelimb (but not hindlimb) disease onset, and slowed declines in forelimb grip strength, motor performance and diaphragm compound muscle action potentials (CMAPs) following phrenic nerve stimulation, a functional electrophysiological assay of diaphragm function. These findings demonstrate the feasibility and efficacy of transplantation-based astrocyte replacement for ALS, and also show that targeted multi-segmental cell delivery to cervical spinal cord is a promising therapeutic strategy, particularly because of it relevance to addressing respiratory compromise associated with ALS. The present studies have extended this therapeutic approach to transplantation of human GRP analogs. Human GRP transplants robustly survived in both gray and white matter, migrated primarily along white matter tracts, and differentiated into astrocytes in SOD1-G93A spinal cord, despite ongoing disease progression. Studies are in progress to examine the therapeutic efficacy of transplanted human GRPs in the SOD1-G93A mouse model. The work was supported by: NIH F32-NS059155 (A.C.L.), The Robert Packard Center for ALS Research (N.J.M.), The ALS Association (N.J.M.), The Maryland Stem Cell Research Fund (N.J.M.).

Name of Principle Investigator: **Jeffrey D. Rothstein** Project Budget: $ **1,010,173.00**

Grantee: **Johns Hopkins University**

Title: **Combinatorial High-Throughput Drug S creening Using Human Astroglial Cell Lines for Small Molecule Compounds that Improve Astroglial Function: Increased EAAT2 Expression/Function**

Description of stem cell research (as submitted by Principle Investigator):

Glutamate is the predominant excitatory amino acid neurotransmitter in the mammalian CNS. To inactivate synaptic glutamate, glutamate is taken up by glutamate transporters localized mainly on astrocytes surrounding the neuronal synapse. The dominant astroglial transporter is,EAAT2 (or GLT1), which is responsible for most of the glutamate uptake in the brain. Animal studies show that EAAT2 dysfunction leads to excessive extracellular glutamate, and thereby contributes to various neurological disorders including amyotrophic lateral sclerosis (ALS). A recent screen of 1040 FDA approved drugs revealed that beta lactam antibiotics protect against neural injury and delay disease in ALS mice by increasing EAAT2/GLT1 protein expression. Similar results have been reported for models of pain, multiple sclerosis, Huntington's disease and depression. Thus, agents that upregulate the EAAT2 transporter might be beneficial in neurological or psychiatric disease by augmenting astrocytic uptake of glutamate. Owing to the complexity of biological networked system, it is desired to employ a new drug discovery approach for identifying effective combinations of compounds. Multi-component therapies through deliberate mixing of drugs have a successful history in a number of medical areas. Here we report our effort to develop a novel combinatorial high-throughput screening (cHTS) paradigm to identify effective compound combinations that upregulate EAAT2 gene expression. We generated fetal derived-human immortalized astroglial cells that are stably expressing a firefly luciferase reporter under the control of the human EAAT2 promoter. Through cHTS of a library of up to 2000 FDA approved compounds and natural products, we discovered a series of candidate drug-pairs that show synergistic effect in activating the EAAT2 promoter. We have quantified synergy in our screening experiments and identified combinations in which one drug enhances the potency or the intrinsic activity of the other drug. One of our lead compounds, JR01, acts not only by increasing the intrinsic activity of its pair, but also mediating a shift in potency. We will advance our lead candidates to more complex in vitro and in vivo assays. Our studies provide potential neurotherapeutics by modulating the activity of glutamate transporters via gene activation.

Name of Principle Investigator: **Elias Zambidis** Project Budget: $ **1,725,000.00**

Grantee: **Johns Hopkins University**

Title: **Human ESC and iPSC-derived erythroid progenitors can be expanded in mass quantities for hemoglobinopathy and malaria studies**

Description of stem cell research (as submitted by Principle Investigator):

Despite improvements in the treatment of beta-hemoglobinopathies such as sickle cell disease (SCD) or beta-thalassemia, that include preventive care, fetal hemoglobin (HbF) induction with hydroxyurea, improved iron chelation during transfusions, and hematopoietic stem cell (HSC) transplantation, life expectancy for such patients remains diminished. Transplantation of genetically-corrected erythroid cells from patient-specific induced pluripotent stem cells (iPSC) would avoid the risk of allogeneic transplantation. Since increased HbF expression ameliorates the clinical symptoms of both beta-hemoglobinopathies and also malaria, we propose that transfusion of long-lived, fetal globin expressing erythroid cells from iPSC may serve as a treatment for these individuals. However, although iPSC possess characteristics of human embryonic stem cells (hESC), their full capacity for differentiation into specific cell types remains untested. In these studies, we quantitatively compared the ability of hESC and iPSC from reprogrammed fibroblasts to differentiate to the hematopoietic lineage. Human iPSC gave rise to various lineages of hematopoietic cells, and produced E-CFU, M-CFU, G-CFU and GEMM-CFU in colony forming assays. Moreover, we demonstrated, that human ESC/iPSC-derived erythroid progenitors can be mass expanded under serum-free conditions in liquid culture for 60-80 days. Primitive iPSC-derived erythroblasts expressed CD71, CD36, and CD235a (Glycophorin A), and also embryonic and fetal globins at expression levels similar to hESC-derived erythroid cells. Next, we showed that hESC/iPSC erythoid cells could be efficiently infected with the malaria parasite Plasmodium falciparum. However, despite efficient infection rates, we observed that malaria parasite development in these infected hESC/iPSC-derived erythroid cells was significantly delayed in comparison with normal adult red blood cells (RBC). These data demonstrate the feasibility of generating erythroid progenitors for hemoglobinopathy and malaria studies and treatment. Further optimization of our methods for mass expansion of hESC and iPSC-derived fetal type erythroid progenitors using bioreactor systems should provide broad applications in molecular and developmental biology, microbiology, gene therapy, pharmacology, and disease modeling.

FY 2008

Exploratory Awarded Research Descriptions

Name of Principle Investigator: **Maria Roselle Abraham** Project Budget: $ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **Molecular engineering of human cardiac-derived progenitor cells and the myocardial micro-environment to improve cardiac regeneration**

Description of stem cell research (as submitted by Principle Investigator):

Heart Failure is an important cause of death in the United States and afflicts more than 5 million Americans. When advanced, these patients have a mortality (>50% at 5 years), exceeding that of many malignant tumors. Stem cell therapy offers the promise of "organ repair on demand" and the potential to help patients avoid heart transplantation.Several groups are focused on a variety of stem cells to treat heart disease, but an important problem encountered in this field is low levels of stem cell survival after transplantation.

We have developed a method to isolate large numbers of stem cells from a patient's own heart tissue; we call them cardiac-derived progenitor cells or CDCs. These CDCs can survive in heart tissue after a heart attack and help grow new heart tissue and improve heart function. Unfortunately, very few cells survive in the heart after cell transplantation, limiting the benefit of this treatment.

In this proposal we plan to develop new methods to improve the numbers of stem cells obtained from patient heart tissue and then discover ways of increasing cell survival in the heart after transplantation, using a combination of gene therapy and stem cell therapy. We expect that if successful, this combination of cell therapy and gene therapy has the potential to greatly increase the success of clinical stem cell transplantation in patients who have suffered heart attacks as well as in patients suffering from advanced heart failure.

Name of Principle Investigator: **Dmitri Artemov** Project Budget: $ **227,948.00**

Grantee: **Johns Hopkins University**

Title: **Targeted Imaging and Therapy of Breast Cancer Stem-like Cells**

Description of stem cell research (as submitted by Principle Investigator):

Recent studies have identified a subpopulation of breast cancer stem-like cells (BCSLC) that contribute significantly to cancer formation, propagation and dissemination and are characterized by their ability to form tumors with low inoculums and resistance to chemo and radiation-therapy. As BCSLC can repopulate the tumor after the bulk of the tumor tissue is destroyed by therapy the emerging paradigm in cancer treatment is that BSCLCs should be one of the primary targets for successful eradication of cancer. Developing of anticancer therapy targeted to BCSLC is thus a critically important goal for breast cancer treatment and identification of stem-like subpopulations in tumors by molecular imaging will be the first step to achieve this goal. Breast cancer cells with high CD44 and low CD24 receptor expression ($CD44^{+}/CD24^{-/low}$ phenotype) have increased tumorogenicity and a range of unique properties that can be interpreted as hallmarks of putative BCSLC. Hyaluronan or hyaluronic acid (HA) is a non-sulfated linear natural glucosaminoglycan and is a highly-specific ligand for CD44 receptor. Due to its excellent safety profile and immunoneutrality HA is an attractive platform to develop specific imaging and therapeutic agents targeted to CD44 expressing BCSLC. A novel HA-based CD44 receptor-targeted agent will enable specific multimodality imaging of BCSLCs *in vivo* as well as specific delivery of chemotherapeutic agents to the BCSL cells. Combination of noninvasive optical and MR imaging probes with cytotoxic drug, paclitaxel, within the targeted construct will provide a unique platform for specific imaging and therapy of BCSLC in preclinical breast cancer models and for future clinical translation. We have synthesized and characterized MR imaging agents using hyaluronic acid (HA) polymer backbones with molecular weights of 16, 31, and 74 kDa. The gadolinium content of HA-(EDA-DTPA-Gd) conjugates was determined by ICP-MS, and typically was close to 10% that corresponds to about 70% modification ratio of the HA carboxyl groups. Fluorescent labeled HA probes were also synthesized and demonstrated efficient labeling of the polymer using anime-reactive dyes and EDC/EDA chemical linker. T1 relaxivity, plasma pharmacokinetics, and biodistribution of the HA-(EDA-DTPA-Gd) conjugates were determined *in vitro* and *in vivo* in animal models of breast cancer at 9.4T small animal MR scanner. Early distribution of the contrast was initially limited to the blood vessels; in addition, HA-(EDA-DTPA-Gd) showed significant uptake by the stomach as early as 1h post injection. Eventually, all agents were readily excreted through the kidney to the bladder and no adverse reactions such as weight loss and death were observed in mice received HA-(EDA-DTPA-Gd). Blood pharmacokinetics were described by a two-compartment model with the fast and slow phases life times of 12.4 min and 141 min, respectively. An increased uptake of 16 kDa and 31 kDa HA-(EDA-DTPA-Gd) agents was detected in CD44-positive MDA-MB-231 tumor xenografts in comparison to CD44-negative MCF-7 models. The synthesized imaging agents demonstrated preferential accumulation in CD44-expressing breast cancer. Based on these results our next step will be development of 16 kDa and 31 kDa HA carriers labeled with both Gd groups for MR imaging and paclitaxel therapeutic moieties as platforms for teragnostic applications in CD44-expressing breast cancer.

Name of Principle Investigator: **Sergei Atamas** Project Budget: $ **224,750.00**

Grantee: **University of Maryland, Baltimore**

Title: **Bone Marrow Stem Cells Heal Tendon via Secreted Factors**

Description of stem cell research (as submitted by Principle Investigator):

PURPOSE: Tendinopathies pose a major biomedical and socioeconomic challenge. Local application of autologous bone marrow aspirate (ABMA) has been suggested as a complement to traditional surgical interventions. It remains unclear whether this new approach is beneficial and, if so, what mechanisms are involved. METHODS: We supplement traditional surgical treatments with local instillations of cells purified from ABMA in patients with tendinopathies. Clinical improvement in these patients is compared with patients treated with traditional surgery alone. Separately, mesenchymal stem cell (MSC) and hematopoietic progenitor (HP) cell cultures from these patients are derived and phenotypically characterized for production of proinflammatory, antiinflammatory, profibrotic, and antifibrotic cytokines and cell surface molecules. Production and activity of matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) in these cultures are also investigated. Additionally, we investigate the effects of MSC and HP cells on tenocytes in co-culture. The effects of stem cells on proliferation and collagen production by tenocytes derived from healthy and disease-involved tendon tissues are compared. RESULTS: Of more than 150 patients treated with ABMA cells so far, most have responded with significantly shortened postoperative recovery time; less swelling, less pain, and less inflammation following surgery; and faster and more complete functional restoration compared to conventionally treated patients. These substantial improvements in recovery are seen as early as day 3 following surgery and continue for months and likely beyond. The very short initial period of time at which the improvement is seen is likely insufficient for differentiation of the injected stem cells into mature cell types and manifestation of their functional effects. In vitro experiments showed that damaged tendon tissues produced elevated levels of mRNA for specific proinflammatory cytokines and MMPs. ABMA cultures from patients showed elevated mRNA and protein levels for several proangiogenic and profibrotic cytokines, and for TIMPs. In tenocyte cell cultures, these recombinant factors promoted proliferation and collagen production. In MSC/tenocyte and HP/tenocyte co-cultures, similar profibrotic effects on tenocytes were also observed. CONCLUSIONS: Tendon inflammation subsides and deposition of connective tissue fibers occurs faster in the presence of adult autologous bone marrow stem cells. These effects are likely mediated by soluble factors such as cytokines and TIMPs produced by bone-marrow stem cells, leading to accelerated tenocyte proliferation and collagen deposition.

Name of Principle Investigator: **Ilia V. Baskakov** Project Budget: $ **230,000.00**

Grantee: **University of Maryland Biotechnology Institute**

Title: **Treatment with normal prion protein delays differentiation and helps to maintain high proliferation activity in human embryonic stem cells**

Description of stem cell research (as submitted by Principle Investigator):

The normal cellular form of prion protein (PrPC) has been shown to exhibit a diverse range of biological activities. Several recent studies highlighted potential involvement of PrPC in embryogenesis or in regulating stem cell self-renewal and proliferation. In the current study, we employed human embryonic stem cells (hESCs) for assessing the potential role of prion protein in early human development. Here we showed that endogenous PrPC was rarely expressed in undifferentiated human embryonic stem cells (hESCs) and its expression was increased during spontaneous differentiated hESCs. The expression of PrPC and neuronal differentiating markers were found to decrease when cells were exposed to recombinant PrP (rPrP) folded into an -helical conformation, whereas the expression of oct-3/4 was upregulated. Furthermore, cells treated with rPrP during the early stages of differentiation displayed substantially higher proliferating activity than non-treated controls and maintained as high proliferation rates as those found in non-differentiated cells. Our data indicated that treatment of hESCs with rPrP in its native conformation delayed the spontaneous differentiation of hESCs and helped to maintain their high proliferation activity during spontaneous differentiation. Together with previous observations, these results support the hypothesis that normal prion protein helps to maintain the undifferentiated pluripotent status for a fraction of embryonic stem cells.[This work was supported by a Maryland Stem Cell Commission grant (to I.V.B)]

Name of Principle Investigator: **Fred Bunz** Project Budget: $ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **Enhancing methods for The Generation of Induced Pluripotent Stem (iPS) Cells: Improving Efficiency and Safety**

Description of stem cell research (as submitted by Principle Investigator):

The recent derivation of pluripotent stem cells from differentiated adult cells represents a technological milestone with enormous therapeutic promise. Studies over the past several years have shown that normal cells can be reprogrammed to an embryonic-like state,thereby bypassing a need for embryonic materials. The factors required for this process remained unknown until recently. In 2007, three labs independently reported the successful derivation of induced pluripotent stem (iPS) cells from human somatic cells with defined genetic elements. The current approach to generate iPS cells is to employ retroviruses engineered to deliver genes that promote pluripotency. Yamanaka and colleagues isolated four genes that were found to be both necessary and sufficient for reprogramming: SOX2, OCT4, KLF4, and C-MYC. Roughly one in 5,000 cells treated with retroviruses expressing these 4 genes were converted to iPS cells. Morphologically and immunologically, iPS cells were shown to be indistinguishable from human embryonic stem cells. Critically, human iPS cells have the capacity to develop into tissues of all three embryonic layers. The reprogramming of differentiated cells into stem cells by the expression of a defined set of genes is an exciting accomplishment that has shed considerable insight into the molecular basis of pluripotency. It now appears that differences in experimental conditions, possibly transgene expression levels or differences in the target cells, can affect the overall efficiency of the reprogramming process. Studies by Jaenisch and colleagues have revealed that the generation of iPS cells is both time-dependent and stochastic in nature, indicating that many undefined cellular changes take place before the acquisition of pluripotency. iPS cells have tremendous translational potential. However, the use of retroviruses in implantable cells may present a significant problem. Retroviruses integrate into genomic DNA and are therefore inherently mutagenic. Furthermore, the retroviral delivery of oncogenes such as C-MYC could also promote tumorigenesis. For these reasons, the use of retroviruses and oncogenes to reprogram somatic cells for subsequent implantation carries a potentia l risk of cancer. Recently, non-retroviral methods for activating the iPS cell transcriptional pathways have been devised. It is hoped that these improved methods will benefit the translation of reprogramming technology into the clinic. Our efforts are focused on continued improvement of iPS technology, and illuminating the stochastic changes that drive reprogramming.

Name of Principle Investigator: **Chi V. Dang** Project Budget: $ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **Time-dependent c-Myc Transactomes Mapped by Array-based Nuclear Run-on Reveal Transcriptional Modules in Human B Cells with a Stem Cell Signature**

Description of stem cell research (as submitted by Principle Investigator):

We report a novel platform to measure genome-wide transcription measured by Array-based Nuclear Run-On (ANRO), which is applicable to commercial microarrays and allows for the definition of the \'transactome.\' Together with changes in total mRNA levels and genome-wide localization of transcription factor binding sites by chromatin immunoprecipitation, the transactome completes the mapping of transcriptional networks. In this regard, ANRO comprehensively defines direct c-Myc target genes in a human Burkitt's lymphoma model. Following c-Myc activation, ANRO also reveals time-dependent transcriptional waves of genes with promoters enriched in distinct transcription factor motifs, thereby uncovering sequential transcriptional responses. A stem cell signature emerges with Gene Set Enrichment Analysis (GSEA) that overlaps with the expression profiles of human embryonic stem cell. ANRO, hence, not only enables the complete mapping of any transcriptional network, but it also reveals a core Myc transcriptional program that has a stem cell signature perhaps revealing a feature of cancer stem cells.

Name of Principle Investigator: **Jennifer Elisseeff** Project Budget: $ **227,601.00**

Grantee: **Johns Hopkins University**

Title: **Examination of Human and Caprine Stem Cell-Tissue Interactions in Response to Changes in Differentiation State, Microenvironment, and Disease Status**

Description of stem cell research (as submitted by Principle Investigator):

Cell and tissue interactions are a vital component of tissue homeostasis. This signaling is disrupted in disease states such as osteoarthritis. Mesenchymal stem cells (MSCs) are under investigation for the treatment of osteochondral defects and diseases given their proven capacity to serve as a cell source and stimulator of tissue repair. However, their ability to serve as a stimulator and cell source for tissue repair in disease states has not been adequately analyzed. Application of MSCs for tissue repair requires an understanding of how factors such as local microenvironment, disease status, and differentiation state and the system in which the communication is being studied affect the observed signaling. This work analyses tissue formation by chondrocytes (Human or Bovine) and MSCs (Human or Caprine) of varying differentiation states both alone and in co-culture with each other. To identify which observations were related to cell differentiation and disease as opposed to those related to the experimental setup, 3 systems were used: traditional transwell co-culture model,bilayered poly(ethylene glycol) (PEG) gels, and a bilayered gel comprised of a layer of PEG polymerized on top of a poly(lactic-co-glycolic acid) (PLGA) scaffold. Results demonstrated that trends of chondrocyte stimulation were depended largely on MSC diffentiation and the disease state of the chondrocytes (healthy vs osteoarthritic). Examination of diseased chondrocytes resulted in altered trends of stimulation. MSC tissue formation was more complex and responsive to factors such as dimensionality in addition to differentiation state. These results may impact future therapeutic use of MSCs.

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Name of Principle Investigator: **Alan D. Friedman** Project Budget: $ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **RUNX1 Phosphorylation by CDK Reduces HDAC Binding and Accelerates Adult Mouse and Human Hematopoietic Stem/Progenitor Cell Proliferation**

Description of stem cell research (as submitted by Principle Investigator):

RUNX1/AML1 is a key regulator of hematopoiesis, as Runx1(-/-) mice do not develop definitive hematopoietic stem cells (HSC). A key roadblock in the successful generation of clinically useful HSC from hESC or iPSC is the difficulty in obtaining adult HSC with multilineage potential. As the only transcription factor whose absence specifically eliminates generation of adult HSC, manipulation of RUNX1 promises to assist in overcoming this roadblock. In addition, the majority of adult HSC exist in a quiescent state in the marrow, with a small number entering cell cycle as needed to provide mature blood elements. Developing means to allow HSC to expand without differentiating or transforming may also assist clinical applications. We have investigated regulation of cell cycle progression by RUNX1. RUNX1 stimulates G1 to S cell cycle progression by inducing cyclin D3 and cdk4 transcription, and inhibition of RUNX1 slows G1 progression. RUNX1 contains three consensus cdk sites, (S/T)PX(R/K), at S48, S303, and S424, and using phospho-specific antisera developed in our laboratory we find that each of these is modified in hematopoietic cells. Mutation of these serines to aspartic acid, mimicking phosphorylation, increases trans-activation of a reporter containing four CBF sites, whereas mutation to alanine reduces trans-activation. p300 interacts similarly with Runx1(tripleA) and Runx1(tripleD). We have also evaluated interaction of HDACs1-8 with these variants and RUNX1 and find that both HDAC1 and HDAC3 have reduced affinity for RUNX1(tripleD), as assessed by co-immunoprecipitation from transiently transfected 293T cells. Evaluation of single serine residue mutants (S48D, S303D, and S424D) demonstrates reduced affinity of HDAC1 or HDAC3 specifically for the RUNX1(S424D) mutant. Thus, cdk phosphorylation of RUNX1 S424 reduces affinity for HDAC1 and HDAC3, increasing RUNX1 trans-activation potency. To assess the functional consequences of RUNX1 cdk phosphorylation in normal hematopoietic stem/progenitor cells, we first compared the ability of RUNX1 or its tripleA or tripleD variants to stimulate proliferation of mouse lin-negative marrow cells. RUNX1 or RUNX1(tripleD) more effectively induced proliferation, compared with RUNX1(tripleA), indicating that RUNX1 phosphorylation stimulates its ability to induce proliferation. We have also observed that RUNX1 potently induces the proliferation of human CD34+ cord blood stem/progenitors, and we are assessing the effects of the tripleA and tripleD variants in this setting. Use of the RUNX1tripleD variant may ultimately assist in the expansion of adult or hESC/iPSC-derived HSC. In addition, RUNX1 cdk modification, for example induced by Wnt or Notch signals, may help regulate adult HSC specification from the hemangioblast, as is also under investigation in our laboratory.

Name of Principle Investigator: **Nicholas Gaiano** Project Budget: $ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **Exploring the Role of NF-kappa-B Signaling in Human Neural Stem Cells**

Description of stem cell research (as submitted by Principle Investigator):

How the brain develops from a proliferating neural stem cell/progenitor pool into the most intricate structure in the human body is a fundamental question facing modern biology. This study is investigating this question in human neural progenitor cells (hNPCs) derived from human embryonic stem cells (hESCs). Specifically, these studies are exploring the role of NF-kappa-B signaling during the maintenance of hNPCs, and during their differentiation into neuronal and glial cells. The NF-kappa-B pathway has been intensively studied primarily in the context of the immune system where it plays a fundamental role during development and in the adult. While numerous studies have examined the role of NF-kappa-B in mature neurons, until recently little was known about the role of this pathway in the neural progenitors. Our recent results, using mice as the model organism, demonstrate that NF-kappa-B signaling is robustly present in neural progenitors, where it regulates the balance between proliferation and differentiation. Much remains to be understood about the role of NF-kappa-B in mammalian neural progenitors in general, and currently nothing is known about the functioning of this pathway in human neural progenitors. The proposed study contains three specific aims. We are characterizing NF-kappa-B pathway activation in hESCs and hNPCs, using gene expression analysis to determine which components of the pathway are present, and reporter strategies to characterize endogenous NF-kappa-B signaling status. In addition, we are determining which exogenous signaling molecules can activate NF-kappa-B signaling in hNPCs. We are also examining the effects of activating or blocking NF-kappa-B signaling on the maintenance and differentiation of hNPCs. These efforts include analyses of cell proliferation, self-renewal, and neuronal and glial cell type specification. Finally, we are using cell transplantation into the mouse brain in utero to test the developmental potential of hNPCs that have had the NF-kappa-B pathway activated or inhibited by molecular manipulations. These studies will greatly enhance our understanding of human NPC regulation, and are likely to facilitate the development of cell-based therapies to treat nervous system damage, including the sort caused neurodegenerative diseases and traumatic brain injury. Neuroregeneration and Stem Cell Program, Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Name of Principle Investigator: **Yoon Young Jang** Project Budget: $ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **Efficient hepatic differentiation of human pluripotent stem cells for liver regeneration**

Description of stem cell research (as submitted by Principle Investigator):

Human embryonic stem cells (hESCs) have been suggested to differentiate into hepatocyte lineage cells in vitro. However, it has not been tested whether these cells have normal liver cell function in vivo, such as regeneration of damaged liver tissue. This is likely due to a low yield of hESCs derived mature hepatic cells for transplantation after differentiation in vitro. These studies are designed to investigate the potential of human pluripotent stem cells for the repair of liver tissue in animal models of liver diseases. To efficiently generate human liver lineage cells from pluripotent stem cells, we have tested multiple hepatic differentiation protocols. To determine whether the human pluripotent stem cell derived liver cells are able to engraft, survive, proliferate and function like normal liver cells in vivo, we have performed transplantation experiments with these differentiated liver lineage cells in mouse models of human liver diseases. We were able to improve and optimize the liver differentiation protocol for human pluripotent stem cells (i.e. hESCs and iPS cells). We have established multiple human iPS cell lines and determined their liver differentiation potential. Completion of this study will provide advanced methodologies to utilize pluripotent stem cell derived functional liver cells for both basic and clinical research. Ultimately, we anticipate th at limitations of current cell therapy (i.e. the shortage of appropriate donor cells) will be improved by safely using differentiated functional cells from hESCs/hiPS cells to transplant for a variety of liver diseases such as Hepatocellular carcinoma; Liver cirrhosis; Chronic hepatitis; Congenital hepatic fibrosis; Glycogen storage disease; Wilson's disease; Crigler Najjar syndrome; and Tyrosinaemia.

Name of Principle Investigator: **Carol L. Keefer** Project Budget: $ **230,000.00**

Grantee: **University of Maryland, College Park**

Title: **Mechanical Phenotyping of Mouse and Human Embryonic Stem Cells using Atomic Force Microscopy**

Description of stem cell research (as submitted by Principle Investigator):

Generation of specific cell lineages during initial differentiation of stem cells in vitro remains a challenge. Common methods used to monitor cell status involve destruction of the cells (mRNA quantification), binding of antibodies (immunocytochemistry) or altering their genetic make-up (transgenic reporter systems). Furthermore, most studies assess cell populations and do not monitor individual cell responses. Consequently, we propose to develop an atomic force microscopy (AFM) based system with haptic feedback capability that can be used to mechanically characterize an individual cell. This system has the capability of measuring forces in nN range and provides a haptic display of the cell indentation forces in real time. Single indentation studies to determine the local elastic modulus of the cell membrane were performed on fixed and live mouse ESC, both undifferentiated and early differentiating (6 days after LIF removal alone or with ascorbic acid treatment). Differentiating ESC had a 2 to 4 fold higher elastic modulus (kPa) than undifferentiated ESC, indicating that they were less supple. This finding was observed for both fixed and live cells. In addition, an increase in Lamin A/C protein expression during differentiation was observed. Lamins and other components of the nuclear scaffold can affect DNA replication, chromatin modification and gene expression. As lamins are structural components, the changes in the elastic modus observed may be reflecting structural modifications within the nuclear scaffold. Therefore, using AFM to detect changes in cellular/nuclear flexibility may allow us to predict the cell's differentiation status. In ongoing studies, live-cell reporter constructs are being used to follow loss or gain of function in a human teratocarcinoma cell line (nTERA) to more clearly define the pluripotency status of the cells being measured by AFM. Two reporter constructs have been made; 1] the Nanog promoter linked to green fluorescent protein (GFP) to indicate pluripotency status, and 2] the Doublecortin (DCX) promoter linked to a red fluorescent protein to indicate early neural differentiation. The Nanog promoter-GFP construct has been transfected into the nTERA cells and has been demonstrated to be functional. The DCX promoter-dsCherry Red construct is being tested for functionality in the nTERA cells. In conjunction with the observed visual phenotype, mechanical measurements, such as cell surface stiffness, will be obtained by AFM through force curves at discrete locations on the cell surface. Patterns associated with appropriate (towards the desired phenotype) and inappropriate differentiation will be identified. This transgenic model will allow us to develop a haptics enabled AFM monitoring system that can be used to detect early changes in cell status. This system could be used to develop improved methods of targeted differentiation for therapeutic purposes and to monitor cellular responses to environmental stimuli.

Name of Principle Investigator: **Candace Kerr** Project Budget: $ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **MicroRNA analysis of pluripotent stem cells**

Description of stem cell research (as submitted by Principle Investigator):

The latest discoveries of new sources for human pluripotent stem cells from parthenogenetic eggs and by genetically-engineered adult fibroblasts has signified the importance of defining the mechanisms involved in regulating pluripotency. Before then, only three types of pluripotent stem cells were derived from human tissue, embryonic stem cells (ESCs), embryonic germ cells (EGCs) and embryonal carcinoma cells (ECCs). Regardless of the stem cell studied, only a few factors controlling the growth of these cells in an undifferentiated state have been identified. As such, defining pluripotency is a major hurdle to utilizing pluripotent stem cells for the treatment of human disease. Recent discoveries in post-transcriptional regulation have shown that most cells, including stem cells express fairly small RNAs called microRNAs (miRNAs) which are used by cells to regulate genes after transcription by silencing complementary messenger RNAs. The purpose of this study is to identify microRNAs that regulate human stem cell pluripotency and self-renewal. This will be performed through a series of comparisons of miRNA profiles among three known sources of embryonic stem cells, ESCs, EGCs and ECCs as well as the progenitor of EGCs and ECCs, primordial germ cells (PGCs). This will be performed using microRNA array analyses, a strategy similar in principle to standard microarray analyses to study gene expression but with significantly fewer targets (<500 miRNAs have been identified in human cells). Candidates will be selected based on strict criteria including miRNAs that are shared among all three pluripotent lines but are not expressed in PGCs or in lines when cultured under differentiating conditions. From this list, miRNAs will also be selected based on sequence complementarity to genes associated with pluripotency including Oct4, Sox2, and Nanog. Following validation assays for expression using quantitative RT-PCR, functional assays will be performed on candidate miRNAs in vitro. Knock-down and knock-in strategies using commercially-available, synthetic miRNAs and their inhibitors will be employed to assess their ability to alter the pluripotent state of EGCs and ESCs in culture. Their ability to influence PGC conversion to EGCs will also be studied. Together, these comparisons will identify miRNAs that regulate genes controlling pluripotency and self-renewal in stem cells and provide mechanisms for understanding pluripotent stem cell derivation and maintenance.

Name of Principle Investigator: **Andre Levchenko** Project Budget: $ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **Enhanced Migration of Neural Stem Cells and Brain Tumor Stem Cells on Nanopatterned Surfaces**

Description of stem cell research (as submitted by Principle Investigator):

Migration is an important behavior of neural stem cells (NSCs) and brain tumor stem cells (BTSCs). Understanding the migration of NSCs is critical to future therapeutic applications for treating neurodegenerative disorders, such as ischemic stroke and Parkinson's disease. Similarly, understanding this activity in BTSCs is crucial to therapeutic approaches that might impede brain tumor invasion into normal tissues. Classic techniques are suboptimal for the study of migration because they allow very limited control over the cellular environment. Specifically, standard flat surfaces present difficulties in quantifying cellular motion due to the random nature of cells' maneuvers. This presents an opportunity to utilize nanobiotechnology such as microfluidics and nanopatterned surfaces to analyze migration. We recently developed a novel directional migration assay using patterned nanogrooves. Using this platform, we previously observed significant increases in cell elongation and bi-directional movement of a variety of cells (NIH3T3 fibroblast, CHO cells, HUVEC, myocytes, etc) along the nanogrooves. In this project, we have adapted the same techniques to NSCs and BTSCs. With cellular motion primarily restricted to a single dimension, we could more easily and accurately measure cell motility parameters such as speed, spindle-shape factor, and cell area. In our initial findings, we observed enhanced migration, characterized by faster migratory speed and more frequent spindle-shapes amongst the NSCs and BTSCs. One mechanism proposed for these differences is an increase in the expression/activity of NKCC1, a cell volume regulatory protein. Future experiments will involve combining patterned surfaces with microfluidic technology to observe cell migration in the presence of gradients of slit proteins, and other signals.

Name of Principle Investigator: **David Litwack** Project Budget: $ **230,000.00**

Grantee: **University of Maryland, Baltimore**

Title: **Regulation of Neurogenesis by Nuclear Factor One Transcription Factors**

Description of stem cell research (as submitted by Principle Investigator):

During central nervous system (CNS) development, progenitor populations produce a large number of neurons possessing distinct identities, a process critical for the acquisition of proper brain function. In many cases these neurons are generated in neuroepithelial compartments that are functionally defined by the combinatorial expression of transcription factors. To determine the molecular mechanisms that regulate neurogenesis and neuronal specification, we have focused on the Nuclear Factor I (NFI) family of transcription factors, which is defined by four genes (Nfia, Nfib, Nfic, and Nfix). Members of this family have been shown to regulate the generation of subpopulations of astrocytes in several regions of CNS and the terminal differentiation of cerebellar granule neurons. We have recently demonstrated that NFI factors regulate neurogenesis during CNS development (Kumbasar et al., 2009, J. Comp. Neurol., 513:98). In particular, Nfib functions during neurogenesis in the rhombic lip, a specialized neuroepithelium that aligns the fourth ventricle in the hindbrain and gives rise to the pontine and other precerebellar nuclei. Progenitors within the rhombic lip, as well as the neurons that they produce, express high levels of NFI proteins. In mice lacking Nfib, the pontine nuclei are virtually absent as a result of a significant decrease in neurogenesis. Experiments examining cell cycle marker expression in the rhombic lip indicate that pontine progenitors are altered in Nfib knockout mice. Therefore, our results show that NFI proteins can directly regulate the ability of neural progenitors to generate specific neuronal populations. The ability to produce specific cell types in culture will be important in developing therapies to treat degenerative diseases and other disorders that affect the brain. Based on our in vivo studies, we hypothesize that NFI proteins will be critical factors in regulating neurogenesis in vitro. Therefore, we are now testing the function of these transcription factors in human embryonic stem cell (hESC)-derived neural precursor cells (NPCs). Using RT-PCR for NFI mRNAs in H9 hESCs and in ENSTEM-A cells, a NPC line derived from H9 cells, we have detected regulated NFI expression. In particular, Nfia is highly expressed in H9 cells but not NPCs, whereas little Nfix expression is observed in either population; Nfib and Nfic are constitutively expressed. Future work will extend the characterization of NFI expression to neurons and astrocytes produced from NPCs. In addition, we will determine how NFI proteins regulate neurogenesis in NPCs by overexpression and knockdown of NFI family members. The results from these experiments will be important in developing hESC-based therapies that target the CNS.

Name of Principle Investigator: **David M. Loeb** Project Budget: $ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **Identification of a Chemotherapy-Resistant Ewing's Sarcoma Stem Cell-Like Population**

Description of stem cell research (as submitted by Principle Investigator):

Ewing's sarcoma family tumors (ESFT) are the second most common bone tumors in children and young adults. Despite being quite responsive to chemotherapy, patients with localized disease have a 30% recurrence rate, and 80% of patients with metastatic disease die within 5 years of diagnosis. The expression of the EWS-FLI1 fusion protein as a result of chromosomal translocation t(11;22)(q24;q12) is crucial for initiation and maintenance of the tumor. The cancer stem cell (CSC) hypothesis provides a framework for explaining the discrepancy between response of ESFT to therapy and the poor survival rate. The CSC hypothesis proposes that most tumors originate from and are maintained by a small subset of chemotherapy-resistant stem cells. Thus, identification of Ewing's sarcoma stem cells will lead to the development of targeted therapies that should improve the treatment of patients with this disease. We attempted to identify a population of Ewing's sarcoma cells with stem cell properties (self-renewal in vitro, clonogenic activity, and tumor initiating activity in immunodeficient mice) based on high aldehyde dehydrogenase (ALDHhigh) activity. We sorted cells from the TC-71 cell line and from primary xenografts grown in immunodeficient mice based on ALDH activity. The ability of ALDHhigh and ALDHlow cells to self-renew in vitro, to form colonies in soft agar and to form tumors in immunodeficient mice was assessed. The ALDHhigh population was enriched for stem cell activity as defined by these assays. As anticipated, ALDHhigh cells were resistant to cytotoxic chemotherapy drugs (doxorubicin and etoposide – two drugs commonly used to treat Ewing's sarcoma), while ALDHlow cells were not. We are currently investigating whether inhibition of key intracellular signaling pathways will augment the sensitivity of the ALDHhigh ESSC population to chemotherapeutics. In conclusion, ESFT cell lines and xenografts contain a small proportion of cells with high level ALDH expression that exhibit stem cell properties. These cells are resistant to standard chemotherapy drugs, but combination therapy with inhibitors of intracellular signaling pathways has the potential to increase their sensitivity and may provide a way to improve the treatment of patients with Ewing's sarcoma family tumors.

Name of Principle Investigator: **Stuart S. Martin** Project Budget: $ **200,000.00**

Grantee: **University of Maryland, Baltimore**

Title: **Epithelial-to-Mesenchymal Transition Promotes Breast Tumor Stem Cell Characteristics, Tubulin Detyrosination and Microtentacles**

Description of stem cell research (as submitted by Principle Investigator):

Understanding the biology of breast tumor stem cells during passage through the bloodstream could provide new therapeutic opportunities to destroy circulating tumor cells. When detached from extracellular matrix, breast tumor cells with increased stem cell characteristics produce unique microtentacles composed of coordinated vimentin intermediate filaments and detyrosinated microtubules. These microtentacles promote the reattachment of circulating tumor cells and are associated with increased invasiveness in vitro and metastatic potential in vivo. Our previous work has shown that microtentacles are increased in Basal B breast tumor cell lines that display hallmarks of epithelial-to-mesenchymal transition (EMT) and stem cell markers (CD44+/CD24-). We report here that the direct induction of EMT by expression of the transcription factor Twist promotes increased stem cell characteristics (CD44+/CD24-) and microtentacle formation in detached human mammary epithelial cells (HMECs). Mechanistically, EMT results in an elevation of alpha-tubulin in which the c-terminal tyrosine residue has been removed (Glu-tubulin), and reorganization of such Glu-tubulin into microtentacle extensions. Conversely, siRNA-mediated downregulation of endogenous Twist in (CD44+/CD24-) breast carcinoma cells decreased Glu-tubulin levels, organization and the frequency of microtentacles. Twist siRNA decreased levels of Glu-tubulin before any changes in either vimentin or E-Cadherin expression, indicating that Twist can have a primary effect on tubulin detyrosination without requiring additional elements of the EMT program. This model is further supported by immunohistochemical staining of 66 patient tissue samples that show a high concordance between Glu-tubulin and Twist staining in invasive ductal carcinomas. In addition, tumor cells co-staining for Twist and detyrosinated tubulin are enriched at the invasive front where tumor cells encounter a changing microenvironment. Given the role of detyrosinated tubulin in cell motility and orientation, these EMT-induced microtubule alterations and stem cell induction may occur as part of an inherent program for cell movement during development or wound healing. However, this program may also prime tumor cells for metastatic success by increasing stem cell charactersitics, stabilizing microtubules, and promoting microtentacles. Functionally, expression of Twist led to an enhanced rate of tumor cell reattachment as monitored over time by electrical impedence or measured by fluorescence over an endothelial layer. Confocal microscopy captures microtentacles engaging and invading through an endothelial layer to facilitate reattachment. These data support a novel model in which the EMT that occurs during tumor invasion increases stem cell characteristics, alpha-tubulin detyrosination and promotes microtentacles which enhance the reattachment of circulating tumor cells during metastasis.

Name of Principle Investigator: **Mervyn J. Monteiro** Project Budget: $ **230,000.00**

Grantee: **University of Maryland, Biotechnology Institute**

Title: **Expression of ubiquilin in human embryonic stem cells**

Description of stem cell research (as submitted by Principle Investigator):

It is now known that several proteins are required to either maintain or reprogram cells into a pluripotent stem cell fate. Interestingly, these studies have revealed that the levels to which many of these and other proteins accumulate in cells is strictly regulated. However, little is known about the molecular mechanisms that modulate protein accumulation in stem cells. One protein that we believe might function in this capacity is ubiquilin. Indeed studies from both other and our laboratories have shown that alteration of ubiquilin expression in cells can affect protein turnover via the ubiquitin-proteasome system. Ubiquilin proteins are characterized by an N-terminal ubiquitin-like domain (UBL), a central more variable domain, and a C-terminal ubiquitin-associated domain (UBA). The UBA domain is known to bind polyubiquitin chains whereas the UBL domain binds the proteasome. These features have led us to propose that ubiquilin functions as a shuttle factor to deliver proteins that are tagged with ubiquitin chains to the proteasome for degradation. We are therefore interested in characterizing expression of ubiquilin genes in stems cells during proliferation and differentiation. We are using real-time PCR to characterize the expression of each of the four different ubiquilin genes found in human embryonic stem cells. In a companion effort we are also quantifying the levels of expression of ubiquilin proteins in the cells. We will report on our results of the expression of the genes during proliferation and differentiation of human H9 stem cells.

Name of Principle Investigator: **Sarah Netzel-Arnett** Project Budget: $ **230,000.00**

Grantee: **University of Maryland, Baltimore**

Title: **Targeting Prosurvival Pathways for Enhanced Stem Cell Based Therapy**

Description of stem cell research (as submitted by Principle Investigator):

Despite substantial advances in the management of ischemic heart disease, it still remains a leading cause of death and morbidity in the United States. Stem cell-based therapies offer an attractive treatment strategy for ischemic heart disease. Both embryonic (ES) and adult stem cells possess the ability to repair or regenerate damaged or injured tissues. Recent human clinical trials have demonstrated the feasibility of adult stem cells, such as skeletal myoblasts, circulating progenitor cells (CPC), and multipotent mesenchymal stromal cells (MSCs) for patients with heart failure. However, current cardiac stem cell therapies still result in low and inconsistent therapeutic efficacy. The goal of the proposed study is to develop a novel strategy to improve post-transplantation survival of stem cells. This strategy is based on repression of cell death signaling pathways by protective intracellular proteins known as Serpins. Recently we identified SerpinB2 as a host survival factor that blocks caspase activation which in turn leads to protection of cells from stress-induced death. Specifically, model cells pre-loaded with SerpinB2 were protected from TNF-induced apoptosis. Others have found that SerpinB3 and SerpinB9 regulate cell death pathways and promote cell survival. We are currently investigating the inhibition of intracellular proteolytic death pathways by Serpins as a major host defense mechanism that can be exploited to rescue donor stem cells from cell death in ischemic and inflammatory environments.

Name of Principle Investigator: **Martin G. Pomper** Project Budget: $ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **Identification of Inhibitors of ABCG2 by a Bioluminescence Imaging-based High-Throughput Assay**

Description of stem cell research (as submitted by Principle Investigator):

As a member of the ATP-binding cassette (ABC) family of transporters, ABCG2 is a known stem cell marker, and its overexpression has been associated with tumor resistance to chemotherapeutic agents. Most cancer patients demonstrate a clinical response to drug treatment and can even achieve a complete remission, but relapse is the rule and promotes the development of MDR. Increasing evidence supports the idea of cancer stem cells as the seeds from which many cancers arise and persist through chemotherapy, and one mechanism by which stem cells become more chemoresistant and can persist through chemotherapy is by overexpression of ABC transporters, particularly ABCG2. Combining ABCG2 inhibitor(s) can potentially improve treatment outcome of chemotherapy, yet no clinically useful ABCG2 inhibitors are available. We developed a bioluminescence imaging (BLI)-based high-throughput assay to screen for ABCG2 inhibitors. This assay exploits our finding that D-luciferin, the substrate of firefly luciferase (fLuc), is a specific substrate of ABCG2, and ABCG2 inhibitors enhance bioluminescence signal by blocking the efflux of D-luciferin and increasing its intracellular concentrations. The Hopkins Drug Library, a library that includes drugs approved by the US Food and Drug Administration (FDA) as well as drug candidates that have entered phase II clinical trials, was screened using HEK293 cells engineered to express ABCG2 and fLuc. Forty seven compounds demonstrated BLI enhancement of five-fold or greater, and ten of them were known ABCG2 inhibitors. For the previously unknown ABCG2 inhibitors, twenty-eight were tested using established in vitro assays (e.g. mitoxantrone resensitization and BODIPY-prazosin assays) and twenty-six were confirmed. Glafenine, a potent new inhibitor, also inhibited ABCG2 activity in vivo. The BLI-based assay is therefore an efficient method to identify new inhibitors of ABCG2. Since the ABCG2 inhibitors uncovered from our screen were derived from an FDA-approved compound library, many of them are ready for clinical testing. Efforts are under way to determine if combining any of these novel ABCG2 inhibitors with chemotherapeutic can potentiate the chemotherapeutic effect and survival of an experimental model of acute myeloid leukemia (AML), a stem cell disease. References: 1. Zhang Y, Bressler JP, Neal J, et al. ABCG2/BCRP expression modulates D-Luciferin based bioluminescence imaging. Cancer research 2007;67(19):9389-97. 2. Zhang Y, Yunzhao R. Ren, Jun O. Liu, John Laterra, and Martin G. Pomper. Identification of Inhibitors of ABCG2 by A Bioluminescence Imaging-based High-throughput Assay. Cancer research 2009. In press.

Name of Principle Investigator: **Yun Qiu** Project Budget: $ **230,000.00**

Grantee: **University of Maryland, Baltimore**

Title: **Regulation of Stemness by Phosphorylation**

Description of stem cell research (as submitted by Principle Investigator):

OCT4 is a key transcriptional factor required for maintenance of the pluripotency and self-renewal of embryonic stem cells. Here, we reported that OCT4 is phosphorylated by a number of protein kinases at Serine and Threonine residues. Mutation of the identified putative phosphorylation sites compromised phosphorylation of OCT4 and altered OCT4 transcriptional activity in the reporter assays. We have also identified the kinase that may be involved in phosphorylating OCT4. The effects of OCT4 phosphorylation on regulation of its target genes will be studied by microarray analysis and its contribution to stemness maintenance in ES cells will also be studied. Our study will provide new insights into the basic mechanisms underlying stem cell maintenance and self-renewal. This will allow us to better understand how to steer human stem cells to proliferate and differentiate in response to specific stimuli, and prevent undesired side effects that are potentially associated with stem cell based therapies.

Name of Principle Investigator: **Alfredo Quinones-Hinojosa** Project Budget: $ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **MIGRATION OF HUMAN BRAIN TUMOR S TEM CELLS IN RESPONSE TO SLIT PROTEINS**

Description of stem cell research (as submitted by Principle Investigator):

PROBLEM, Glioblastoma multiforme (GBM) is the most common and devastating intracranial malignant tumor. Diffuse infiltration of cells into the brain parenchyma makes complete surgical resection impossible, leading to 99% recurrence. BTSCs, like their non-cancerous counterparts, have self-renewal and differentiation properties with the added ability to initiate tumor formation. Increasing evidence suggests that Brain Tumor Stem Cells (BTSCs) are specifically responsible for brain tumor invasion. The mechanisms responsible for BTSC migration are unknown. BACKGROUND, Slit and Robo proteins function as a chemorepellant system. This system exhibits aberrant methylation in various cancers, including gliomas. In vitro experiments have shown that Slit-2 specifically affects the migration of some medulloblastoma and glioma cell lines. We HYPOTHESIZE that Slit proteins will have a chemorepellant effect on GBM-derived BTSC migration. Methods, We studied the effect of the Slit/Robo system on the migration of human GBM-derived BTSCs in vitro and ex vivo using BTSCs cultured from intraoperative tissue samples of human GBMs. The expression of Slit and Robo genes was evaluated by RT-PCR and Western Blot in response to a de-methylating agent (5-Aza). The effect of Slits on BTSC migration was evaluated in transwell migration and Matrigel® assays. Purified Slit-2 protein was obtained by dialysis of culture media conditioned by transfected Hek293 cells. Robo-N, a decoy receptor that acts as a specific inhibitor for the Slit-Robo interaction was used as control. Migration of BTSC in human brain organotypic cultures was tracked with GFP. In vivo rodent tumor models are being used to determine the role of Slit proteins in tumor formation and invasion. RESULTS, three BTSC lines have been obtained from intraoperative tissue. These cells form neurospheres, differentiate into mature neural cells and form tumors in vivo. Our characterized BTSCs exhibit a basal expression of Slits and Robo proteins. The expression of Slit mRNA increases after the cells are treated with 5-Aza ($p<0.05$). Slit-2 showed a chemorepellant effect on BTSC migration ($p<0.05$). We are currently examining organotypic cultures and in vivo tumor models with co-injection of BTSC and Slit-2-releasing cells. CONCLUSIONS, our results show that human GBM-derived BTSCs show a basal level of Slit protein expression that increases in response to a de-methylating agent. Slit proteins exert a chemorepellant effect on the migration of human BTSCs in vitro. Ongoing ex vivo and in vivo experiments will provide us with more information on the effect of Slit/Robo have on brain tumor invasion.

Name of Principle Investigator: **Venu Raman** Project Budget: $ **229,609.00**

Grantee: **Johns Hopkins University**

Title: **TWIST Modulates the Breast Cancer Stem Cell Phenotype**

Description of stem cell research (as submitted by Principle Investigator):

The cancer stem cell paradigm postulates that dysregulated tissue specific stem cells or progenitor cells are the precursors for cancer biogenesis. Consequently, identifying cancer stem cells is crucial to our understanding of cancer progression and for the development of novel therapeutic agents. In this study, we demonstrate that the over-expression of Twist in breast cells can promote the generation of a breast cancer stem cell phenotype characterized by the high expression of CD44, little or no expression of CD24, and increased ALDH activity, independent of the epithelial mesenchymal transition. In addition, Twist over-expressing cells exhibit high efflux of Hoechst 33342 and Rhodamine 123 as a result of increased expression of ABCC1 (MRP1) transporters, a property of cancer stem cells. Moreover, we show that transient expression of Twist in multiple breast cell lines can induce the stem cell phenotype and that decreasing Twist expression by shRNA in Twist over-expressing transgenic cell lines MCF 10A/Twist and MCF-7/Twist as well as in MDA-MB-231 partially reverses the stem cell molecular signature. Importantly, we show that inoculums of only twenty cells of the Twist over-expressing CD44+/CD24-/low sub-population are capable of forming tumors in the mammary fat pad of SCID mice. Finally, with respect to mechanism, we provide data to indicate that Twist transcriptionally regulates CD24 expression in breast cancer cells. Taken together, our data demonstrates the direct involvement of Twist in generating a breast cancer stem cell phenotype via down-regulation of CD24 expression and independent of an epithelial mesenchymal transition.

Name of Principle Investigator: **Linda Resar** Project Budget: $ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **HMGA1 in Adult Refractory Leukemia and Human Leukemic Stem Cells: A Potential Therapeutic Target Down-Regulated by Flavopiridol**

Description of stem cell research (as submitted by Principle Investigator):

Problem: Emerging evidence indicates that refractory leukemia is initiated and maintained by stem-like cells (leukemic stem cells or LSCs) that are resistant to therapy. Thus, targeting LSCs is needed for successful treatment. Background: Previous work from the Resar laboratory indicates that the HMGA1 oncogene drives leukemic transformation in cultured hematopoietic cells and transgenic mice. Our preliminary results also indicate that HMGA1 correlates with relapse in some forms of leukemia. Recent findings also implicate HMGA1 in the stem cell phenotype. First, we showed that HMGA1 is enriched in human embryonic stem cells and expression falls with differentiation. Second, HMGA1a transgenic mice develop aggressive lymphoid malignancy, and the tumors cells can be transplanted in serial transplant experiments, indicating that at least some leukemic cells have long-term self-renewal like stem cells. Hypothesis: We hypothesize that HMGA1 induces refractory leukemia by activating transcriptional networks that enforce the stem cell phenotype and a therapy that blocks HMGA1 expression will also target LSCs and lead to remissions. Flavopiridol (FP) is a prototypical cyclin dependent kinase (cdk) inhibitor that induces long-term remissions in many patients, suggesting that it targets LSCs. Research: In refractory or high-risk leukemia patients, we assessed (1) the HMGA1 mRNA in leukemic stem cells compared to bulk tumor cells (2) the molecular signature, before and after treatment with flavopiridol, in leukemic blasts from refractory patients to determine if FP targets HMGA1 and LSCs. (3) the clinical outcome of flavopiridol therapy. Observations: In our preliminary pilot study, we found that HMGA1 is enriched in the leukemic stem cells in a subset of patients. In our experimental model of hematopoietic differentiation from human embryonic stem cells, high expression of HMGA1 was detected in the hematopoietic stem cells and the expression falls as the cells differentiate into mature blood cells from all lineages. Thus far, we found that FP down-regulates HMGA1 in primary leukemic blasts from most patients (11/12 or 92%). We previously discovered that STAT3 is a transcriptional target of HMGA1 in hematopoietic malignancy. Moreover, STAT3 is an important hematopoietic stem cell survival factor. We found that STAT3 is down-regulated in the leukemic blasts from most patients (10/12 or 83%) following FP treatment. Of these refractory, very high-risk patients treated with FP-based therapy, five achieved complete remission (CR) and the remaining seven did not respond (NR). Studies are underway to determine if down-regulation of HMGA1 or STAT3 correlate with pharmacologic data or clinical responses. Conclusions: Although further study is needed, our preliminary results indicate that HMGA1 could drive the stem cell phenotype in refractory leukemia. Moreover, our findings suggest that FP targets HMGA1 and could be a useful therapy for refractory leukemia and LSCs.

Name of Principle Investigator: **Charles M. Rudin** Project Budget: $ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **The Roles of Notch Signaling in the Development of Non-small Cell Lung Cancer**

Description of stem cell research (as submitted by Principle Investigator):

Lung cancer is the most common cause of cancer related deaths in the United States, accounting for 29% of all cancer deaths. Approximately 85% of all lung cancers are of non-small cell histology. Although current therapies have marginally improved survival and quality of life for patients with Non-Small Cell Lung Carcinoma (NSCLC), this remains a nearly universally fatal disease. New approaches to drug development and further studies of the biology of this disease are critically needed. We hypothesize that high level Notch signaling defines a subpopulation of NSCLC cells with markedly elevated clonogenic potential, and that inhibition of Notch signaling can inhibit NSCLC tumorigenesis. To test these hypotheses and to define the potential utility of Notch inhibitors in NSCLC, we proposed 4 specific aims: (1) To evaluate temporal and spatial activation of Notch signaling in a murine model of NSCLC tumorigenesis; (2) To assess the requirement of Notch signaling in murine NSCLC tumorigenesis through use of a dominant negative transgenic model; (3) To assess whether Notch activation correlates with clonogenic potential in human NSCLC; (4) To analyze whether human NSCLC clonogenic and tumorigenic potential is affected by a selective Notch inhibitor. Down regulation of Notch signaling using a selective γ-secretase inhibitor (Mrk 003; Merck) was evaluated in two NSCLC cell lines (NCI-H1299, and -H1993). Cells were serum starved for 24hrs and incubated for 72 hours with Mrk 003 at concentrations ranging from 0.1uM to 3.0μM. After 72 hours, a 60% suppression of Hes 1 expression was observed. We have also begun preliminary experiments with a double transgenic model, LSL-K-rasG12D X DN-MAML (dominant negative mastermind) mice. Expression of the DN-MAML gene results in inhibition of the Notch pathway. We successfully administered adenoviral CRE intranasally to these mice and have generated intraparenchymal lung tumors in both KRAS and KRAS/DN-MAML mice. These results do not represent what we originally hypothesized, however it has led us to believe that mastermind-1 may play an unknown role in other pathways (i.e. p53). We are currently in the process of characterizing these tumors both qualitatively and quantitatively. Characterization of this stem-like cell subpopulation in NSCLC, and definition of the roles of Notch signaling in promoting tumorigenesis, may aid in the development of more effective therapeutics. Traditional chemotherapeutics agents may be used to debulk the larger mass of tumor cells in combination with drugs, possibly including γ–secretase inhibitors, which will target and deplete the cancer stem cell population. Such combination strategies should promote both tumor regression and reduce to probability of recurrent disease due to survival, proliferation, and differentiation of tumor progenitor population.

Name of Principle Investigator: **Nitish Thakor** Project Budget: $ **229,190.00**

Grantee: **Johns Hopkins University**

Title: **The Formation of Neuromuscular Junction in the Novel Compartmentalized Platform**

Description of stem cell research (as submitted by Principle Investigator):

The objectives of this research to culture and differentiate well-defined populations of motor neurons derived from embryonic stem cells and myoblasts to form neuromuscular junction in a novel compartmentalized neuron culture platform. Problem: In order to study the local event of the formation of neuromuscular junction between embryonic stem cell derived motor neuron and muscle cells, subcellular part of neurons should be fluidically isolated. Background: Traditionally, compartmentalized \'Campenot' chambers and other chambers have been used to study the neuroscience in vitro. However, these chambers were cumbersome to fabricate and reproduce the chambers. Research: Using microfabrication method and soft lithography, a novel compartmentalized platform was developed to study the formation of neuromuscular junction. The platform has two compartments to mechanistically study the cellular and molecular events leading to formation of neuromuscular junctions between embryonic stem cell derived motor neurons and myoblasts by neurotrophic factors. In order to improve the buffering of media, cell viability, and cell loading in the platform, most of compartments were open to air by removing the top polydimethyl siloxane (PDMS). Observation: The myoblasts were differentiated into myotubes successfully in the compartmentalized platform. Axons of motor neurons extended to the myoblast compartment through microchannels. Conclusion: We have developed the novel compartmentalized platform to culture embryonic stem cell derived motor neuron and myoblasts in a fluidically isolated manner. The novel platform we developed will be very useful to study the event of the formation of neuromuscular junction in a mechanistic manner.

Name of Principle Investigator: **Leslie Tung** Project Budget: $ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **Human Embryonic Stem Cell Derived Cardiomyocytes Ameliorate Vulnerability to Arrhythmias in an In Vitro Model of Cardiac Fibrosis**

Description of stem cell research (as submitted by Principle Investigator):

Problem: In this study, we characterize the therapeutic benefits of human embryonic stem cell-derived cardiomyocytes (hESC-CMs) in an in vitro model of cardiac fibrosis. Background: Cardiac fibrosis is a pathological condition found in aging, heart failure, and myocardial infarction. Fibrosis reduces the electrical coupling between cardiomyocytes via fibroblast-deposited collagenous septa that electrically separate cardiac cells and greatly diminishes the speed of action potential propagation. Hypothesis: We hypothesize that hESC-CMs can improve abnormal conduction in a model of cardiac fibrosis. Research: Human embryonic stem cells (hESCs) were differentiated into beating embryoid bodies (EBs) using established protocols. Beating or non-beating EBs were dissected and dissociated into single cell suspensions at 28 or 15 days, respectively, and 100,000 hESCs were added to 21 mm diameter fibrotic monolayers consisting of neonatal rat ventricular myocytes (NRVMs) treated with transforming growth factor-beta. Three days later, the co-cultures were analyzed for hESC-CM survival and engraftment, functional coupling, and electrophysiology using optical mapping. Observations: Increased collagen I in fibrotic monolayers was confirmed by picrosirius red stain (n=15) and collagen type I immunostaining (n=15). Longitudinal (LCV) and transverse conduction velocity (TCV) decreased in fibrotic monolayers (14.3±0.8 (SEM) and 4.2±0.3 cm/s, respectively; n=21) compared with control monolayers (25.4±0.9 and 8.2±0.3 cm/s, respectively; n=21). Immunostaining with human mitochondrial protein, cardiac troponin T, and connexin43 showed hESC-CM survival, engraftment and heterocellular gap junctions. hESC-CM pre-treatment with DiI and calcein-AM confirmed functional coupling between hESC-CMs and NRVMs. LCV and TCV of fibrotic monolayers were augmented to 39.3±2.7 and 12.5±1.3 cm/s, respectively (n=6), following engraftment of hESC-CMs from beating EBs, but in contrast, were suppressed to 6.5±1.3 and 2.1±1 cm/s, respectively (n=4), with addition of hESCs from non-beating EBs. Spiral wave vulnerability decreased from 70% to 50%, and cycle length to initiate spiral waves decreased from 303±38 ms to 183±13ms (fibrotic, n=10 vs. hESC-CM treated monolayers, n=6, respectively). Conclusions: hESC-CMs reverse the loss of conduction velocity and reduce the incidence of spiral waves in this fibrosis model. This finding is significant in that it demonstrates the possibility that hESC-CMs participate in electrical propagation through gap junction coupling and can ameliorate abnormal conduction in our fibrotic monolayers. Future research includes silencing hESC-CM connexin43 expression before addition to the fibrotic monolayer to provide direct evidence for gap junction mediated improvement in electrical propagation.

Name of Principle Investigator: **Piotr Walczak** Project Budget: $ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **Non-Invasive Imaging and Evaluation of Glial Differentiation and Engraftment of Skin-Derived Pluripotent Stem Cells**

Description of stem cell research (as submitted by Principle Investigator):

Stem cell therapy for brain diseases is currently considered very promising, as functional improvement has been observed in both animal disease models and patients. With significant progress being made in developing stem cell therapies, it is important to focus on the key features important for treatment of a large population of patients. First, the cells must be accessible in large quantities, sufficient for therapy for all patients affected by the disease. Second, the cells must be identical immunologically to the cells of the individual patient otherwise the cells are eliminated by an immune response, which is only hindered by toxic treatments that are directed at protection of the transplanted cells by suppressing an immune response. Presently scientists are pursuing methods that generate embryonic stem cells from normal adult human cells and several laboratories have reported a great deal of success in doing so. If the properties of these induced stem cells are truly the same as those of the embryonic stem cells, it would not only allow help to resolve the ethical controversy associated with obtaining human embryonic stem cells, but it could also provide an unlimited source of stem cells without the need for immunosuppression.

In this project, we will use in vivo cellular imaging to evaluate whether properties of induced stem cells are similar to those of human embryonic stem cells. In addition, we will evaluate their therapeutic potential in a mouse myelin disease model.

Name of Principle Investigator: **Katherine Whartenby** Project Budget: $ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **Gene modification of CD34+ cells for transplant to induce tumor immunity**

Description of stem cell research (as submitted by Principle Investigator):

We have been investigating the utility of gene modification of various forms of stem cells to engineer the development of immune cells, with an ultimate translational goal of developing a novel tumor immunotherapy. Specifically, we have been developing a system in which gene-modified hematopoietic stem cells (HSC) for blood or marrow transplant (BMT) are genetically modified to express a tumor antigen (Ag). Following hematopoietic recovery from transplantation, systemic administration of Flt3 ligand (FLT3L) is used to differentiate the transduced HSCs into DCs such that a large percentage of DCs in the body express the tumor Ag in the secondary lymphoid organs, which is critical for an effective anti-tumor response. These DCs potently expand adoptively transferred tumor-specific T cells, leading to the cure of a significant percentage of tumor-bearing mice. In our previous studies, adoptively transferred CD8+ but not CD4+ T cells were required for cure, suggesting that exogenous CD4+ T cell help was not required for the initial clonal expansion of tumor-specific, CD8+ cytotoxic T cells. We have been conducting studies geared towards optimizing this approach for clinical translation by developing this system in human stem cells and extrapolating our findings to the human system while further evaluating the mechanism of action of the anti- tumor effect.

Name of Principle Investigator: **Huakun Xu** Project Budget: $ **200,000.00**

Grantee: **University of Maryland, Baltimore**

Title: **Human Mesenchymal and Umbilical Cord Stem Cell Delivery via Calcium Phosphate Scaffolds for Bone Repair**

Description of stem cell research (as submitted by Principle Investigator):

Due to its moldability and excellent osteoconductivity, calcium phosphate cement (CPC) is highly promising for craniofacial and orthopedic applications. However, the CPC setting reaction may be cytotoxic to human mesenchymal stem cells (hMSCs). The aim of the present study was to encapsulate hMSCs in alginate hydrogels and combine them with CPC, CPC-chitosan and CPC-chitosan-fiber scaffolds. Cell viability and osteogenic differentiation were measured. At 1, 7, 14, and 21 days, cell viability was quantified via live/dead and a Wst-1 assay. Cell viability at 1, 7, and 21 d was similar between FDA-approved CPC-control and reinforced CPC-chitosan and CPC-chitosan-fibers, while only CPC-chitosan-fiber exhibited a significant decrease in viability between 1 and 21 d according to Wst-1. ALP activity for all materials reached a maximum at 14 d, decreasing to 7 d levels at 21 d. Mineral staining with xylenol orange, SEM microscopy and powder x-ray diffraction all confirmed the presence of poorly-crystalline hydroxyapatite-like mineral deposited by the cells. On the basis of these results, it is concluded that encapsulation of hMSCs in alginate hydrogel protect the cells from the CPC setting reaction and allow them to undergo osteogenic differentiation.

Name of Principle Investigator: **Paul Yarowsky** Project Budget: $ **230,000.00**

Grantee: **University of Maryland, Baltimore**

Title: **Targeted Migration of Magnetically Labeled Stem Cells as a Therapeutic Delivery Method for Neurodegenerative Diseases**

Description of stem cell research (as submitted by Principle Investigator):

Problem: Stem cell therapy in the nervous system is considered among the most promising strategies for the treatment of neurodegenerative disorders. The feasibility of this strategy depends upon the extent to which transplanted neural stem cells will survive, differentiate, and migrate to their appropriate positions and act as biological pumps for delivering vital growth factors or proteins that are lost in these disorders. In distributed neurodegenerative disease such Alzheimer's disease (AD), damage occurs in cortical and subcortical areas, so local injection of stem cells is highly invasive. Background: In this project, we characterized the migration of human neural progenitor cells (hNPC) cells before and after Feridex labeling in the presence of a magnetic field. Feridex (generic name ferumoxides injectable solution) is a sterile aqueous colloid of superparamagnetic iron oxide associated with dextran. It is an FDA approved MRI contrast agent. It has been known that that the external magnets influence the movement of Feridex labeled stem cells in the circulation such that the cells were retained in the region of interest (Arbab et al., Hum Gene Ther 15:351-60). Hypothesis: We will determine in control rats whether transplanted Feridex-labeled hNPCs can be localized to the contralateral cortex and hippocampus by injection into the ipsilateral lateral ventricle by the presence of an external magnetic field. Research: We characterized the migration of hNPC cells in vitro before and after Feridex labeling in the presence of a magnetic field. Observations: Feridex is introduced into hNPC cells by incubating cells with Feridex/poly-l-lysine (FE-PLL). Incubation of hNPC with FE-PLL for 2 or 3 days gives the highest labeling rate, 85 ± 1.8% cells (n = 4 experiments). Further analysis of FE-PLL labeled hNPC cells with propidium iodine (to label cell death) and Hoechst dye (to label live cells) showed that viability of Feridex labeled hNPCs is 92.3 ± 1.0%. Next we showed that hNPC cells labeled with FE-PLL solution for 2 days and then dissociated with Accutase, for 10 min respond to magnetic fields. hNPC cells were plated onto 6-well plates and a disc magnet (K&J Magnetics) was placed beneath the well. Observations: We found that at 2 days post-plating and thereafter, Feridex-labeled NPC cells accumulated right above the magnet disc, forming compact "cell ball." These "cell balls" displayed intense Perl's staining. Currently, we are preparing animal (rat) experiments for unilateral injection of Feridex labeled hNPCs into the lateral ventricle and testing the influence of external magnets on their migration in the brain. Conclusions: These observations support the hypothesis that the introduction of a local magnetic field enhances directed stem cell migration.Supported by Maryland Stem Cell Research Fund.

Name of Principle Investigator: **Srinivasan Yegnasubramanian** Project Budget: $ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **Genome-Wide Characterization of Gene Expression Patterns Distinguishing Human Normal Hematopoietic Stem Cells, Chronic Myelogenous Leukemia (CML) Stem Cells, and Their Differentiated Counterparts**

Description of stem cell research (as submitted by Principle Investigator):

Background and Problem: CML stem cells are a small fraction of the cancer cells in patients with CML, but these cells are capable of giving rise to the entire tumor burden in patients with CML. Current therapies, while capable of effectively killing the bulk of the CML cells, inadequately eliminate the CML stem cells, and consequently, these cells can cause recurrence of the CML after withdrawal of therapy. Understanding the gene expression patterns that differentiate normal hematopoietic and CML stem cells and progeny can lead to identification of targets for identification and elimination of the CML stem cells. Hypothesis: We hypothesize that the identification of gene expression signatures that can distinguish CML stem cells from normal stem cells and from their differentiated counterparts will yield targets for identification and elimination of the CML stem cells. Research: We collected bone marrow samples from 5 individuals with chronic phase CML and 5 normal marrow donors, and collected DNA and RNA from the following four categories of stem cells and their progeny from these marrow samples, obtained by FACS enrichment: (i) highly enriched normal HSC (CD34+/CD38-/ALDHhigh) from normal marrow donors; (ii) more differentiated progeny of normal HSC, isolatable as (CD34+/CD38+) cells; (iii) highly enriched CML cancer stem cells from CML patients (CD34+/CD38-/ALDHhigh, with Philadelphia chromosome); and (iv) more differentiated progeny of CML cancer stem cells identifiable as (CD34+/CD38+, with Philadelphia chromosome). These total RNA samples (20 total) were then processed and hybridized to Affymetrix Human Exon 1.0 tiling arrays, which tile oligonucleotide probes across all known exons in the human genome, allowing assessment of both differential gene expression as well as differential exon usage and alternative splicing. Observations: Principal components analysis of gene expression/exon usage data showed that normal hematopoietic stem cells formed a relatively tight cluster significantly separated from their differentiated counterparts. CML stem cells were more closely related to normal hematopoietic stem cells than to the differentiated cells. Interestingly, unsupervised hierarchical clustering largely distinguished each of the four groups described above, and combined with one way and two way ANOVA models, allowed identification of several genes that distinguished CML stem cells from normal hematopoietic stem cells, and from their differentiated progeny. Next steps include verification of top hits identified in the microarray data by real time RT-PCR. Then, we can test the utility of these genes for identification and targeting of CML stem cells. Conclusions: We have identified novel gene expression signatures that distinguish CML stem cells from normal hematopoietic stem cells and from their differentiated counterparts. These genes may serve as novel targets for identification and/or elimination of CML stem cells.

Name of Principle Investigator: **Steven Zhan** Project Budget: $ **230,000.00**

Grantee: **University of Maryland, Baltimore**

Title: **Cortactin is Implicated in the Asymmetric Division of Oocytes**

Description of stem cell research (as submitted by Principle Investigator):

Problem: Clinical application of stem cells requires ultimately directional differentiation of stem cells that can be conducted in a controllable manner. However, there is little known about the mechanics for the establishment of the stem cell polarity and subsequent asymmetric division. Background: One of the best characterized cell polarities is the meiotic division of oocytes that generates two minor cells or polar bodies. Previous studies have demonstrated that the earliest polarity of oocytes is achieved by the formation of an apical layer of actin cytoskeleton. In mammalian cells, the dynamics of the actin cytoskeleton involves cortactin, an intracellular protein that is enriched in the cell cortex and subjected to regulation by various signaling pathways, in particular that involved in the Src tyrosine kinase. Hypothesis: We hypothesize that the cortactin mediated actin dynamics is implicated in the cellular polarity of oocytes and asymmetric division and differentiation of human stem cells. Research: We analyzed the distribution of cortactin in oocytes arrested at metaphase II, and microinjected two cortactin antagonists into oocytes. We also analyzed the distribution of cortactin in MCF-10A, a human mammary stem cell line, and effect of cortactin knockdown on the differentiation of the mammary stem cells. Observations: Cortactin forms a polarized cap in oocytes and is co-localized with the actin cap, the structure that specifies the position for the asymmetric division. Injection of cortactin antagonists diminished the extrusion of the second polar bodies of oocytes. We also found that cortactin is enriched in the junction of human mammary stem cells MCF-10A. A preliminary has found that suppression of cortactin impaired the formation of acini-like structures under a three-dimensional matrix culture condition. Conclusions: Our data suggests an important role of cortactin in the establishment of embryo polarity and asymmetric division. Thus, cortactin may represent a mechanic pathway that can be used for stem cell manipulation to achieve directional differentiation.

FY 2008

Post Doctoral Fellowship

Awarded

Research Descriptions

Name of Principle Investigator: **Jonathan Alder** Project Budget: **$ 110,000.00**

Grantee: **Johns Hopkins University**

Title: **Genetic Rescue of Short Telomere Defect in Hematopoietic Stem Cells**

Abstract of stem cell research (as submitted by Principle Investigator):

Telomerase is critical for stem cell function. Telomerase is composed of two components; telomerase reverse transcriptase (TERT), the catalytic component, and telomerase RNA (TR), which provides a template for telomere addition. Mutations in either component of telomerase lead to a stem cell failure disorder, dyskeratosis congenita. In dyskeratosis congenita and related disorders, the stem cell failure leads to premature mortality from aplastic anemia. Mice with short telomeres also develop fatal bone marrow failure similar to humans. To test whether telomerase replacement therapy can rescue the stem cell defect in hematopoietic stem cells, we cloned the essential telomerase components into lentiviral vectors. In murine cells deficient for telomerase components, transduction with mTR and mTert containing lentiviruses restored telomerase activity. Importantly, when both components of telomerase were exogenously expressed, telomere lengths increased dramatically. These studies set the stage for in vivo experiments that will test whether telomere elongation can rescue the hematopoietic stem cell failure in mice and in human cells with the dyskeratosis congenita defect.

Name of Principle Investigator: **Michael Bonaguidi** Project Budget: $ **110,000.00**

Grantee: **Johns Hopkins University**

Title: **Isolation of Adult Human Neural Stem Cells From Temporal Lobe Biopsies**

Abstract of stem cell research (as submitted by Principle Investigator):

Neural stem cells (NSCs) are present throughout life and continuously give rise to new neurons and glial cells in the mammalian central nervous system. Despite the growing understanding of factors regulating adult murine neurogenesis, little is currently known about the mechanisms underlying human neurogenesis. This is due primarily due to technical limitations in working with human adult neural tissue. We show here that tissue removed from the medial temporal lobe region, during surgery to alleviate intractable epilepsy, acts as a source of human adult hippocampal and SVZ stem cells (hANSCs). We have been collecting fresh surgical tissues of human hippocampus and developed protocols to isolate and establish NSC lines. These hANSCs are multipotent, capable of giving rise to neurons and glia cells, and can be genetically modified using retrovirus-based strategies. However, hANSC lines remain limited in their capacity for self-renewal and can only be passaged 4 or 5 times. We are currently modifying the extrinsic environment of the hANSCs to optimize their self-renewal based on our studies with adult mouse hippocampal NSC. This system should allow for adequate cell numbers to study human adult neurogenesis and facilitate hANSC use toward pharmacological and clinical applications.

Name of Principle Investigator: **Selen Muratoglu-Cantania** Project Budget: **$ 110,000.00**

Grantee: **University of Maryland, Baltimore**

Title: **Regulation of Mesenchymal Stem Cell Differntiation by Wnt Signaling: The Role for LRP-1**

Abstract of stem cell research (as submitted by Principle Investigator):

The central role of Wnt during development has been well established. More recently Wnts have been implicated to act in cell fate determination of mesenchymal stem cells (MSCs), however, currently the mechanism of this regulation is unknown. Multipotency of MSCs strongly indicates multiple pathways dictating cell fate decisions in a context dependent manner. Low density lipoprotein receptor related protein 1 (LRP1) is a multifunctional surface receptor that binds and endocytoses a variety of structurally and functionally distinct ligands. In addition to its endocytic function, LRP1 has been increasingly identified as playing a role in cell growth regulation through signal transduction. Our preliminary results demonstrated the involvement of LRP1 with Wnt/b-catenin signaling. In initial experiments, we measured the levels of LRP1 expression in MSCs and showed the high abundance of expression both in mouse and human bone marrow (BM) MSCs by immunoblotting and immunofluorescence. Based on its structural similarity with LRP5/6, we addressed the question whether LRP1 binds directly to Wnt ligands. We tested the binding of Wnt3a and Wnt5a to LRP1 employing surface plasmon resonance measurements. Our data showed that there is no direct interaction between these Wnt ligands and LRP1. We next used shRNA gene silencing to study a potential role of LRP1 in Wnt signaling during cell fate commitment. Our current data revealed that shRNA silencing of LRP1 in human BMMSCs resulted in decreased accumulation of β-catenin, attenuating Wnt signaling. LRP1 -/- and non-target shRNA transduced BMMSCs were induced with Wnt3a and analyzed with a quantitative RT-PCR array including Wnt signaling target genes, as well as the genes involved in Wnt signal transduction. Thus far, our experiments suggest that elevated levels of protein phosphatase 2a may be responsible for repression of Wnt signaling in LRP1 -/- cells. We are in the process of reproducing this experiment. Finally, we addressed cell fate choice preferences of BMMSCs by ablating LRP1 function with a receptor agonist. BMMSCs were initiated for adipocyte differentiation in the presence or absence of the receptor agonist RAP. We detected less lipid accumulation in the presence of RAP. This exciting result may be due to blockade of LRP1's contribution to the Wnt signaling, or alternatively, may result from a loss of lipid uptake since LRP does function as a lipoprotein receptor. To differentiate between these two possible mechanisms, we will be repeating the adipogenic differentiation in LRP1 silenced cells. Overall, our results suggest that LRP1 is capable of modulating the Wnt signaling pathway, and is important for adipocyte differentiation of MSC. It will be important to identify specific mechanisms of how this occurs to understand the role of LRP1 in modulate MSC fate and choices between self-renewal or lineage commitment.

Name of Principle Investigator: **Jessica Carmen** Project Budget: $ **110,000.00**

Grantee: **Johns Hopkins University**

Title: **Glial Progenitor Cell Function And Neurodegeneration**

Abstract of stem cell research (as submitted by Principle Investigator):

Glial cells (non-neuronal cells) play a major role in the normal functioning of the healthy central nervous system (CNS). The two main classes of glial cells in the CNS are astrocytes and oligodendrocytes. In the disease Amyotrophic Lateral Sclerosis (ALS) dysfunctional astrocytes contribute to the motor neuron loss (and ultimately death) associated with the disease. Glial progenitor cells are an important reservoir of stem cells in the adult CNS. These cells maintain the potential to become either astrocytes or oligodendrocytes. This ability to generate new glial cells is particularly important in the diseased CNS as we can devise therapeutic strategies to promote the differentiation of these cells into new glial cells. In this study human embryonic stem cells (H9) as well as human neural progenitor cells (derived from human fetal brain) are used. We are transfecting both types of stem cells(and the downstream neural and glial derivatives) with mutant superoxide dismutase-1 (mSOD1), the molecule that has been shown to reproduce familial (genetic) ALS in the mouse model. We are studying the ability of the pluripotent cells to differentiate into neural progenitors, glial progenitors, and finally astrocytes. We are also testing the effects of mSOD1 transfection at each of these intermediary steps in order to determine at which stage of astrocyte differentiation astrocytes become dysfunctional. Ultimately we will determine the steps that result in the development of diseased astrocytes and to devise therapeutic strategies that promote the establishment of healthy astrocytes in ALS patients.

Name of Principle Investigator: **Jon Gerber** Project Budget: **$ 110,000.00**

Grantee: **Johns Hopkins University**

Title: **Expression of Potential Therapeutic Targets by Leukemia Stem Cells**

Abstract of stem cell research (as submitted by Principle Investigator):

Problem: Remission in leukemia often does not translate into cure. Background: This phenomenon might be due to the failure of existing therapies to reliably eradicate leukemia stem cells (LSC). Numerous candidates have emerged as possible therapeutic targets in leukemia; among the most notable are: WT1, Proteinase 3 (PR3), Survivin, hTERT, and PRAME. However, their expression in LSC is largely unknown. Hypothesis: LSC will have a unique profile of expression of putative therapeutic targets, which is distinct from both their normal counterparts and their own, more differentiated, bulk leukemic progeny. Research: Three CD34+ cell fractions were isolated from blood and/or bone marrow of 10 patients with chronic phase (CP) chronic myeloid leukemia (CML), 4 patients with blast crisis (BC) CML, 10 patients with acute myeloid leukemia (AML), and 12 normal donors by magnetic bead/column selection and FACS: total CD34+ (unfractionated), CD34+CD38-ALDH- (an intermediate population), and CD34+CD38-ALDH+ (most enriched for stem cells). Expression of PR3, Survivin, hTERT, WT1, and PRAME mRNA was quantified by real time RT-PCR and normalized to GAPDH expression. Protein expression of PR3, WT1, and PRAME was quantified by flow cytometry in an additional 3 normal controls and the K562 cell line. Observations: Expression of PR3, Survivin, and hTERT mRNA in both CML and AML paralleled that of normal controls, with lowest levels in the CD34+CD38-ALDH+ cells, intermediate levels in the CD34+CD38-ALDH- cells, and highest levels in the total CD34+ fraction. In fact, expression was highest of all in the normal, total CD34+ fraction. In contrast, WT1 was expressed at 2 to 5-fold higher levels in all cell fractions from CP and BC CML patients, as compared to their normal counterparts. PRAME expression was very low to undetectable in the normal samples, especially the CD34+CD38-ALDH+ population. All CP CML CD34+ cell fractions expressed >4-fold higher levels of PRAME than any normal fraction; >10-fold higher levels than the normal CD34+CD38-ALDH+ cells. PRAME was even more highly expressed in BC CML; >50-fold higher than the corresponding CP CML fractions and >300-fold higher than any normal fraction. Expression of WT1 and PRAME was more heterogeneous in AML than in CML, though on average, levels were similar to those in BC CML. Protein expression of PR3, WT1, and PRAME correlated closely with mRNA expression. Conclusions: Expression of a putative target by the leukemic bulk does not guarantee expression by the LSC. PR3, Survivin, and hTERT are all expressed at low levels in the LSC, actually lower than in normal CD34+ cells, suggesting that these antigens would constitute poor targets on LSC. Conversely, WT1 and PRAME are selectively expressed in LSC versus their normal counterparts and hence represent promising potential therapeutic targets in myeloid leukemias. Work remains to be done to functionally validate these as viable targets.

Name of Principle Investigator: **Hugo Guerrero** Project Budget: $ **110,000.00**

Grantee: **Johns Hopkins University**

Title: **Migration of Human Brain Tumor Stem Cells in Response to Split Proteins**

Abstract of stem cell research (as submitted by Principle Investigator):

PROBLEM, Glioblastoma multiforme (GBM) is the most common and devastating intracranial malignant tumor. Diffuse infiltration of cells into the brain parenchyma makes complete surgical resection impossible, leading to 99% recurrence. BTSCs, like their non-cancerous counterparts, have self-renewal and differentiation properties with the added ability to initiate tumor formation. Increasing evidence suggests that Brain Tumor Stem Cells (BTSCs) are specifically responsible for brain tumor invasion. The mechanisms responsible for BTSC migration are unknown. BACKGROUND, Slit and Robo proteins function as a chemorepellant system. This system exhibits aberrant methylation in various cancers, including gliomas. In vitro experiments have shown that Slit-2 specifically affects the migration of some medulloblastoma and glioma cell lines. We HYPOTHESIZE that Slit proteins will have a chemorepellant effect on GBM-derived BTSC migration. Methods, We studied the effect of the Slit/Robo system on the migration of human GBM-derived BTSCs in vitro and ex vivo using BTSCs cultured from intraoperative tissue samples of human GBMs. The expression of Slit and Robo genes was evaluated by RT-PCR and Western Blot in response to a de-methylating agent (5-Aza). The effect of Slits on BTSC migration was evaluated in transwell migration and Matrigel® assays. Purified Slit-2 protein was obtained by dialysis of culture media conditioned by transfected Hek293 cells. Robo-N, a decoy receptor that acts as a specific inhibitor for the Slit-Robo interaction was used as control. Migration of BTSC in human brain organotypic cultures was tracked with GFP. In vivo rodent tumor models are being used to determine the role of Slit proteins in tumor formation and invasion. RESULTS, three BTSC lines have been obtained from intraoperative tissue. These cells form neurospheres, differentiate into mature neural cells and form tumors in vivo. Our characterized BTSCs exhibit a basal expression of Slits and Robo proteins. The expression of Slit mRNA increases after the cells are treated with 5-Aza ($p<0.05$). Slit-2 showed a chemorepellant effect on BTSC migration ($p<0.05$). We are currently examining organotypic cultures and in vivo tumor models with co-injection of BTSC and Slit-2-releasing cells. CONCLUSIONS, our results show that human GBM-derived BTSCs show a basal level of Slit protein expression that increases in response to a de-methylating agent. Slit proteins exert a chemorepellant effect on the migration of human BTSCs in vitro. Ongoing ex vivo and in vivo experiments will provide us with more information on the effect of Slit/Robo have on brain tumor invasion.

Name of Principle Investigator: **Maged Harraz** Project Budget: $ **110,000.00**

Grantee: **Johns Hopkins University**

Title: **MicroRNAs in Human Neural Stem Cells Differentiation**

Abstract of stem cell research (as submitted by Principle Investigator):

Neural stem cells (NSCs) differentiate readily into glia or neurons. NSCs potential use in the clinic is to replace lost neurons or glia in neurodegenerative, neuro-traumatic and/or cerebrovascular diseases. In this context, the current model for stem cell therapy relies on expansion of NSCs in vitro, induction of differentiation and then injection of these committed cells to the affected region in the nervous system. Controlling the fate of NSCs in vitro is the limiting step in such model and is still under investigation. MicroRNAs (miRNAs) are conserved ~ 21 nucleotide long non-coding regulatory RNA molecules. miRNAs play major roles in embryonic and adult stem cell differentiation. miRNAs carry a high potential as therapeutic tools due to their small size and the relative ease of delivery to cells. While multiple studies demonstrate the importance of certain miRNAs in neurogenesis, the role of miRNAs in human NSCs differentiation remains unclear. Our preliminary results demonstrate that upon induction of differentiation of human NSCs in vitro, neurons expressed the neuronal marker Tuj-1 but not the glial marker GFAP and glia were GFAP positive but Tuj-1 negative as determined by immuno-cytochemistry. The expression of the neurogenic miRNAs, miR-124, miR-132 and miR-200 were upregulated in differentiated cells compared to undifferentiated NSCs assayed using quantitative real time PCR (qPCR). We used qPCR-based microarray to determine the expression profile of miRNAs in differentiated and undifferentiated NSCs. The miRNAs that are different between the undifferentiated and differentiated states will be further validated individually. We anticipate that our results will identify important molecules in hNSCs differentiation. This will serve as a resource for developing new tools to direct hNSCs fate for therapeutic intervention in neuroregenerative medicine.

Name of Principle Investigator: **Biju Joseph** Project Budget: $ **110,000.00**

Grantee: **Johns Hopkins University**

Title: **HMGA1: A Potential Stem Cell Factor Involved in the Maintenance or Proliferation of the Stem Cells**

Abstract of stem cell research (as submitted by Principle Investigator):

Problem: While recent studies have identified a few genes and microRNAs important in "stemness", the molecular circuitry responsible for this state is largely unknown. Moreover, further study has the potential to provide valuable insight relevant to normal stem cells, tumor stem cells, cancer therapy, and regenerative medicine. Background: We are investigating molecular pathways that direct the stem cell phenotype. Our focus is the HMGA1 oncogene, which encodes the HMGA1a and HMGA1b chromatin remodeling proteins. HMGA1 is expressed at high levels during murine embryogenesis. We and others recently showed that HMGA1 is also highly enriched in human embryonic stem cells (hESCs) and refractory, high-grade (poorly differentiated) cancers. In addition, we engineered transgenic mice overexpressing HMGA1a and all mice develop aggressive leukemia. The leukemic cells can be serially transplanted into recipient mice, indicating that the tumors include a subpopulation of tumor-initiator cells capable of long-term renewal, or leukemic stem cells. Hypothesis: Based on these findings, we hypothesis suggest that HMGA1 is important in stem cell survival or renewal. Because HMGA1 proteins function in modulating gene expression, we hypothesize further that they contribute to "stemness" by inducing specific genes and microRNAs. In fact, some putative transcriptional targets of HMGA1 include genes and microRNAs that are known to be important in maintaining the stem cell phenotype, such as STAT3. Research: To test these hypotheses, we are investigating HMGA1 expression and function in hESCs during differentiation. Observations: We found that HMGA1 mRNA falls when hESCs differentiate, indicating that it may function in the maintenance of stem cell populations. In fact, its expression pattern closely parallels that of NANOG and SOX2, two genes important in stem cell renewal and pluripotency. Forced overexpression of HMGA1 in hESCs accelerates proliferation rates in our preliminary studies. We also found that HMGA1 is highly expressed in pluripotent hESCs compared to unipotent primordial germ cells. HMGA1 is also enriched in leukemic stem cells. Studies are underway to identify downstream mRNA and microRNAs induced by HMGA1 in hESCS. Conclusions: Although further study is needed, our results suggest that HMGA1 is an important stem cell factor that could promote the stem cell phenotype, both in cancer and normal stem cells.

Name of Principle Investigator: **Tarja Juopperi** Project Budget: **$ 110,000.00**

Grantee: **Johns Hopkins University**

Title: **Neural Differentiation of Human Embryonic and Induced Pluripotent Stem Cells**

Abstract of stem cell research (as submitted by Principle Investigator):

Stem cells are primitive, long-lived cells capable of self-renewal and the ability to differentiate into multiple specialized cell types. The pluripotent human embryonic stem cell (hESC) has become an intensively studied target for cell-replacement therapy due to its unique ability to generate any cell in the body. The successful reprogramming of human somatic cells into cells exhibiting a pluripotent phenotype (induced pluripotent stem cells-hiPSC) has opened up the possibility of custom designed cells derived from individual patients for specific disease treatment. Cell-based therapies are currently being investigated for a variety of neurodegenerative disorders where pharmacologic and surgical treatments are inadequate to prevent disease progression such as Parkinson's and Huntington's disease. Generating mature functional neurons from both hESCs and hiPSCs is essential for these cell types to be used for neuroregenerative studies. We have been successful in differentiating three hESC lines into neural progenitors and mature neurons utilizing two diverse approaches: a feeder-free method employing an ES cell aggregate intermediate to form neural progenitors and a protocol using co-culture with the stromal cell line PA6 to initiate differentiation. Using both methods, human ES cell lines H1, H9 and HSF-6 were differentiated into neural progenitors that were capable of being maintained in culture. HESC-derived neural progenitor cells could generate neurons expressing neuronal markers TUJ1 and MAP2ab. HESCs could also be directed to produce neurons expressing the enzyme tyrosine hydroxylase, a feature of dopaminergic neurons, the neural subtype lost in Parkinson's disease. In addition to using hESC, we have derived pluripotent stem cells from dermal and foreskin fibroblasts. The hiPSCs generated exhibit characteristic hES cell-like morphology and express typical pluripotency markers. In vitro germ layer differentiation and in vivo teratoma formation have revealed that our hiPSCs have a similar developmental potential to hESCs. Furthermore, hiPSC-derived neural progenitors can be formed that yield mature neurons upon differentiation. Future studies will evaluate the functional capabilities of hiPSC-derived neurons and evaluate whether they can be used interchangeably with hESCs for generating neurons for cell based therapies.

Name of Principle Investigator: **Vasiliki Machairaki** Project Budget: $ **110,000.00**

Grantee: **Johns Hopkins University**

Title: **Human Neural Stem Cell-Biomatrix Preparations As Tools In Reconstructing Neural Pathways**

Abstract of stem cell research (as submitted by Principle Investigator):

The ability of transplanted Human Neural Stem Cells (HuNSCs) to elaborate axons, engage in short- and long-distance projections and innervate both local and remote targets in host rodents as demonstrated in recent work from our us and other laboratories is a remarkable experimental development. However, the fact that most of the observed connections involve either local circuits or large host pathways shows the substantial challenges when dealing with the regeneration of circuits. We have recently used biodegradable polymeric scaffolds to optimize neuronal differentiation, dendrite/axon formation and directed axonal growth. Studies of HuNSCs on aligned and non-aligned fibrous meshes of poly(ε-caprolactone), poly-ornithine/Laminin-pretreated nanofibers demonstrate that HuNSCs adopt an elongated and polarized cell morphology along the axis of fiber, in contrast to HuNSCs on two-dimension (2-D) or non-aligned fiber substrates that exhibit non-polarized neurite networks. HuNSCs cultured on aligned fibrous substrates show a modest improvement in neuronal differentiation over the other substrates, as shown by levels of expression of the early neuronal marker Tuj1.We have used the callosal window model as a first approach to explore the importance of alignment of HuNSC-derived axons in vivo. Preparations of HuNSCs-aligned nanofibers were encased in calcium alginate hydrogels and grafted along cortico-cortical fibers in the anterior corpus callosum of nude rats. The survival and fate of HuNSCs were assayed with human-specific and neuronal markers. Early data show an extensive growth of newly formed axons along the trajectory of callosal axons. In sum, we demonstrate that scaffolds can serve as an artificial ECM, providing cells with a microenvironment necessary for tissue repair and regeneration.

Name of Principle Investigator: **Celine Plachez** Project Budget: **$ 110,000.00**

Grantee: **University of Maryland, Baltimore**

Title: **Role of Metalloproteinases in the Migration of Transplanted Human Stem Cells**

Abstract of stem cell research (as submitted by Principle Investigator):

The transplantation of stem cells into the central nervous system has the potential for delivering exogenous gene products or a cell replacement therapy. In many cases when stem cells are transplanted into the brain they migrate extensively from the site of engraftment. Regulating the intrinsic capability of stem cells to migrate within the brain is an essential step for targeting stem cell transplantation therapy for maximum effectiveness. In order for cells to penetrate the complex microenvironment of a mature brain, the extracellular matrix must be modulated and matrix metalloproteinases (MMPs) are thought to play key roles in extracellular matrix remodeling. We are exploring the role of MMPs in regulating migration of transplanted stem cells. Our preliminary data show that ES stem cells can be efficiently transfected with MMP expression constructs (MMP2, MMP9, MT5-MMP and TIMP2). Transfected ES cells in vitro display 100-fold increased expression/secretion of MMP9 protein compared to non-transfected control, with commitment increases of enzymatic activity. Transfection with the membrane inserted MT5-MMP construct increased expression in transfected cells by ~4x compared to control ES stem cells. We next transplanted those transfected ES stem cells into mature brain and found that those transplanted and transfected stem cells survive and migrate in adult brain. Preliminary observations suggest that MMP9 transfected ES stem cells were capable of migrating out of the injection sites at great distances, even traversing into the other hemisphere following the corpus callosum pathway. MMP9 transfected cells were co-transplanted with control cells and interestingly the lead/most distant cells were the ones expressing MMP9 expression construct. These preliminary transplants suggest transfected cells may migrate with higher efficiency than non-transfected. Understanding and potentially regulating the intrinsic capability of stem cells to migrate within the brain is an essential step for targeting stem cell transplantation therapy. MMPs represent a novel target to address the fundamental problem of uncontrolled migration that is currently inherent in any transplanted stem cell applications.

Name of Principle Investigator: **Marina Pryzhkova** Project Budget: **$ 110,000.00**

Grantee: **Johns Hopkins University**

Title: **Fetal Erythroblasts from Human Pluripotent Stem Cells for Treatment of Hemoglobinopathy and Malaria**

Abstract of stem cell research (as submitted by Principle Investigator):

Despite advances in the treatment of sickle cell disease (SCD), which include better preventive care, fetal hemoglobin (HbF) induction with hydroxyurea, improved iron chelation during transfusion, and hematopoietic stem cell (HSC) transplantation, life expectancy for these individuals remains diminished. Transplantation of genetically-corrected HSC derived from patient-specific induced pluripotent stem cells (iPSCs) would avoid the risks of allogeneic transplantation, and has been proposed as curative therapy for SCD. However, the generation of engraftable HSC from iPSC currently remains an elusive goal. Herein, we alternatively demonstrate that SCD-affected human embryonic stem cells (hESCs) and normal iPSCs efficiently differentiated into engraftable fetal-type erythroblasts expressing predominantly HbF. Since increased HbF expression ameliorates the clinical symptoms of both beta-hemoglobinopathies and severe malaria, we propose that transfusion of long-lived, HbF-expressing fetal erythroblasts from ABO-genotyped hESC or patient-specific iPSC represents an alternative therapeutic approach for these important diseases. Additionally, the large-scale production of HbF-expressing hESC/iPSC-derived erythrocytes will provide a valuable resource for experimental hematology.

Name of Principle Investigator: **Kara Scheibner** Project Budget: $ **110,000.00**

Grantee: **Johns Hopkins University**

Title: **Mir-27a is dysregulated in leukemias and functions like a tumor suppressor in hematopoietic cells**

Abstract of stem cell research (as submitted by Principle Investigator):

MicroRNAs (miRNAs), non-coding ~22 nucleotide RNAs, are important regulatory molecules in many biological systems, including in cancer pathobiology. Based on expression of miRNAs and their predicted target mRNAs in human CD34+ hematopoietic stem-progenitor cells (HSPCs), we hypothesized that certain HSPC-expressed miRNAs (HE-miRs) can down-regulate key hematopoietic proteins and thereby regulate hematopoiesis. Additionally, we looked for differences in miRNA expression levels between normal CD34+ HSPC cells and cancer cells as clues to dysregulation of human leukemias. We observed that miR-27a appeared to be expressed in normal HSPCs, but absent or expressed at lower levels in several human leukemia cells lines examined. These data were a rough first hint that lack of expression of miR-27a might be associated with leukemia, speculatively, in a tumor suppressor role. We investigated the action of miR-27a via ectopic overexpression of this miRNA in 4 human leukemia cell lines REH (B-precursor acute lymphoblastic leukemia), HL60 (acute promyelocytic leukemia), TF1 (acute myeloid leukemia), and K562 (chronic myeloid leukemia blast crisis) which all have low levels of miR-27a. Lipofection of synthetic miR-27a mimic or lentiviral expression of miR-27a decreased human leukemia cell proliferation, and increased cell death. These drug-resistant human leukemia cell lines also exhibited increased spontaneous apoptosis and became more susceptible to drug- and growth factor withdrawal-induced apoptosis upon enforced expression of miR-27a. Using luciferase assays, we confirmed that the anti-apoptotic molecules YWHAQ, the drug-resistance pump ABCC4, and several Wnt pathway family members were targets of miR-27a. Leukemia cells with enforced miR-27a expression also had reduced proliferation and decreased percentages of cells in the G1 cell cycle phase. Thus, based on its expression, functional effects, and targets, we concluded that miR-27a may function as a tumor suppressor miR--lack of miR-27a expression in cases of leukemias may contribute to their development and/or progression, and may also offer a potential therapeutic target.

Name of Principle Investigator: **Farhad Vesuna** Project Budget: **$ 110,000.00**

Grantee: **Johns Hopkins University**

Title: **TWIST Modulates the Breast Cancer Stem Cell Phenotype**

Abstract of stem cell research (as submitted by Principle Investigator):

The cancer stem cell paradigm postulates that dysregulated tissue specific stem cells or progenitor cells are the precursors for cancer biogenesis. Consequently, identifying cancer stem cells is crucial to our understanding of cancer progression and for the development of novel therapeutic agents. In this study, we demonstrate that the over-expression of Twist in breast cells can promote the generation of a breast cancer stem cell phenotype characterized by the high expression of CD44, little or no expression of CD24, and increased ALDH activity, independent of the epithelial mesenchymal transition. In addition, Twist over-expressing cells exhibit high efflux of Hoechst 33342 and Rhodamine 123 as a result of increased expression of ABCC1 (MRP1) transporters, a property of cancer stem cells. Moreover, we show that transient expression of Twist in multiple breast cell lines can induce the stem cell phenotype and that decreasing Twist expression by shRNA in Twist over-expressing transgenic cell lines MCF 10A/Twist and MCF-7/Twist as well as in MDA-MB-231 partially reverses the stem cell molecular signature. Importantly, we show that inoculums of only twenty cells of the Twist over-expressing CD44+/CD24-/low sub-population are capable of forming tumors in the mammary fat pad of SCID mice. Finally, with respect to mechanism, we provide data to indicate that Twist transcriptionally regulates CD24 expression in breast cancer cells. Taken together, our data demonstrates the direct involvement of Twist in generating a breast cancer stem cell phenotype via down-regulation of CD24 expression and independent of an epithelial mesenchymal transition.

Name of Principle Investigator: **Jizhong Zou** Project Budget: **$ 110,000.00**

Grantee: **Johns Hopkins University**

Title: **Gene Targeting in Human Induced Pluripotent Stem Cells and Embryonic Stem Cells**

Abstract of stem cell research (as submitted by Principle Investigator):

Recently described patient-specific induced pluripotent stem cells (iPSCs) and traditional embryonic stem cells (ESCs) hold enormous promise for personalized cell replacement therapy as well as for research of various human diseases. One key approach to fulfill these promises is via gene targeting, a precise and permanent genome modification approach which utilizes the cell's natural DNA repair mechanism – homologous recombination (HR). Engineered zinc finger nuclease (ZFN) has been shown in multiple organisms to create sequence-specific double strand break and therefore stimulate HR. We demonstrated high-efficiency ZFN-mediated gene targeting was achieved at the endogenous PIG-A gene, which is required for the retention of dozens of glycosyl-phosphatidyl-inositol (GPI)-anchored proteins on the cell surface and is mutated in hematopoietic stem cells from patients with the rare clonal blood disorder paroxysmal nocturnal hemoglobinuria (PNH). Candidate pairs of PIG-A targeting ZFNs were first tested in 293T and Jurkat cells for their potential to enhance gene targeting. We confirmed targeted insertion of a drug-selectable gene by PCR analysis and detected the loss of GPI-anchored proteins by FACS analysis, thus validating these ZFNs. We then targeted the endogenous PIG-A locus in karyotypically normal human iPSCs and ESCs and achieved high efficiencies of HR-mediated donor DNA insertion, with PIG-A specific ZFNs enhancing HR efficiency by >200-fold boost in both types of pluripotent stem cells. Clonal PIG-A knockout human ESCs and iPSCs with normal karyotypes were readily obtained. Targeted insertion of a single copy of the donor sequence in the PIG-A locus was confirmed by Southern blot. We also explored ZFN-induced mutagenic non-homologous end-joining (NHEJ) in human ESCs and obtained clones with defined PIG-A null genetic mutations. PIG-A knockout human pluripotent stem cells retained undifferentiated morphology and markers when being cultured as undifferentiated cells, but showed defects upon differentiation induction in vivo and in vitro. They are also sensitive to complement-mediated killing, a phenotype resembling that of PIG-A null blood cells in PNH patients. The phenotypic and biological defects were rescued by PIG-A transgene expression in all the PIG-A knockout iPSC or ESC lines. Our study provides the first demonstration of gene targeting in human iPSCs, and the power of ZFNs for inducing specific genetic modifications in human iPSCs as well as ESCs. Efficient gene targeting in patient/disease-specific iPSCs also open the doors to gene correction in genetic diseases such as sickle cell anemia (SCA) and b-thelassemia. We have derived multiple lines of iPSCs from SCA fibroblasts of an adult patient as well as fetal tissues. We will report our efforts to correct its single-nucleotide mutation, and in vitro differentiate into hematopoietic progenitors for transplantation and disease treatments.

FY 2009

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Research Descriptions

Name of Principle Investigator: **Angelo All** Project Budget: **$ 733,400.00**

Grantee: **Johns Hopkins University**

Title: **Efficient differentiation of human embryonic stem cells into oligodendrocyte progenitors for application in a rat contusion model of spinal cord injury**

Abstract of stem cell research (as submitted by Principle Investigator):

Problem: Spinal cord injury (SCI) is a devastating condition with a significant socio-economic burden due to the loss of mobility experienced by the patients. SCI is characterized by rapid development of necrosis in the damaged tissues, followed by a secondary degeneration of surrounding neural tissue, including oligodendrocytes, leading to paralysis. Human embryonic stem cells (hESCs) provide novel prospects for cellular replacement strategies due to their ability to provide seemingly unlimited stem cell numbers in vitro, their amenability to genetic engineering, and their broad developmental capacity. To determine the utility of hESCs in SCI, it will be essential to understand their survival and migration in damaged tissues and their ability to remyelinate. Hypothesis: Transplant of hESC-derived oligodendroglial progenitors (OPs) can repair lesions in the spinal cord when injected 24 hours after injury. The anatomical and functional benefits of the cell transplant can be assessed with imaging and electrophysiology respectively. Research: To investigate the utility of OPs in vivo, we used a rat model of contusive SCI followed by OPs transplant. One day post-injury, hESC-derived OPs were transplanted into rats' spinal cord. The time frame was chosen for clinical relevance. A combination of clinically relevant measures was used for characterization including a) electrophysiology with somatosensory and motor evoked potentials (SEP/MEPs) for changes in electrical conduction; b) diffusion tensor MRI (DTMRI) for imaging anatomical changes to detect the progress of injury and identify the spared fibers in the parenchyma surrounding the epicenter; and c) postmortem analysis for studying survival and migration of transplanted cells. Observations: Prior to transplant, in vitro immune-fluorescence revealed that most hESC-derived OPCs expressed oligodendrocyte markers, including CNPase, NG2, O1, O4, and Olig1. Results showed that OP's survived when injected at the center of injury and began to migrate away from the injection sites after one week. Within a few weeks after cell transplant, the electrophysiology indicated some degree neuroelectrophysiological improvement. Standard behavioral tests also supported these results. This is a promising result that needs further investigation. T2 weighted MRI images revealed a significant damage to the grey matter at the epicenter while white matter injury could be detected more readily with the DTMRI images. Histological sections revealed integration of OPCs into the spinal cord without disruption to the parenchyma. Postmortem analysis also showed that the OPCs survived without tumor formation. Conclusions: hESC-derived OPCs integrate into host tissue weeks following injection into an injured spinal cord. Recovery of neural function can be detected by electrophysiology as well as by behavioral studies. Future studies will be required to determine the mode of recovery after treatment.

Name of Principle Investigator: **Linzhao Cheng** Project Budget: **$1,695,570.00**

Grantee: **Johns Hopkins University**

Title: **Derivation and use of patient-specific iPS cells for modeling acquired blood diseases**

Abstract of stem cell research (as submitted by Principle Investigator):

Human embryonic stem cells (hESCs) are normally derived from surplus early embryos obtained from in vitro fertilization clinics. They are called pluripotent stem cells because they are capable of being molded into all cell types. Unlike adult stem cells (such as blood stem cells) that are only capable of generating limited cell types (such as blood and immune cells) and difficult to expand, hESCs can be expanded indefinitely in laboratory while retaining their potential to generate any cell type under the right conditions. Thus, pluripotent stem cells like hESCs provide unprecedented opportunities to develop new cell replacement therapies and other treatments for human diseases. In the past few years, we, along other scientists, developed a way to establish pluripotent stem cells from adult tissues such as skin biopsies using molecular biology techniques. These induced pluripotent stem (iPS) cells that are derived from skin or blood cells of healthy donors or patients can be near identical to hESCs: they can also proliferate forever in the laboratory while retaining their capability to generate any cell types including blood and immune cells. The derivation of iPS cells from a given patient not only alleviates the problem of immune rejection, but also offers the unique ability to investigate the underlying defects in the patients. In the past scientists quite often lacked adequate research systems to investigate the exact role of culprit genes in patients and to recapitulate the disease process in laboratory, which would help them to find drugs or appropriate treatments. The formation of iPS cells from a given patient that immortalizes patient's cells and DNA offers unprecedented opportunities to investigate the biological consequences of the culprit gene and recapitulate in laboratory the disease process. In this project, we will focus on a group of chronic blood diseases called myeloproliferative disorders or MPDs. The rate of MPDs increases dramatically with age. It is widely believed that certain genes are altered many years before when MPD patents show symptoms. However, the nature of the altered genes remains elusive. Because MPDs are acquired diseases occurring many years after birth, we could not closely monitor and study the clinical onset as we can do with an inherited disease. In this project, we, as an interdisciplinary team of leading stem cell researchers and hematologists, will develop a method to convert MPD patient's blood cells back to forever-young iPS cells. After obtaining enough iPS cells, we will then allow them to form blood cells in the lab and find out what goes awry in MPDs. This study will help us establish a novel research system to pinpoint and correct the genetic alternations in MPDs, ultimately leading to improved diagnosis and treatment. This study will also establish a universal method for studying other acquired as well as inherited blood diseases.

Name of Principle Investigator: **Valina Dawson** Project Budget: **$ 1,725,000.00**

Grantee: **Johns Hopkins University**

Title: **Neuroprotective Pathways in iPS Derived Human Neuronal Cultures**

Abstract of stem cell research (as submitted by Principle Investigator):

This application meets the stated goals of MDSRF program for "The development of new medical strategies through human stem cell research on the prevention, diagnosis and treatment of human diseases and conditions is a high priority for the State of Maryland. Stem cell research offers immense promise for new medical therapies and a better understanding of debilitating human diseases and conditions." In this project we will realize the potential of stem cell technology by characterizing, standardizing and studying human neuronal cultures in the setting of glutamate neurotoxicity. This form of neuronal injury is thought to play a primary role in brain injury following stroke and trauma and epilepsy, as well as play major roles in neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis (ALS). Developing and standardizing a human neuronal cell culture model paves the way for future work on human neurons in studying cell death signaling in human brain injury and disease. Additionally this project will identify and study neuroprotective signaling molecules expressed in human neuronal cultures and thus likely to be expressed in human brain. We will investigate mechanisms to activate neuroprotection as a potential strategy to be used in the future to treat patients that suffer the burden of neurologic injury and disease.

Name of Principle Investigator: **Ricardo Feldman** Project Budget: **$ 1,725,000.00**

Grantee: **University of Maryland, Baltimore**

Title: **Generation of patient-specific iPS cells for modeling and treating Gaucherâ€™s disease**

Abstract of stem cell research (as submitted by Principle Investigator):

Human embryonic stem (hES) cells are capable of differentiating into virtually any cell type, making them very promising tools for regenerative medicine. There are many genetic diseases for which there is no cure. In these cases, it would be very desirable to have patient material to study the cause of the disease, and for testing novel therapies. However, the cell types that are affected in these patients are not always available. In these cases, having hES cells harboring the same inherited defects would be very useful. This would allow us to induce them to differentiate into the same types of cells that are affected in the patients, so that they can be studied. Until recently, there were no good sources of disease-specific hES cells. One of them is discarded embryos from IVF clinics. However, these embryos are not available for a large number of diseases. Another option is nuclear transfer (NT) using somatic cells obtained from patients. However, in humans, this approach is fraught with many ethical and technical hurdles that have not yet been solved. Fortunately, a recent new technology was developed, which allows conversion of adult differentiated cells such as fibroblasts into hES-like cells. These cells, which are called induced pluripotent stem (iPS) cells can be differentiated into virtually any tissue. In this proposal we will create a panel of iPS cells derived from skin fibroblasts of patients that harbor the most frequent mutations that cause Gaucher's disease, and we will establish the conditions for repairing the defect. In these patients, there is an enzyme (glucocerebrosidase) that is missing and as a result, the macrophages in various tissues become engorged with undigested lipids. As a consequence, these patients have anemia, enlarged livers and spleens, and bone disease. At the present time the most effective therapy is enzyme replacement, using an enzyme produced in the laboratory. However, this therapy involves bi-weekly intravenous infusions for life, at an annual cost of hundreds of thousands of dollars per patient. We think that iPS cells may one day help to provide a cure for these patients. Once we generate Gaucher-specific iPS cells we will induce them to differentiate into macrophages, which will be studied to see if they exhibit similar defects to macrophages from Gaucher patients. Then we will assess if by reintroducing the missing enzyme into iPS cells we can repair the resulting macrophages. Another use of the mutant iPS cells will be to screen for drugs that can help the mutant protein to become active again. The availability of an unlimited source of mutant macrophages will speed up the development of new treatments for the disease. Gaucher's disease was the first for which enzyme replacement therapy was used successfully and has served as a model for other inherited diseases. We think that this work to find a cure for Gaucher's disease will also have applications for other genetic diseases as well.

Name of Principle Investigator: **Roger Johns** Project Budget: **$ 1,725,000.00**

Grantee: **Johns Hopkins University**

Title: **A novel stem cell therapy for spinal cord injury-induced chronic neuropathic pain**

Abstract of stem cell research (as submitted by Principle Investigator):

Patients with spinal cord injury (SCI) often suffer from severe chronic pain that is difficult to treat. The goal of this research project is to determine whether human embryonic stem (ES) cell-derived oligodendrocyte progenitor cells (OPCs) transplanted into the injured spinal cord can relieve SCI-induced chronic neuropathic pain, and if so, to understand how. We hypothesize that transplantation of human ES cell-derived OPCs into the injured spinal cord could reduce SCI pain by diminishing abnormal sprouting of pain nerve fibers, increasing myelin production (myelin is the insulation around a nerve), and enhancing specific cell communication pathways in the injured spinal cord. To prove these hypotheses, we will transplant human ES cell-derived OPCs into the spinal cord of rats to determine the effect on pain behaviors in two different SCI models (specific aim 1); we will determine whether an increase in myelin production and prevention of abnormal pain fiber sprouting are key central mechanisms by which spinally transplanted human ES cellderived OPCs relieve SCI-induced chronic neuropathic pain (specific aim 2); and we will determine whether a particular signaling pathway (NRG1/ErbB signaling) contributes to the anti-pain effect of the transplanted OPCs by regulating myelin production (specific aim 3). The overall goal of this proposal is to optimize stem cell transplantation strategies in the treatment of SCI-induced chronic neuropathic pain and provide experimental evidence for human clinical trials of SCI pain treatment. These studies will demonstrate that transplantation of human ES cell-derived OPCs can treat SCI-induced chronic neuropathic pain by increasing myelin production and preventing abnormal pain fiber sprouting in the injured spinal cord and that the particular NRG1/ErbB signaling could contribute to the anti-pain effect of the transplanted OPCs by regulating myelin production. The knowledge gained from this research project will provide the justification for us to perform clinical trials of human stem cells in human SCI patients. This objective is consistent with the goals of the Maryland Stem Cell Research Fund.

Name of Principle Investigator: **Guo-Li Ming** Project Budget: **$ 1,725,000.00**

Grantee: **Johns Hopkins University**

Title: **Understanding functions of schizophrenia susceptibility genes using pluripotent human stem cells**

Abstract of stem cell research (as submitted by Principle Investigator):

Schizophrenia and bipolar affective disorder are chronic and generally disabling brain diseases with a prominent genetic basis. These illnesses affect a large portion of the world's population and have devastating consequence for the sufferers, their family and for the society as a whole. For example, Schizophrenia affects 1% of population world-wide and ranked 9th in the World Health Organization estimates for disease-related lifetime disabilities, costing over $60 billion annually in the United States alone. The available symptomatic treatment is only partially effective, thus development of rational therapeutics based on understanding of the etiology and pathogenesis of the disease is imperative. Increasing clinical, pharmacological and genetic data suggest that the pathogenesis is neurodevelopmental in nature. Disrupted-in-schizophrenia 1 (DISC1) was originally identified at the breakpoint of a balanced chromosome translocation that co-segregates with schizophrenia and other major affective disorders in a large Scottish family. DISC1 has emerged as one the best supported susceptibility genes for major mental illness, including schizophrenia, bipolar disorders, major depression and autism. Little is known about the functions of DISC1 in human development due to a lack of experimental systems. Advance in the stem cell field have led to successful derivation of human embryonic stem cells (hESCs) and more recently human induced pluripotent stem cells (hiPSCs) from skin fibroblasts. These pluripotent stem cells have the capacity of unlimited self-renewal and differentiation into all cell types of a human body, thus provide opportunities to model human diseases using relevant human cell types for understanding the biology of the diseases and for drug discovery with higher predictability of their effects in humans. The current collaborative project, building upon the expertise of several laboratories and ongoing collaborations with researchers, aims to establish a platform using hESCs and hiPSCs and their neuronal progeny to understand functions of normal and mutant schizophrenia susceptibility genes for disease development. Our proposed study using DISC1 and its binding partners as an entry point may provide fundamental and critically needed information on functions and mechanisms of these susceptibility genes in human neurodevelopment and a better understanding of the etiology and pathogenesis of schizophrenia and other related major mental illnesses. Importantly, our study may lead to new targets for future diagnostics, prevention and development of therapeutic treatments, as well as generation of new models of human psychiatric diseases to be tested with potential therapeutic molecules.

FY 2009

Exploratory

Awarded

Research Descriptions

Name of Principle Investigator: **Jonathan Auerbach** Project Budget: **$ 230,000.00**

Grantee: **Global Stem, Inc.**

Title: **Scale-Up Manufacturing, Authentication, and Banking of Human Pluripotent Stem Cells.**

Abstract of stem cell research (as submitted by Principle Investigator):

Human pluripotent stem cells (hPSC) are those cells that can be grown indefinitely in the laboratory and can be differentiated to give rise to any cell type in the human body. These cells can be derived either from pre-implantation embryos or from adult cells which have been induced to become pluripotent. The potential and promise held by these cells for the treatment of degenerative disease and the amelioration of the human condition are tremendous. Relative to the diversity of the human population, there are very few cell lines available today. Those that are available are not suited for clinical applications, since they are difficult to handle, they are unstable, and have been exposed to animal components. In order to move the field of hPSC research forward and to begin to realize the great promise of these cells, we propose to develop a reliable, reproducible method to scale-up the propagation of hPSCs. Because no such method is available, researchers do not have access to large banks of hPSCs which have been authenticated, well-characterized, and produced under standardized conditions. Here we propose to create an optimized system for the expansion, authentication, characterization, and banking of hPSC lines. Throughout this project, we will collect and analyze data to determine the effects of the optimized conditions on the genome of the hPSC lines. This project is very responsive to the Maryland Stem Cell Commission's request for applications. The translational potential is clear – both the methodology and the cell banks created will have important clinical implications.

Name of Principle Investigator: **Alexey Belkin** Project Budget: **$ 229,964.00**

Grantee: **University of Maryland, Baltimore**

Title: **The role of transglutaminase in adhesion-dependent and growth factor-mediated responses of mesenchymal stem cells**

Abstract of stem cell research (as submitted by Principle Investigator):

Stem cells (SCs) represent a unique population of cells that reside in various body tissues and organs and are characterized by the two key features: (i) their limitless ability for self-renewal and (ii) their capacity to develop into all the principal types of cells that comprise muscle, brain as well as other tissues and organs. In particular, mesenchymal stem cells (MSCs) that are typically isolated from the bone marrow, are capable of developing into the cells of muscle, tendon, cartilage, fat, etc. This ability makes MSCs an invaluable source for treating various human disorders for which other approaches fail and when a replenishment of cellular pool in the damaged or injured organ appears necessary. Hence, the use of human MSCs derived from human bone marrow, often from the same patient, is becoming a preferred strategy for treating a number of devastating diseases, including the heart disease. However, despite this progress, a low viability of the transplanted MSCs remains an important drawback. This is particularly true in the case of heart disease, when transplantation of these cells into the damaged heart muscle produces only relatively few cells that are able to survive and thrive after this procedure. Hence, the two important capabilities of these cells: (i) to attach to other cells / tissues and (ii) to survive in the new unreceptive microenvironment have both to be improved in order to increase their therapeutic efficiency. Our laboratory is studying a protein that is produced by MSCs, termed tissue transglutaminase (tTG), which endows cells with both these important advantages. Specifically, this protein physically interacts with integrin and platelet derived growth factor receptor (PDGFR), the two regulatory proteins on the surface of various cells that control many pivotal cellular processes, including their ability to attach to various substrates, to survive, grow, and migrate. We propose that tTG enhances the function of integrin and PDGFR in MSCs and by doing so, improves their attachment, survival, growth and migration. The outlined study is set to test this hypothesis. The acquired knowledge will permit us to elucidate the functions of tTG in human bone marrow derived MSCs and thereby to design and implement new therapeutic approaches based on altering the level and function of this protein in these cells for their use in tissue repair and regeneration.

Name of Principle Investigator: **Michael Betenbaugh** Project Budget: **$ 230,000.00**

Grantee: **Johns Hopkins University**

Title: **A Translational Study to Define Cell Culture Conditions For Human Embryonic Stem Cell Expansion on Collagen**

Abstract of stem cell research (as submitted by Principle Investigator):

Embryonic stem cells represent a potential source for cell and tissue therapies needed to treat debilitating diseases including Parkinson's disease, diabetes, liver disease, and heart failure. However, before these therapies can become a reality, cell culture processes must be developed that are defined, reproducible and relatively inexpensive for producing the very large quantities of stem cells needed for the clinic. In addition, reproducible embryonic stem cell culture conditions will permit a controlled comparison of research findings from different laboratories in order to advance scientific knowledge in the field. A major recommendation of the International Stem Cell Forum is to develop a fully defined culture system that could be used throughout the field and serve as a rational foundation for both future scientific and clinical advancements. Current culture systems are not chemically defined. This means that the culture systems used in one laboratory may differ from those in another laboratory. This variability in stem cell culture conditions makes it hard to compare findings from different laboratories and difficult to develop a defined clinical process that can be approved by the regulatory authorities. As with any commercial process, it is highly desirable to define all the components so that the system can be applied reproducibly for small or large scale production of stem cells and be used safely in the clinic. Indeed, acceptance of stem cell therapy by the public will depend on defining all the components of a culture system. The two main components of the stem cell culture system include the biomaterial or "matrix" that the cells attach to and the liquid formulation or medium that provides the nutrients for stable growth and renewal of hESC without differentiation. This translational project will lead to a novel human embryonic stem cell (hESC) culture system that consists of a new liquid formulation and a defined collagen matrix. Using collagen as a matrix would be a major advance over current systems because it is well defined, inexpensive, and approved by the FDA for clinic. Most current matrices do not have a defined chemical composition, making them difficult to meet regulatory guidelines for implementation in a clinical setting. In addition to implementing a defined matrix, this project will develop a new chemically defined liquid formulation that allows stem cell growth and expansion on collagen which is not possible with the current liquid formulations. The medium will be made available to other researchers and clinics around the world so that research findings can be compared in a format that is compatible across all laboratories. Furthermore, the development and implementation of such a defined stem cell culture system may form the basis of a new commercial enterprise for stem cell processing in Maryland. A defined culture system will be ideal for implementing less expensive, reproducible, and safe processes essential for producing large quantities of stem cells needed for use in the emerging cell therapies industry.

Name of Principle Investigator: **Maria Canto-Soler** Project Budget: **$ 230,000.00**

Grantee: **Johns Hopkins University**

Title: **Development of iPS technology for the treatment of retinal degeneration**

Abstract of stem cell research (as submitted by Principle Investigator):

Retinal dystrophies are diseases in which the death of the photoreceptor cells (the cells of the retina in charge of processing the visual input) leads to vision loss, and some times, total blindness. Although significant effort has been directed toward understanding these diseases and the development of therapeutic treatments, there is still no cure for them. Stem cell transplantation has emerged as a potential strategy to restore vision in patients affected by these diseases, particularly for advanced stages in which most of the photoreceptors have been already lost. Unfortunately, there are still several limitations that need to be overcome before stem cells can be safely and efficiently used in the clinic. One of these limitations is the lack of a source of stem cells from the patient that could be used for his/her own treatment, thus avoiding potential immunological complications. In this project we will investigate the possibility of generating "patient-specific" stem cells that could in turn be "instructed" to differentiate into photoreceptor cells and then transplanted back into the patient in order to replace the cells that have been lost. "Patient specific" stem cells can be generated from skin cells obtained through a routine skin biopsy. In the laboratory, these cells are then forced to express a group of specific genes that instruct them to "go back on time" and acquire an "embryonic stem cell-like" status. These cells can then potentially be re-directed to differentiate into any cell type of the adult body and used for cell transplantation therapies. In this study we will focus in the generation of photoreceptor cells from skin cells obtained from patients affected by retinal dystrophy. The results from these experiments are expected to help us establish the conditions for the generation of patient-specific stem cells that could be potentially used for retinal transplantation. In addition, the development of this technology will also provide an ideal biological system for the study of these diseases, thus further contributing to the discovery of possible alternative therapeutic treatments.

Name of Principle Investigator: **Chunzhang Cao** Project Budget: **$ 230,000.00**

Grantee: **University of Maryland, Baltimore**

Title: **Activated Protein C protects mesenchymal stem cells from ischemia-induced death**

Abstract of stem cell research (as submitted by Principle Investigator):

Heart attacks can lead to congestive heart failure which affects approximately 5 million Americans annually. This disease remains a formidable health challenge due to limited treatment options. Recent advances in stem cell research offer hope as these cells have potential to replace damaged heart muscle cells and replenish the hearts blood supply. Stem cells that have shown significant potential for therapy are human mesenchymal stem cells (hMSCs), which can be isolated readily from bone marrow, liposuction-derived adipose tissue, or from human umbilical cords. Recent clinical trials demonstrate that hMSCs exhibit the best therapeutic activity with the least number of complications in patients with congestive heart failure. However, despite encouraging results, the clinical application of hMSCs is limited by poor viability of MSC transplanted into the infarcted heart. One of the reasons could be due to significant death of implanted MSCs within the injured heart. Therefore, better preparations of hMSCs that are capable of resisting to ischemia-induced cell death are critical to improving the efficiency of stem cell therapy. Our recent discoveries provide a rationale for developing human MSCs that are likely to be more effective for cellular-based therapy. First, activated protein c (APC), a physiologic anticoagulant, has been reported to prevent apoptotic death of human brain endothelium under hypoxia; second, we found APC also protected human MSCs from H2O2-induced apoptosis; third, the ischemic/reperfusion injury can generate a lot of reactive oxygen species including H2O2 which may trigger apoptotic cell death in infarcted heart. In this application we propose to determine that APC treatment would enhance the survival of human MSCs in the infarcted heart, so that improve therapeutic efficacy of hMSCs for patients with heart failure. We anticipate that the information gained from these studies will advance the stem cell field in innovative directions outside of prevailing views, and open up new avenues for accelerating the progress of stem cell therapies for treating human disease. If successful, this work will bring new opportunities to further enhance the economy of Maryland and position Maryland as a leader in stem cell biotechnology, and most importantly will advance our ability to improve the quality of life for patients with congestive heart failure.

Name of Principle Investigator: **Curt Civin** Project Budget: **$ 230,000.00**

Grantee: **University of Maryland, Baltimore**

Title: **The Hippo pathway in control of hematopoietic organ size and stemness**

Abstract of stem cell research (as submitted by Principle Investigator):

A new cascade of molecules called the Hippo pathway was discovered recently to control the size of several organs in fruit flies. An analogous pathway exists in higher species. In mice, it has been shown that experimental manipulation of the Hippo pathway has massive effects on the size of the liver, intestine, and pancreas (Other tissues and organs have not yet been studied intensively). This and other evidence implicates the Hippo pathway in controlling the balance between stem cell renewal (stem cells giving rise to exact copies of themselves) versus differentiation (stem cells giving rise to one or more types of more differentiated cells) in multiple organ systems. For example, experimental manipulation of the Hippo pathway in mouse intestine results in massive expansion of early undifferentiated (stem-progenitor) cells and concomitant loss of more mature, differentiated cells. Since it appears likely, but has not yet been shown, that manipulation of the Hippo pathway can regulate hematopoiesis (blood cell formation), we propose to investigate the effects of the Hippo pathway on human and mouse hematopoiesis. A major translational objective will be to determine whether manipulation of the Hippo pathway facilitates expansion (growth) of human stem-progenitor cells in the test tube for research and potential clinical use.

Name of Principle Investigator: **Anne Comi** Project Budget: **$ 199,951.00**

Grantee: **Hugo W. Moser Research Institute at Kennedy Krieger, Inc.**

Title: **Human Cord Blood Stem Cell in a Mouse Model of Neonatal Stroke**

Abstract of stem cell research (as submitted by Principle Investigator):

Background: Neonatal stroke occurs in 1 per 4000 live term births and causes cerebral palsy, developmental delays and seizures. Diagnosis of neonatal stroke is delayed and therefore the development of effective treatments has been difficult. New strategies are needed to improve recovery after neonatal stroke. Goals: Our goals are to 1) to begin to determine the usefulness of human cord blood derived stem cells for the treatment of neonatal stroke, 2) to develop new hypotheses regarding cord blood stem cell biology and therapeutic targets for neonatal stroke and 3) to determine how human cord blood stem cells impact the birth of new brain cells after neonatal stroke. Approaches: Human cord blood stem cells will be injected into a mouse model of neonatal stroke. State-of-the-art techniques will be used to determine proteins that are increased or decreased in the injured neonatal mouse brain after stroke in response to the stem cells, and assess their impact upon new cell birth during recovery. Significance and Relevance: This work will lay a firm foundation for multiple important future research studies to develop new therapeutic targets, generate novel hypotheses about how human cord blood stem cells function in the injured immature brain, and produce pre-clinical data that will guide future clinical studies in neonatal stroke. This work will also establish new biotech resources in the Maryland in the forms of new investigators to the field of stem cell translational research, new technicians trained, and new large publicly available datasets on protein expression from the injection of these human cord blood stem cells into a model of neonatal stroke.

Name of Principle Investigator: **Jenice D'Costa** Project Budget: Project Budget**: $ 230,000.00**

Grantee: **Virxsys Corporation**

Title: **Developing safer methods for induced Pluripotent Stem (iPS) cell generation using Spliceosome-mediated RNA trans-splicing (SMaRTTM) technology**

Abstract of stem cell research (as submitted by Principle Investigator):

Pluripotent stem cells have the unique ability to develop into different cell types in the body. They have therapeutic applications for the regeneration of damaged organs or replacement of defective tissues with genetically corrected stem cells. The discovery that differentiated cells or cells which are specialized for a particular function such as skin or hair follicle cells can be reprogrammed into pluripotent stem cells has presented many applications for their use in the clinic. These induced pluripotent stem (iPS) cells can potentially circumvent the use of ethically controversial human embryonic stem cells for therapeutic applications. iPS cells can be patient-specific derived stem cells and used as cell-based therapies for diseases such as Hemophilia A, Parkinson's disease, or Type I diabetes. iPS cells are derived by expressing four "pluripotency factors" in differentiated cells. The expression of these factors reprograms these differentiated cells to become iPS cells. iPS cells generated from patients with genetic diseases can be corrected for the genetic defect and then used to replace the damaged tissue or regenerate new tissue for damaged organs. Current methods to derive iPS cells include elements that may cause insertional mutagenesis and transformation. The pluripotency factors used to generate iPS cells are expressed using gene delivery systems that integrate within the DNA of the cells, hence retaining the possibility that they may be reactivated to produce the factors inappropriately. Our proposal describes our proprietary technology for safer derivation of iPS cells using a delivery system that does not permanently modify the genomic configuration of the cell. Instead, the delivery system is transient and will be lost as the cells divide and grow. An additional safety feature is that the expression of the pluripotency factors will be linked to endogenous genes which are turned on only at the relevant stage of reprogramming, and then shut off once iPS are generated. Our specific aims for this proposal are 1. Design and test the delivery system for expression of the "pluripotency factors" and 2. Optimize safer and more efficient methods to derive iPS cells using our gene delivery system.

Name of Principle Investigator: **Alan Friedman** Project Budget: **$ 200,000.00**

Grantee: **Johns Hopkins University**

Title: **RUNX1 Role and Epigenetics in Development of HSC from Human ES/iPS**

Abstract of stem cell research (as submitted by Principle Investigator):

The focus of this application is developing an improved understanding of the regulation of human hematopoietic stem cell (HSC) development from human embryonic stem cells (hESC) or from induced pluripotent stem cells (iPSC) obtained from adult cells, with the ultimate goal of obtaining sufficient numbers of HSC to benefit patients. Achieving this goal would increase our ability to offer stem cell transplantation or autologous transfusions to patients with cancer ormarrow failure syndromes, will serve as a source of white blood cells and platelets to prevent serious infections or bleeding in patients receiving intense chemotherapy and in patients with aplastic anemia, and will serve as a vehicle for improving gene therapies for genetic diseases such as sickle cell disease. A protein termed RUNX1 controls mouse HSC formation. Aim 1 of this proposal is to determine whether RUNX1 is also required for human HSC formation and whether increasing RUNX1 levels can stimulate hESC or iPSC to develop into HSC. Aim 2 of this proposal will study the gene controlling RUNX1 expression, to obtain information that will help us devise strategies to increase RUNX1 expression to favor HSC formation. In the long-term we expect to utilize our findings to benefit patients by generating HSC from immunologically matched hESC or from a patient's own iPSC.

Name of Principle Investigator: **Da-Wei Gong** Project Budget**: $ 230,000.00**

Grantee: **University of Maryland, Baltimore**

Title: **Novel vector systems to reprogram human adipose stromal vascular cells into insulin-producing cells and transplantable adipocytes**

Abstract of stem cell research (as submitted by Principle Investigator):

Stem cell research offers a great promise for cell-based therapy for diabetes, Parkinson's disease, spinal cord injury and other degenerative diseases. However, the research on human embryonic stem cells (ESCs) has been limited by ethical issues and availability of ESCs. The situation has changed when a group of Japanese scientists discovered that somatic cells can be reprogrammed into induced pluripotent stem (iPS) cells by delivering a combination of four protein factors into fibroblast cells. The iPS cells are indistinguishable from ESCs in terms of their appearance and biological function. Thus, iPS cell are potentially a great source for patient-specific cell replacement therapy, becauase they can give rise to pancreatic insulin-producing beta-cells, neurons, heart cells or fat cells under appropriate conditions. However, current delivering methods for those protein factors are inconvenient and inefficient, and may cause tumor. This research group is developing a novel vector to replace the currently-being used vectors in the reprogramming process. This new vector will be a safe and powerful laboratory tool to produce iPS cells, a significant step toward the application of embryonic stem cell-like cells for clinical purposes. Using this vector tool, we will first generate the iPS cells from human fibroblasts of adipose tissues, and then turn the iPS cells into insulin-producing cells by introducing protein factors that are important for the cell transformation. These cells will ultimately be used for cell replacement therapy for diabetes patients who cannot make insulin because of the destruction of pancreatic islets. Another planned use of the iPS cells is for breast reconstruction after mastectomy after breast cancer surgery. Currently, breast reconstruction is done by implants of exogenous materials (e.g. silicone) or autologous transplantation; each method has its own disadvantages. We propose to turn iPS cells into fat cells, which are then mixed with a matrix of biomaterial called hydrogel and grown in cell culture dishes. This mixture of fat cells and hydrogel will be then implanted to animal, where the fat cells will grow into fat tissues but hydrogel will be absorbed naturally. Breast cancer inflicts one in ten women in the U.S. in life time, and about 56,000 breast reconstruction surgeries were conducted in 2007. If we can prove this technology working, it would offer a safe, effective, natural and durable breast reconstruction, superior to currently available breast reconstruction methods. In summary, this proposal aims to develop new methodologies to facilitate the clinical application of iPS cells in diabetes and cancer fields and is expected to have significant social and economic impact.

Name of Principle Investigator: **Jordan Green** Project Budget**: $ 229,812.00**
Grantee: **Johns Hopkins University**

Title: **Non-Viral Nanoparticles for Creation of Human Induced Pluripotent Stem Cells**

Abstract of stem cell research (as submitted by Principle Investigator):

There is a tremendous medical need for a safe, ethical, and effective means of generating patient specific human induced pluripotent stem cells (iPS). The range of diseases that show promise for finding new treatments through the use of iPS cells include spinal muscular atrophy, Lou Gehrig's disease (ALS), Parkinson's disease, sickle cell anemia, and many others. Reprogramming human differentiated cells into undifferentiated, pluripotent cells could potentially enable a patient to receive a customized cell therapy of any cell type that is a perfect genetic match. It has been demonstrated that human skin cells can be reprogrammed to iPS cells through the use of integrating viruses and are essentially indistinguishable from embryonic stem cells. However, unlike embryonic stem cells, iPS cells are created without requiring an embryo. The current method requires the use of viruses to deliver DNA and poses a significant clinical safety risk. To improve safety, non-viral methods for reprogramming are needed. There is no non-viral gene delivery system specifically designed to efficiently create iPS cells. Due to a lack of effective non-viral delivery systems, human iPS cells have never been created non-virally. Non-viral gene delivery efficacy generally remains dramatically lower than those achieved using viruses. To tackle this challenge, we have developed highly effective non-viral gene delivery systems for gene transfer to hard to transfect cell types including human primary endothelial cells and human embryonic stem cells. For some human primary cells, these materials are comparable to viruses for gene delivery efficacy. While these materials have been shown to be superior to commercially available reagents, the lead structures are cell-type specific, and efficacy can be improved further with improvements to biomaterial design. Building on this experience, our lab will design a more effective library of safe, biodegradable polymers to use to form gene delivery nanoparticles. We will use high-throughput synthesis and screening methodology to select for novel biomaterials highly efficient for gene delivery to human fibroblasts and for non-viral reprogramming of human fibroblasts to human iPS cells. This research aims to create new, transformative technology that is able to safely and effectively reprogram human skin cells into human induced pluripotent stem cells without the use of viruses. These reprogrammed human cells could then potentially be used as new stem cell lines for research purposes or directly in patients for many regenerative medicine applications.

Name of Principle Investigator: **John Harmon** Project Budget**: $ 230,000.00**

Grantee: **Johns Hopkins University**

Title: **Platform Technology to Promote Angiogenesis and Healing in Burn Wounds Using Hydrogels and Stem Cells**

Abstract of stem cell research (as submitted by Principle Investigator):

Burn injuries remain a major public health concern, requiring medical attention for more than one million Americans annually. Despite therapeutic advances, normal human skin is not regenerated after burn wound healing. Non-healing burn wounds and excessive scarring cause significant long-term morbidity. Although superficial burns heal with minimal scarring, deep second and third degree burns often heal with a dense fibrotic scar that impairs function and can be disfiguring. These burn scars heal with fibrosis, scarred dermis, effaced epidermis, and lack sweat glands, oil glands and hair follicles. Deeper burns may require skin grafting for wound closure; these are thin and vulnerable to re-injury. Therapies which improve burn wound healing and skin regeneration would significantly improve outcomes for burn patients. Regenerating blood vessels in the burn wound is a strategy to improve healing of these wounds. Bone marrow-derived circulating endothelial progenitor cells (EPCs) have been recently identified. EPCs are mobilized from bone marrow in response to tissue ischemia from burn wounding, and migrate to the wounded area where they participate in tissue revascularization and repair. EPCs may also be obtained from umbilical cord blood, with a recent study finding that blood vessels derived from these cells are more functional and stable than those from bone marrow-derived EPCs. (Au, Daheron et al Blood 2008). The identification of EPCs presents an outstanding opportunity to impact the healing of burn wounds. To unlock the full potential of stem cells in burn wound healing, it is crucial to mimic aspects of the dynamic in vivo environment in which they function. In the body, stem cells are surrounded by an extracellular matrix which mediates cell to cell communication and provides spatial domain. A hydrogel scaffold is a three-dimensional construct which incorporates bioactive molecules, allows better spatial control of stem cells, is itself vascularizable, and may direct the use of these cells for in vivo conditions. Given their accessibility, burn wounds are an excellent target for hydrogel therapy. Dextran is a biodegradable and nontoxic polysaccharide which exists in human tissues, and can be modified to create a hydrogel construct. Our laboratory has developed a murine burn wound model in which uniform, graded severity of thermal injury can be reliably reproduced. This model can be utilized to establish a precise relationship between burn wound healing and experimental therapies.In this exploratory grant, we aim to develop a method to utilize umbilical-cord-derived human EPCs encapsulated in a three-dimensional dextran hydrogel to promote angiogenesis and healing in burn wounds. We will combine the expertise of Dr. John Harmon's laboratory in evaluating healing and angiogenesis in burn wounds in a murine model, with that of Dr. Sharon Gerecht, in combining stem cells and hydrogels to promote angiogenesis.

Name of Principle Investigator: **Yoon-Young Jang** Project Budget**: $ 230,000.00**

Grantee: **Johns Hopkins University**

Title: **FUNCTIONAL ISOLATION OF NICHE SPECIFIC HUMAN STEM CELLS**

Abstract of stem cell research (as submitted by Principle Investigator):

Hematopoietic stem cells (HSCs) have been widely used for treatment of many blood diseases. Graft failure after stem cell transplantation is a serious and ultimately fatal complication that can only be circumvented by a second stem cell transplant. It is difficult to find another matched donor graft for second transplant and especially in patients receiving an umbilical cord transplant, the same cord blood unit is not available. The cause of graft failure is mostly associated with immune mediated rejection and disease relapse, but for some patients who develop late graft failure, the cause remains unknown. This could be due to the limitation of current isolation technology for selecting "very primitive human HSCs residing within a highly specialized marrow environment (i.e. niche)", which provide a more sustained blood cell generation after transplantation. It has been evidenced that primitive mouse long-term engrafting HSCs (LT-HSCs) reside preferentially within the bone niche (rather than the vascular niche) and we have recently established the novel isolation method for purifying the bone niche associated primitive mouse HSCs by taking advantage of niche specific properties. Although isolation of human HSCs has been improved during recent decades by using cell surface marker selection, the functions of these surface markers regarding the fate of human HSCs remain highly controversial. Therefore, it is important to develop isolation technologies for human HSCs by utilizing a more reliable and functional selection method. Based upon our experience and expertise in mouse stem cell isolation and transplantation, we propose a new project to functionally purify a primitive human HSC subset(s) residing within the niche compartment and to rescue the hematopoietic function in a myeloablated mouse model (i.e. a mouse model with host marrow cells removed). We will first apply the mouse HSC isolation protocol that has been successfully established in our laboratory by utilizing differential metabolic features of niche stem cells, to selecting human HSCs. We also plan to isolate human HSC subsets with other attributes associated with distinct stem cell niches.This study will improve human HSC isolation, and increase our understanding of a) the biology of the human stem cell microenvironment and b) the pathogenesis of stem cell related diseases such as leukemia, aplastic anemia and myeloproliferative disorders. Ultimately, our studies will provide stem cells with functional long-term hematopoiesis potential which is required for successful bone marrow transplantation for various blood diseases.

Name of Principle Investigator: **Feng Jiang** Project Budget**: $ 230,000.00**

Grantee: **University of Maryland, Baltimore**

Title: **A MicroRNA Signature of Cancer Stem Cell as Prognostic Biomarker for Lung Cancer**

Abstract of stem cell research (as submitted by Principle Investigator):

Non-small cell lung cancer (NSCLC) is the number on cancer killer in the USA and the State of Maryland. In men the mortality of NSCLC exceeds the combined total of prostate, colon and pancreatic cancer and in women, the combined total of breast and colon cancers. A critical issue in the management of lung cancer is the ability to identify early stage NSCLC patients at high risk of recurrence who would benefit most from adjuvant therapies, while sparing patients at low risk of recurrence from unnecessary cytotoxic chemotherapy. The current staging classification does not accurately distinguish between the patients who will benefit from therapy and those who will not. In the proposed two-year translational project, we will develop a novel molecular genetic signature of cancer stem cells (CSCs) as prognostic biomarker for early stage NSCLC. The proposed study will greatly contribute to accomplishment of the goals of this RFA "Exploratory Research Grants for Basic and Translational Stem Cell Research In Support of Medical Therapies", because the following reasons:
Firstly, this project has translational potential in cancer patient care, as it integrates advances of tumor stem cells and into clinical diagnosis of lung cancer, and hence drives the basic scientific discoveries from bench to bedside. Furthermore, it addresses obstacles that hinder the clinical use of molecular genetic technique and further brings the advancements of biomarkers into the laboratory settings. In addition, the research will be carried out by a multidisciplinary research team combining complementary expertise in molecular genetics, cell biology, histopathology, clinical oncology, and biostatistics and bioinformatics. Secondly, the two-year research will result in large clinical trails, because based on the generated data, we will perform multicenter clinical trials designed to prospectively confirm its utility in additional patient series. At the completion, the biomarker could be rapidly and widely adopted in routine clinical practice. Furthermore, we will collaborate with industry laboratories to bring the resulted technology to the marketplace. We will also be able to develop anti-cancer agents that can specifically kill the CSCs by targeting the identified abnormalities for efficient treatment of lung cancer. Thirdly, and most importantly, because the developed biomarker can potentially be applied for monitoring the prognosis and prediction of treatment response of lung cancer patients, the research will lead to personalized treatment, and thus reduce lung cancer mortality. Therefore, the study will have great impact on biotechnology in Maryland and ethics. The application is highly responsive to RFA-MD-09-2. The application also meets the mission of MSCRF established under the Maryland Stem Cell Research Act of 2006 to promote state-funded human stem cell research and medical treatments through grants to public and private entities in the state.

Name of Principle Investigator: **Mihoko Kai** Project Budget**: $ 230,000.00**

Grantee: **Johns Hopkins University**

Title: **DNA Damage Response in Glioblastoma Stem Cells**

Abstract of stem cell research (as submitted by Principle Investigator):

Glioblastomas are among the most lethal brain tumors. In majority of cases, the tumors regrow even after surgery, chemotherapy, radiation, immunotherapy and combinations of these existing therapies. The patients newly diagnosed glioblastoma have a survival rate of about one year after the therapies. Because the existing therapies are not effective enough to destroy all tumor cells. A small population of cells, so called "cancer stem cells", are believed to be the cause of the resistance to the cancer therapies. New therapies that target cancer stem cells are needed to cure cancer completely. If we can find a way to attack the cancer stem cells as well as the bulk of tumor, the survival rate of patients will significantly improve. Ionizing radiation represents the most effective therapy for glioblastoma, but radiotherapy remains only palliative. It has shown recently that the radiation resistance of glioblastomas is due to the cancer cell population because cancer stem cells have ability to repair DNA lesions caused by the ionizing radiation. Taking advantage of this fact, we proposed to discover cancer stem cell specific factors, identifying factors which are responsible for radiation resistance by looking at the upstream of DNA damage response pathways in the cancer stem cell population. If successful, we will be able to develop new cancer stem cell specific therapies. This prpposal holds great promise for improving treatment outcomes and preventing relapse in malignant disease. In addition, besides opening up new possibilities for the specific targeting of cancer stem cells, this proposal will make clear for the first time links between cancer stem cell regulation and DNA damage-response pathways.

Name of Principle Investigator: **Joelle Hillion** Project Budget**: $ 230,000.00**

Grantee: **Johns Hopkins University**

Title: **The Role of HMGA2 in Normal & Leukemic Stem Cells**

Abstract of stem cell research (as submitted by Principle Investigator):

Human embryonic stem cells (hESCs) are defined by 2 basic characteristics: 1) Self-renewal or the capability to form new stem cells, and, 2) Pluripotency or the potential to develop into any mature tissue. Because of these unique properties, hESCs have the extraordinary potential to replace tissues lost by damage, defective genes, or cancer. Thus, elucidating the cellular pathways that give rise to hESC properties should enable us to ultimately harness these cells for use in regenerative medicine and cancer therapy. Our proposed studies are directed at understanding the genes that regulate stem cell properties. Our focus is the HMGA2 gene because preliminary studies suggest that it plays an important role in this process. HMGA2 is expressed at high levels during development of the human embyo and in hESCs. Moreover, HMGA2 expression falls when human stem cells are induced to differentiate or form mature tissues. HMGA2 expression also decreases with aging, indicating that it could be responsible for the loss of functional stem cells with advancing age. These findings suggest that HMGA2 functions in the survival or growth of hESCs. Like other genes involved in this process, HMGA2 is overexpressed in aggressive, refractory cancers arising from diverse tissues, including the blood, lung, breast, prostate, and pancreas. In fact, our group recently showed that HMGA2 protein levels correlate with more advanced disease in both lung and pancreatic cancer. These results are consistent with the idea that cancer cells with stem cell properties (or cancer stem cells) lead to aggressive cancers that are refractory to therapy because of their inherent stem cell properties, including high levels of proteins involved in the removal of toxic agents and low baseline growth rates. This latter characteristic could confer resistance to treatment because most chemotherapy is directed at cells that are actively dividing. Thus, we hypothesize that HMGA2 functions in the survival or growth of stem cells, both in normal development and cancer. We hypothesize further that this property contributes to the refractory nature of cancers overexpressing HMGA2. To study HMGA2, we developed genetically engineered cell lines that express high levels of HMGA2 and techniques to block its function in stem cells. Using our unique reagents, we now propose the following Specific Aims: 1.) Define the role of HMGA2 in the survival and growth of normal hESCs, and, 2.) Determine if HMGA2 and downstream pathways are dysregulated in cancer stem cells. Results from these studies will advance our knowledge of normal hESCs and stem cells important in cancer. A better understanding of the cellular pathways that underlie stem cell properties should lead to the discovery of novel therapies directed at cancer stem cells and the capability to use stem cells in regenerative medicine for diseases affected by abnormalities or damage to normal stem cells.

Name of Principle Investigator: **Mariusz Karbowski** Project Budget**: $ 230,000.00**

Grantee: **University of Maryland Biotechnology Institute**

Title: **Maintenance of Mitochondrial Fusion and Fission as a Critical Factor in Stem cell viability, Differentiation Capacity and Differentiation-induced Mitochondrial Biogenesis**

Abstract of stem cell research (as submitted by Principle Investigator):

Mitochondria are intracellular structures essential for a variety of cellular functions, including energy conversion, synthesis of lipids and buffering of calcium. Moreover, a number of major cell signaling pathways, including programmed cell death, require mitochondria. Within a single cell, the mitochondria exist as two interconverting forms, long tubules and small round vesicles, which are balanced in a dynamic organizational equilibrium (also denoted as mitochondrial network dynamics) through fusion and division processes. Consistent with the major role of mitochondrial network dynamics in the control of cell function, mutations or deletions of proteins regulating mitochondrial fusion and division result in severe early developmental abnormalities. Based on the published studies it is likely that proteins vital for merging (via mitochondrial membrane fusion) and dividing mitochondria also regulate mitochondrial function in stem cells and contribute to early transitions during embryonic development. However, despite this potential importance, the role of mitochondrial structural dynamics and mechanisms controlling conversion of stem cell mitochondria into specialized mitochondria in differentiated cells, as well as the impact of mitochondrial network dynamics on stem differentiation are largely unknown.The present proposal seeks to elucidate the functions of mitochondrial fusion- and division-regulating proteins in human embryonic and mesenchymal stem cell fitness and differentiation capacity, as well as their roles in formation of "adult" cell mitochondria, a process inescapably associated with stem cell differentiation. Biochemical and cellular studies, live-cell imaging investigations, modern fluorescent tools (including photoactivable fluorescent proteins), and a variety of molecular genetic methodologies will be utilized to address the following two problems:

1) We will explore the basic status of mitochondrial fusion and fission (division), and proteins regulating these processes in human embryonic and mesenchymal stem cells, and how they change upon stimuli inducing distinct modes of stem cell differentiation.

2) We will also explore the extent to which mitochondrial fusion and fission contribute to stem cell differentiation into distinct types of "adult" cells. The role of mitochondrial fusion and fission in transitions of "stem" mitochondria into "specialized" mitochondria in differentiated cells will be also investigated here.

Addressing these questions will improve the general understanding of mitochondrial function in stem cell fitness and differentiation capacity, and in the long term, are likely to contribute to the development of novel pharmacological approaches to treat diseases stemming from mitochondrial dysfunctions and to improve efficiency of stem cell-based therapies. The proposed studies are part of our long-term effort to understand the normal functions of mitochondria and how mitochondrial defects contribute to disease.

Name of Principle Investigator: **Irina Kolosova** Project Budget**: $ 230,000.00**

Grantee: **Johns Hopkins University**

Title: **Role of hypoxia-induced mitogenic factor in regulating proliferation and differentiation of human stem cells**

Abstract of stem cell research (as submitted by Principle Investigator):

Hypoxia-induced mitogenic factor (HIMF) is a protein specifically produced by lungs. In healthy, normal lungs HIMF is present in minimal amounts, but its production is increased during certain lung diseases, including pulmonary hypertension, allergic inflammation, lung fibrosis, and asthma. HIMF stimulates division of lung cells, inhibit cell death, causes growth of new blood vessels, and sometimes causes pathological thickening of small blood vessels in the lung, leading to breathing abnormalities. We have discovered that HIMF attracts stem cells from bone marrow into lungs of laboratory mice and rats, where they become part of the small blood vessels. Stem cells have the potential to develop into many different cell types in the body (process called "differentiation") serving as a repair system for the body. However, sometime these cells may contribute to undesirable, pathological growth, such as cancer and atherosclerosis. Possibly, HIMF causes stem cells recruited to blood vessels to divide faster, thereby contributing to pathological thickening of a vessel wall. The effect of HIMF on marrow stem cells in humans is unknown. We propose to investigate role of HIMF in human stem cell growth and transformation into different specialized types of cells, composing lung tissue. For this purpose, isolated human bone marrow cells together with human lung tissue will be grown in laboratory conditions, and then "stimulated" by adding HIMF to the system. We will be able, using microscopy, and other techniques, to see stem cell differentiation and growth within the lung tissue. These studies will help us to understand effect of HIMF on marrow stem cells fate in lungs, and will contribute to future development of treatment options for common lung diseases.

Name of Principle Investigator: **Gerard Lutty** Project Budget**: $ 230,000.00**

Grantee: **Johns Hopkins University**

Title: **Progenitor cell based therapies for ocular vasculopathies.**

Abstract of stem cell research (as submitted by Principle Investigator):

The human retina consumes more oxygen per gram of tissue than other tissues in the body because of continuous activity in processing images. It requires a lush blood supply to provide oxygen and nutrients to perform these normal functions. If blood supply is compromised (ischemic) and oxygen levels decline, it becomes unhealthy. When ischemic, it makes factors that stimulate growth of new blood vessels that are leaky and fragile. This scenario occurs in branch vein occlusion (BVO) where a major vein in retina becomes blocked. This results in death of adjacent small blood vessels that carry oxygen to the tissue. Both cell types in capillaries, endothelial cells and pericytes, die creating acellular capillaries. This scenario in diabetic subjects is called diabetic retinopathy (DR). In DR, high glucose kills pericytes then endothelial cells, resulting in acellular capillaries. If one could repopulate acellular capillaries with new cells and restore blood flow to affected areas, ischemia and complications of DR and BVO might be prevented. Branch vein occlusion and DR are the major causes of new blindness in the US. Vascular progenitors hold the promise of being able to differentiate into pericytes and endothelial cells and regenerate viable capillaries from acellular ones. There are two kinds of progenitors that actually make blood vessels of the eye during development, angioblasts and hemangioblasts. We have shown that retinal angioblasts become both pericytes and endothelial cells and they differentiate to form blood vessels in fetal human. The choroidal vasculature, lies under retina and serves outer retina, forms by hemo-vasculogenesis, the differentiation of blood vessel cells and blood cells from hemangioblasts. Our collaborators Drs. Zambidis and Park have generated both of progenitors from human embryonic stem cells (hESCs). They will also make for our studies both progenitors from induced pluripotent (iPS) cells, which can be generated from a patient's skin cells. In our proposed studies we will inject either angioblasts or hemangioblasts into vitreous or liquid in eyes of mice. The mice will undergo an ischemia/reperfusion event (block blood flow and restart it), a model for BVO, or experimental diabetes. Both insults result in acellular retinal capillaries. Our experiments demonstrate which progenitor can repopulate acellular capillaries, make functional blood vessels, and return blood flow to ischemic areas. We will investigate which progenitor can become endothelial cells, pericytes or both. To make functional blood vessels, both cell types may need to be present. These studies hold the promise of using a person's skin cells to make iPS cells that become vascular progenitors, which could repopulate acellular retina capillaries. If successful, a BVO or DR patient's cells could be injected into their vitreous and degenerated capillaries regenerated, relieving ischemia in retina and preventing the blinding complications of BVO and DR.

Name of Principle Investigator: **Hai-Quan Mao** Project Budget**: $ 230,000.00**

Grantee: **Johns Hopkins University**

Title: **Matrix-Assisted Schwann-Cell Differentiation from Human Induced Pluripotent Stem Cells for Peripheral Nerve Regeneration**

Abstract of stem cell research (as submitted by Principle Investigator):

Schwann cells play a pivotal role in the maintenance and regeneration of axons in the PNS. They can be used for treating neural demyelinating diseases and traumatic injuries in the peripheral nervous system, for example, Charcot-Marie-Tooth hereditary peripheral neuropathy, and spinal cord injuries. To realize this potential, a reliable Schwann cell source and a robust method for generating sufficient and high quality functional Schwann cells. Recent studies have demonstrated the feasibility of generating Schwann cells from human pluripotent stem cells. Here we propose to develop a nanotechnology-based cell culture method to enhance the efficiency in generating Schwann cells from human induced pluripotent stem (iPS) cells, which are derived from human skin fibroblasts. We choose human iPS cells because of the potential of generating clinically applicable and patient-specific cells.
This proposed research builds on our novel technology to enhance the oligodendroglial differentiation of neural stem cells using biomimetic nanofiber matrices. We will test the hypothesis that nanofiber topographical cues can similarly promote the differentiation of iPS cell-derived neural crest stem cells and enhance the maturation of Schwann cells.
To achieve this goal, we will first characterize the effect of nanofiber matrix cues on differentiation of hiPS cell-derived neural crest stem cells, develop a more effective method to generate Schwann cells, and characterize myelination ability of the generated Schwann cells in vitro. These experiments will demonstrate the feasibility to improve the directed differentiation of functional Schwann cells from human iPS cells using nanofiber matrix.
We will then characterize the effect of nanofiber matrix cues on the maturation of the generated Schwann cells, and demonstrate the advantage of seeding iPS-derived Schwann cells in nanofiber nerve guide for peripheral nerve regeneration in a rat sciatic nerve transection model. This study promises to develop a more effective method to generate Schwann cells from human iPS cells by using functional nanofiber matrix, and to demonstrate functions of these iPS cell-derived Schwann cells in vitro and in vivo. If successful, this study will provide a key technology to generate patient-specific autologous Schwann cells, and demonstrate the potential of using iPS cell-based therapies in treating neurodegenerative diseases and traumatic injuries in the peripheral nervous system.

Name of Principle Investigator: **Nicholas Maragakis** Project Budget**: $ 230,000.00**

Grantee: **Johns Hopkins University**

Title: **Development of iPS cells for ALS research**

Abstract of stem cell research (as submitted by Principle Investigator):

Amyotrophic lateral sclerosis (ALS) is the most common form of adult motor neuron disease in which there is progressive degeneration of both the upper motor neurons in the cortex and the lower motor neurons in the brainstem and spinal cord. The majority of ALS cases are apparently sporadic (SALS), accounting for greater than 90% of reported cases. The remainder of these cases are inherited--classified as familial ALS (FALS). In 1993 FALS cases were found to be associated with mutations in superoxide dismutase (SOD1. It now appears that SOD1 mutations account for about 10% of all familial cases of ALS. Since then several SOD1 transgenic mouse models of ALS have been developed. While these animal models recapitulate the relative selective loss of motor neurons resulting in limb paralysis and death, they may only represent a small proportion of relevant ALS pathobiology—familial ALS. Because modeling ALS has been largely limited to the use of the SOD1 animal models, a significant proportion of ALS biology (particularly relevant to sporadic ALS) has gone largely unstudied.
Recently, an exciting technique called induced pluripotent stem cell (iPSC) methodology has allowed for the generation of pluripotent stem cells from skin fibroblasts. This methodology holds great promise in medicine. Most basically, the ability to produce pluripotent cells from somatic cells obviates the need for obtaining human embryonic stem cells and at least easing this ethical dilemma. The technique is also powerful because we can obtain tissue not only from a few ALS patients, but the hundreds that are seen in our clinic at Johns Hopkins University each year. This allows us to create cell lines of patients with a variety of ALS presentations and allows us to ask questions regarding differences in speed of disease progression, location of disease onset, and the prominence of one feature over another. We can also sample tissue from patients with both familial ALS (particularly those with the SOD1 mutation) as well as subjects with sporadic ALS. This will now allow us to compare how those diseases differ. For the induction of pluripotent stem cells, we will use a technique utilized in the laboratory of Dr. Hongjun Song. After iPS cells have been created, these cell lines can be used to generate two of the most important cell types in ALS—motor neurons and astrocytes. This will allow investigators to understand how these cells interact with each other in ALS. Additionally, when adequate samples of iPS cells from ALS patients are derived they can be put to other uses including screening drug candidates in a highly efficient manner using existing technologies. Other potential used for these iPS cell-derived motor neurons and astrocytes is for potential use in autologous transplantation into subjects with ALS. This proposal represents the first step in the further development of this emerging technology.

Name of Principle Investigator: **Avindra Nath** Project Budget**: $ 230,000.00**

Grantee: **Johns Hopkins University**

Title: **Inflammatory cells and Neuronal Stem Cell Functions: A New Frontier for Therapeutic Stem Cell Transplantation in Neurodegenerative Diseases**

Abstract of stem cell research (as submitted by Principle Investigator):

If stem cell therapy is to become a reality for treatment of neurological disorders, close attention would be needed to the environment within the brain in which these cells will be transplanted. Neurological diseases are often accompanied with an inflammatory process which could be detrimental to the survival and proper functioning of the stem cells. Unless we are able to control this inflammatory process, stem cell therapy may not be viable. Preliminary data from our laboratory suggests that when the brain stem cells are exposed to molecules released as a result of the inflammation they cause the production of a unique protein on the surface of the cells. This protein results in the leak of ions into the cells triggering a cascade of events causing the cell to become dysfunctional. In this proposal we will further explore how the cells that form the part of the inflammatory process alter the expression of this protein and if compounds that block this protein can protect the brain stem cells from the effects of the activated immune system. Such an approach is likely to identify novel modes of drug treatment that could eventually be used in conjunction with stem cell therapy to protect the transplanted cells.

Name of Principle Investigator: **Sridhar Nimmagadda** Project Budget**: $ 230,000.00**

Grantee: **Johns Hopkins University**

Title: **Brain Tumor Stem Cell Imaging With Radiolabeled Antibodies**

Abstract of stem cell research (as submitted by Principle Investigator):

Brain cancers, particularly glioblastoma, are often associated with high recurrence and poor survival rates. Recent findings indicate that, a sub population of cells within these tumors (called Brain Tumor Stem Cells, BTSCs) play a critical role in cancer recurrence and do so by evading the conventional radiation and chemotherapeutic treatments. Identification of these cells in vivo has significant therapeutic and prognostic value. CD133 expression is one of the criteria for the identification of BTSCs. We hypothesize that radiolabeled antibodies of CD133 could be used to detect BTSC population in vivo. We intend to achieve this goal using highly sensitive imaging techniques such as Positron Emission Tomography aided by signal amplification methods. Such non-invasive and repetitive detection will be useful in identifying and specific targeting of BTSCs, which in combination with conventional chemotherapy may reduce the tumor stem-cell burden and minimize the recurrence of the tumor. More importantly, the same strategy could be extended to other cancers to detect the cancer stem cell population in vivo. If successful, i.e., selective stem cell imaging can be achieved in vivo, the path to therapeutic applications such as functionalization of the antibodies with a therapeutic radionuclide such as 131I, 90Y or 213Bi or immunoconjugates – including clinical translation – will be vigorously pursued.

Name of Principle Investigator: **Sridhar Nimmagadda** Project Budget**: $ 230,000.00**

Grantee: **University of Maryland, Baltimore**

Title: **Ligand presentation directs neural stem cell fate decisions**

Abstract of stem cell research (as submitted by Principle Investigator):

The promise of stem cells to cure human disease is tempered by our lack of scientific knowledge about stem cell development and few tools for producing specific cells in vitro. These problems are interconnected, for if we had the proper tools to expand and differentiate stem cells, then we would have a better understanding of stem cell biology. This proposal outlines biomaterial scaffolds to study human neural stem cells in vitro. The scaffolds will serve as a research tool for studying cells in three-dimensions and also as a platform for the amplification and targeting of specific cell types for clinical use. This proposal combines state of the art biomedical neural engineering technology with the forefront of developmental biology. The project translates basic science research in the mouse to the human neural stem cell and investigates new materials to produce neurons for clinical applications.

Name of Principle Investigator: **Linda Resar** Project Budget: **$ 230,000.00**

Grantee: **Johns Hopkins University**

Title: **Molecular Pathways Induced by HMGA1 in Embryonic Stem Cells**

Abstract of stem cell research (as submitted by Principle Investigator):

Human embryonic stem cells (hESCs) are characterized by 2 basic properties: 1) Self-renewal or the ability to form new stem cells, and, 2) Pluripotency or the potential to develop into any mature tissue. Because of these unique properties, hESCs have the extraordinary potential to replace tissues lost by damage, defective genes, or cancer. Thus, identifying the cellular pathways that give rise to stem characteristics should enable us to harness these cells for use in regenerative medicine and cancer therapy. Our proposed studies are directed at understanding the genes that regulate stem cell properties. Our focus is the HMGA1 gene, which is expressed at high levels during development of the human embyo. We also showed that it is highly expressed in hESCs and its expression falls when hESCs are induced to differentiate or form mature tissues. In fact, its expression parallels that of 2 other genes known to be important in stem cell renewal. Experiments that increase HMGA1 levels in hESCs accelerate the growth rates. These findings suggest that HMGA1 functions in the survival or growth of hESCs. Like other genes involved in this process, HMGA1 is overexpressed in aggressive, refractory cancers arising from diverse tissues, including the blood, lung, breast, prostate, and pancreas. We found high levels in leukemic cells with stem cell properties (or leukemic stem cells). Based on these findings, we hypothesize that HMGA1 functions in the survival or growth of normal and cancer stem cells. To study HMGA1 in cancer, we developed genetically engineered (transgenic) mice that express high levels of HMGA1 and all mice succumb to aggressive blood cancer (leukemia) that closely resembles refractory human leukemia. The leukemia can be transferred to other mice in serial transplant experiments, indicating that at least a subpopulation of leukemia cells possess the self-renewal properties of stem cells. Taken together, these results suggest that HMGA1 is important in the maintenance and renewal of stem cells and our proposed studies are directed at elucidating its role in this process. In fact, we have already identified some pathways induced by HMGA1 that appear to be important in stem cell survival. Using our unique reagents, we now propose the following Specific Aims: 1.) Identify genes regulated by HMGA1 and important in stem cell survival, 2.) Identify naturally-occurring, small regulatory molecules (microRNAs) regulated by HMGA1 and important in stem cell survival, and, 3.) Determine if HMGA1 can promote the induction of pluripotency (or stem cell characteristics) in normal, adult human cells. Results from these studies will advance our knowledge of normal hESCs and stem cells important in cancer. A better understanding of the cellular pathways that underlie stem cell properties should lead to capability to harness stem cells for use in regenerative medicine and the discovery of novel therapies directed at cancer stem cells.

Name of Principle Investigator: **Lewis Romer** Project Budget**: $ 230,000.00**

Grantee: **Johns Hopkins University**

Title: **Harnessing Induced Pluripotent Cells for Pulmonary Microvascular Restitution Using the Focal Adhesion Kinase**

Abstract of stem cell research (as submitted by Principle Investigator):

Respiratory failure is the most prevalent problem encountered in intensive care medicine in America today. Dramatic advances in resuscitation and life support technologies have improved short-term survival for increasing numbers of people whose lungs are severely damaged by causes ranging from premature birth, to infection, cancer treatment, trauma, and house fires. Unfortunately, these advances have only delayed death in the sickest among them, without improving hope for long-term recovery. We propose to develop a novel regenerative therapy that will reverse the course of respiratory failure. A multidisciplinary team with expertise in stem cell biology, blood vessel growth, and clinical medicine will design a new strategy for creating new blood vessels from a critically ill patient's own cells. This will be done through a process called induced pluripotency that gives adult cells from skin or other tissues the capacity to behave like stem cells. The work will proceed in two steps. In the first step we will optimize the process of turning cells with induced pluripotency into cells that can make blood vessels. The second step will use cellular and genetic engineering techniques to produce functional blood vessels from these cells. If we succeed, this research could provide a pathway for recovery for thousands of people facing certain demise from respiratory failure.

Name of Principle Investigator: **Michael Shamblott** Project Budget**: $ 230,000.00**

Grantee: **Hugo W. Moser Research Institute at Kennedy Krieger**

Title: **ECM, Integrins and Endocrine differentiation**

Abstract of stem cell research (as submitted by Principle Investigator):

Type 1 diabetes (T1D) is caused by autoimmune destruction of pancreatic beta-cells and is characterized by insulin insufficiency, poor glycemic control and significant morbidity. ES cells could be an unlimited source of cells for these therapies if a process to generate a safe and effective population of beta-cell substitutes can be established. Most importantly, these cells would be capable of sensing blood glucose and responding with the production, processing and release of physiologically relevant levels of mature insulin. Differentiation of pluripotent stem cells into glucose-responsive insulin producing (GRIP) cells has been challenging. Recent studies have used a wide variety of methodologies to establish the capacity of mouse and human ES cells to generate cells with some capacity to produce insulin. Although promising, the current processes by which GRIP cells are derived from human pluripotent stem cells must be improved before they are ready for therapeutic use. In general, these insulin producing cells or multi-cellular structures are inefficiently produced, making the production of therapeutically viable numbers of cells difficult and expensive. Our long-term goal is to generate cells capable of authentic GRIP function in vitro and restoration of glycemic control in vivo. As a step towards this goal, we evaluated the capacity of human ES cells (hESC) to differentiate into three-dimensional structures containing cells capable of insulin C-peptide synthesis and release, termed islet-like structures (ILS). Like others, we noted the differentiation process results in very high levels of cell death and ultimately, in a low efficiency conversion to C-peptide expressing cells. Loss of contact between cells and extracellular matrix (ECM) is a well established signal for cell death in many developmental contexts and cell types. Cell death due to the loss of ECM contact has been extensively investigated by interfering with the relationship between ECM components and cell surface receptors called integrins. We hypothesize the cell death observed during hESC differentiation is an integrin-dependent process triggered by loss of cell-ECM contact. We propose to test our hypothesis with two independent but complementary approaches. By blocking/neutralizing both ECM components and integrins expressed during the differentiation process we seek to assign roles (i.e. blocking cell death, signals for cell division, effects on cellular composition) to specific ECM-integrin relationships. These findings will provide mechanisms by which ECM compounds improve hESC differentiation into endocrine cells as well as a model to investigate the role of integrins during endoderm, pancreatic and endocrine development.

Name of Principle Investigator: **Hong Song** Project Budget**: $ 229,988.00**

Grantee: **Johns Hopkins University**

Title: **Targeting metastatic pancreatic cancer stem cells by alpha particle radiation**

Abstract of stem cell research (as submitted by Principle Investigator):

Patients with pancreatic cancer suffer a very high rate of mortality with five-year survival of only 5%. The main reason for the poor prognosis of pancreatic cancer is that, in majority of the patients, cancer cells have already metastasized to other vital organs, such as liver, when patients are diagnosed. Very few treatment options are available for these patients. It will be very useful to find out what makes cancer cells metastasize and which fraction of the cancer cells can form metastases in other organs. Recent studies have found that not all pancreatic cancer cells are able to form tumors. Only a small fraction of pancreatic cancer cells, identifiable by some unique proteins they express on their surface, are able to form the whole tumor. These cells are also more resistant to chemotherapy and radiation therapy and they are considered to be the culprit of relapse. Since these small fraction of cells are able to renew themselves and able to grow into a variety of cancer cells within a tumor, they are given the name pancreatic cancer stem cells and are usually identified by their unique surface markers. One study has shown that some pancreatic cancer stem cells, expressing the unique surface marker CD133+CXCR4+, are more likely to form metastases. If this is the case, we want to test a novel strategy to kill these metastatic pancreatic cancer stem cells. We will use a monoclonal antibody that can specifically target and bind one of the unique surface markers cancer stem cells express. We will then attach radioactive atoms emitting alpha particles to the monoclonal antibody. Alpha particles are helium nuclei that deposit very high energy along the track they travel. When they hit cell nucleus, they will cause severe damage to the DNA double strand helix; the level of damaged caused by alpha particles is too great for cancer cells to repair and the cells will be killed. Since cancer stem cells are usually more resistant to low energy radiation, alpha particle radiation provides a unique approach to kill these pancreatic cancer stem cells. We have demonstrated in a metastatic breast cancer model that alpha particle labeled monoclonal antibody is very effective to control metastatic growth. In this proposal, we aim to answer these questions: 1. Are pancreatic cancer stem cells responsible for metastasis? 2. Can pancreatic cancer stem cells be targeted by their surface markers? 3 Are alpha radiations more effective at killing cancer stem cells than external beam radiotherapy? The answers to these questions will improve our understanding of the role pancreatic cancer stem cells play in metastasis and also assess the effectiveness of alpha radiation to kill cancer stem cells.

Name of Principle Investigator: **Karl Womer** Project Budget**: $ 230,000.00**

Grantee: **Johns Hopkins University**

Title: **Immunoregulatory Function of Pancreas Mesenchymal Stem Cells**

Abstract of stem cell research (as submitted by Principle Investigator):

In this project, we will study the ability of a certain type of stem cell in the pancreas (Mesenchymal Stem Cell) to dampen the body's immune system in a way that prevents damage to islet cells transplanted in patients with type-1 diabetes. Type-1 diabetes is cause by destruction of islet cells in the pancreas by the body's immune system. Since these cells produce insulin for the body, patients with this condition must go on life-long insulin treatment to keep their blood glucose levels normal. Alternative therapies to reverse elevated glucose levels are to transplant a whole pancreas or simply to transplant the islet cells alone. Islet cell transplantation is plagued by problems with early islet damage due to responses in the recipient called innate immune responses. Furthermore, immunosuppressive medications are required to prevent later damage due to responses called allogeneic immune responses (i.e. rejection) that see the islet cells as foreign invaders. Both of these types of immune responses shorten the life of transplanted islet cells, forcing the patient to resume insulin therapy.
Stem cells have the ability to regenerate damaged tissue in the body. A special type of adult stem cell, called mesenchymal stem cells (MSC), also has the ability to dampen immune responses. MSC have been found in virtually every organ in the body and may be important in preventing inflammation after organ injury to allow healing to occur. In our proposal, we plan to isolate MSC from adult human pancreas tissue to study their ability to dampen immune responses of human blood cells in culture. Next we will test their ability to dampen immune responses from isolated human islet cells in culture. These experiments are "test tube" equivalents to the events that likely occur during actual islet transplantation. Finally we will use a "humanized" mouse model that has a defective immune system. Because of this defect, human immune cells injected into this mouse are free to function as they would in a normal human. Furthermore, human islet cells can be transplanted into this mouse and will be rejected by the human immune cells as would occur in a human without immunosuppressive medications. This model allows us to study rejection of islet cell transplants without harming actual human patients. We will transplant pancreas MSC with the islet cells and expect that their ability to dampen the immune response will prevent early islet damage due to innate immune responses and later rejection. Our findings, because they are performed in "humanized" mice, can be directly applied to patients and will hopefully lead to therapies to improve the survival of islet cell transplants in human patients and therefore prevent the long-term consequences of type-1 diabetes, including kidney disease, blindness, heart disease and nerve damage. Such therapy would likely stimulate jobs and promote further scientific collaborations in the state of Maryland.

Name of Principle Investigator: **Shuli Xia** Project Budget**: $ 230,000.00**

Grantee: **Hugo W. Moser Research Institute at Kennedy Krieger**

Title: **Differentiation Therapy of Glioblastoma Stem Cells**

Abstract of stem cell research (as submitted by Principle Investigator):

Malignant gliomas are amongst the most devastating and intractable of all cancers. Glioblastoma multiforme (GBM) is the most malignant glial neoplasm. Despite considerable progress in modern tumor therapy including aggressive surgery, radiotherapy and chemotherapy, the median survival duration for patients with GBM is about 14 months; and the majority (>70%) die within 2 years. Understanding the cellular and molecular basis for the resistance of these cancers to current therapies is critical to improving patient care, quality of life and survival. Recent studies have demonstrated the existence of glioblastoma stem cells within gliomas. When these cells grow, they are able to replace themselves and generate more differentiated cancer cells. If implanted to rodent brain, these cancer stem cells are capable of forming aggressive tumors that mimic real human GBM. When placed under special conditions in the laboratory, these cells can also "differentiate" to resemble specific neuronal cell types (e.g. astrocytes, neurons). It is currently believed that GBM stem cells are required to maintain GBM growth and it is possible that a single surviving GBM stem cell can cause the cancer to recur. According to this, tumors have a cellular hierarchy consisting of resting cancer stem cells and more proliferating progenitor cancer cells. Conventional radiotherapy and chemotherapy mainly target the proliferating cancer cells and spare cancer stem cells because these cells are normally protected from cell death induced by conventional therapy due to their special features. This predicts that curing glioblastoma will require therapy that either kills the malignant stem-cells or permanently prevents them from growing. In this proposal, we will determine if strategies that specifically target GBM stem cells (GBM-SCs) will efficiently inhibit tumor growth in animals. We have found that all-trans retinoic acid (RA) induces differentiation and growth arrest of GBM-SCs. If combined with other cell death-inducing agents, RA induces cell death of GBM-SCs. In addition, RA pretreatment reduces tumor growth from GBM-SCs. Here, we will determine the molecular and cellular responses to RA in multiple GBM stem cell models. For animal studies, we will rigorously examine the effect of different RA treatment regimens on tumor propagation in animals and optimize the conditions to treat animals bearing tumors generated by GBM-SCs. Positive results could lead to the development of new anticancer strategies. Identifying the molecular basis of RA response will contribute to the discovery of clinically translatable biomarkers for predicting the cancer stem cell pools likely to be responsive to RA-based differentiation therapy. These discoveries will lead to strategies for manipulating the pathways to therapeutic advantage.

Name of Principle Investigator: **Shuli Xia** Project Budget**: $ 230,000.00**

Grantee: **University of Maryland Biotechnology Institute**

Title: **P-QD hESC Labeling and Its Application on Cell Transdifferentiation**

Abstract of stem cell research (as submitted by Principle Investigator):

Stem cell holds tremendous promise for treating a wide variety of human diseases. However, recent research has yielded conflicting results in both experimental and clinic fields. These inconsistent results are especially prominent in cardiovascular stem cell therapy. In this proposal, we propose a unique hESC labeling approach, the peptide-mediated quantum dots (P-QDs) hESC labeling system. By using cell membrane and nuclear specific fusion peptides with the QD features of antiphotobleaching and strong fluorescence, this approach promises the best specificity and sensitivity for immunostaining analysis which will lead to more accurate identification of trans-differentiated cells. The peptides driven by heart specific promoters (MHC) and non-tissue specific pCMV promoter will provide an extremely sensitive tag to track fate of the stem cells in cell differentiation to reveal the detailed mechanism of tissue repair in stem cell therapy. In this application, we propose to: (1) Generate cell membrane specific targeted hESC and nuclear membrane specific targeted hESC by using fusion peptides of FLAG/c-myc, and transmembrane domain (TMD) of E-cadherin (Ecad) / TMD of Lamin B receptor (LBR); (2) Characterize the pluripotency of the cell and nuclear membrane specific labeled hESC by analyzing the expressions of Oct-3/4, rex-1 and Nanog genes and describing the differentiation in teratomas with mouse testis transplantation assay; (3) Cell differentiation studies in the cardiosphere formed by the labeled hESC will be performed to explore the possibility of trans-differentiation from hESC to heart cell using cardiomyocyte differentiation assay. Subsequently, application of the cell and nuclear membrane specifically labeled hESC will be performed in ischemic heart models to track the transdifferentiation from hESC to heart cells. The plasmalemma and nucleolemma labeled hESCs present a strong capability to distinguish cell differentiation in vivo with QD fluoresced plasmalemma and nucleolemma via double immunostaining analysis. The P-QD labeled mouse ESC (mESC) and adult stem cells (mASC) will be generated through the transgenic approach as well. Establishment of the P-QD targeting approach is not restricted to just heart tissue. When different tissue-specific promoters are used, the approach can be adapted to many fields of molecular biology. Therefore, P-QD approach would yield a series of labeled hESC, mESC and mASC that have great marketing potential as biotechnology products as well.

FY 2009

Post Doctoral Fellowship

Awarded

Research Descriptions

Name of Principle Investigator: **Julio Altamirano** Project Budget**: $ 110,000.00**

Grantee: **University of Maryland Biotechnology Institute**

Title: **Calcium signaling in human embryonic stem cells-derived cardiac myocytes and their functional interactions with acutely isolated rat ventricular myocytes in primary culture.**

Abstract of stem cell research (as submitted by Principle Investigator):

In the adult human heart, differentiated muscle cells display a limited or null capacity to replicate, and the healthy myocardium responds to increased metabolic demands by modifying gene expression leading to hypertrophy and enhancing cardiac performance. However, when pathological conditions cause cardiac cell injury (e.g., myocardial infarction; MI), gene regulation is altered, and this causes further myocardium deterioration, heart failure (HF) and death. It has been repeatedly suggested that stem cells (SC) from various sources (e.g., mesenchymal or embryonic)1,9,13 and delivered by different methods (e.g. intracoronary or intramyocardial) can engraft in the heart, and help to improve cardiac performance or diminish HF progression after myocardial injury. However, it is still unclear whether SC transform into functional myocytes, with normal Ca2+ cycling and with permanent electrical and mechanical connections with the host tissue, or whether they fuse with existing cells, or act through paracrine actions on the surrounding native cardiac cells enhancing their survival and performance1,9, 13. This controversy arises in part because the individual functional properties of engrafted cells are hard to study in situ7,9,13, and also because, particularly in cardiac myocytes differentiated from human SC in vitro, the molecular characterization of Ca2+ signaling is still incomplete7,9,11,13. Available human embryonic (hE) SC lines propagate indefinitely in culture, retaining pluripotency12, however, differentiation into cardiac myocytes can be induced4,7-9,11,13, as evidenced by specific cardiac muscle molecular markers (α and βMHC, MLC-2v, etc)7-9,11, and they might represent a reliable source for cell therapy4,7,12. Mouse- (mESC) and hESC-derived cardiac myocytes (dCM) in vitro resemble native cardiac ventricular or atrial myocytes at early developmental stage or pace maker sinoatrial node (SAN) cells. These cells develop spontaneous action potentials, contract and present sub-cellular and whole cell Ca2+ cycling events, similar to those in native cardiac cells4,8,10,13. However, the molecular determinants underlying the Ca2+ events in hESC-dCM have not been thoroughly investigated8,11,13. Since engrafted hESC-dCM are expected to replace damaged contractile ventricular myocardium, where normally intrinsic pacemaker activity is null, then ideally their electrical and Ca2+ signaling properties should match those of the surrounding ventricular tissue. Otherwise, spontaneous or heterogeneous electrical activity of engrafted cells might lead to ventricular arrhythmia13. Furthermore, these myocytes should establish permanent mechanical contact and electrical continuity (gap junctions) with native cells to synchronize myocardial activity4,7,13. These issues will be assessed in this proposal by studying the functional properties of hESC-dCM in vitro, cultured alone and by co-culturing them with acutely isolated rat ventricular myocytes under various experimental conditions. The translational potential of the proposed basic research studies resides on the fact that for the effective use of hESC-dCM in therapeutic procedures, aimed to enhance or restore human cardiac function after myocardial injury, it is extremely important to understand at the molecular level the intrinsic functional properties of the derived cardiac myocytes. Equally important is also to understand how those hESC-dCM interact with the host tissue, and whether those interactions are permanent and have feedback effects on the functions of both cell types; host and engrafted cells. Therefore, the proposed studies will help us to: A) understand in greater detail the properties, time course of development and regulatory factors of Ca2+ signaling, Ca2+ cycling proteins expression and electrophysiological properties of hESC-dCM, and B) assess the potential of hESC to engraft within the injured heart and transform into viable muscle cells that interact electrically and mechanically with the surrounding host muscle.

Name of Principle Investigator: **Amy Belton** Project Budget**: $ 110,000.00**

Grantee: **Johns Hopkins University**

Title: **The Role of HMGA1 in Intestine and Colon Cancer Stem Cells**

Abstract of stem cell research (as submitted by Principle Investigator):

Problem: The molecular underpinnings that give rise to stem cell properties in the intestinal crypt cells are poorly understood. To enhance our understanding of intestinal stem cells, we are investigating molecular pathways that contribute to the maintenance of stem cells in the gastrointestinal tract. Background: Our focus is the high-mobility group-A1 (HMGA1) oncogene, which is highly expressed in high-grade colon cancer, human embryonic stem cells (hESCs), and during embryologic development. The HMGA1 gene encodes the HMGA1a and HMGA1b protein isoforms, which are members of a family of non-histone, chromatin remodeling proteins. These proteins bind to the minor groove of AT-rich regions in the B-form of DNA and recruit additional transcriptional factors. Together with other factors, HMGA1 proteins function in modulating gene expression. We showed that HMGA1 expression falls as hESCs differentiate, indicating that it may function in the maintenance of stem cell populations. In fact, its expression pattern mirrors that of NANOG and SOX-2, two genes important in stem cell self-renewal. Hypothesis: Based on these findings, we hypothesize that HMGA1 has a role in stem cell maintenance or proliferation. Research: To test this hypothesis, we engineered HMGA1a transgenic mice, all of which succumb to aggressive lymphoid tumors; the females also develop uterine sarcomas. Observations: We found that the HMGA1a transgenics develop gastrointestinal polyps and proliferative changes in the intestinal epithelium. Histopathologically, the transgenic intestines are characterized by a thickened mucosal surface, hyperproliferative changes, and hamartomatous polyps. Immunohistochemical analysis demonstrates an increase in the population of intestinal cells expressing the colon stem cell marker Lgr-5 (or leucine-rich repeat-containing G protein-coupled receptor 5) in the transgenic compared to wildtype mice. These findings suggest that HMGA1 is driving expansion of the crypt stem cell niche in the gastrointestinal tract. We and others have also shown that HMGA1 is overexpressed in high grade colon cancer. Our study found high levels of HMGA1 in >70% of high-grade tumors. Our preliminary results also show that inhibiting HMGA1 expression interferes with tumorigenicity in colon cancer xenografts. Studies are underway to determine if knock-down of HMGA1 alters the number of colon cancer cells with stem cell-like properties. Conclusions: Although further studies are needed, our findings suggest that HMGA1 promotes expansion of the stem cell compartment in the gut. Our results also demonstrate that HMGA1a promotes polyp formation in mice and possibly colon cancer in humans by conferring a stem cell phenotype in colonic cells.

Name of Principle Investigator: **Paul Burridge** Project Budget**: $ 110,000.00**

Grantee: **Johns Hopkins University**

Title: **Differentiation of Human Pluripotent Stem Cells to Mesoderm and Cardiomyocytes**

Abstract of stem cell research (as submitted by Principle Investigator):

Cardiovascular disease is the leading cause of death in the industrialized world. Cardiomyocyte generation from human pluripotent stem cells offers unique opportunities for cellular therapies that replace damaged heart muscle. However, this strategy requires a greater understanding of the genetic programs that control early human cardiac development, which has been impeded in part due to the inaccessibility of the post-implantation human embryo. To better clarify the genetic programs associated with human cardiogenesis, we used microarray technology to identify genes that are specifically expressed during the development of human embryonic stem cells (hESC) to the cardiac lineage. We utilized a novel and highly efficient hESC cardiomyocyte differentiation system that has been demonstrated to closely mimic in vivo cardiovascular developmental ontogeny. Using this optimized human embryoid body (hEB) differentiation protocol that employs chemically defined and animal product-free media, we produced beating hEBs after 9 days of differentiation with greater than 90% efficiency. We initially established the developmental kinetics for expression of pluripotency markers, cardiac mesoderm, and committed cardiac progenitors using real-time quantitative PCR methods. We then performed microarrays to compare the transcriptomic profiles for each of these populations. These data allowed identification of novel transcripts that likely direct the mesodermal differentiation of human cardiogenesis from a pluripotent state. A cross-comparison to similar data from mouse embryonic stem cell cardiac differentiation, as well as whole mouse embryos will allow the confirmation of in vivo biological relevance to correlative cardiac structures during murine development. These data also demonstrate the power of integrating genomic approaches with efficient methods for human pluripotent stem cell differentiation.

Name of Principle Investigator: **Wen-Chih Cheng** Project Budget**: $ 110,000.00**

Grantee: **University of Maryland, Baltimore**

Title: **Hippo Signaling Pathway in Control of Human Hematopoiesis**

Abstract of stem cell research (as submitted by Principle Investigator):

The Hippo pathway is a well-conserved signaling pathway that controls organ size from Drosophila to mammals. Composed of a kinase cascade, the Hippo kinase (Hpo) cooperates with the WW domain-containing Salvador (Sav) to phosphorylate and activate the Warts kinase (Wts). In turn, the activated Warts phosphorylate and inactivate Yorkie (Yki), a transcription coactivator that partner with the TEAD family transcription factor Scallopad (Sd). Since Yki and Sd transactivate genes including cell cycle regulators (e.g. cyclin E) and cell death inhibitors (e.g. inhibitor of apoptosis), inactivation of Hpo or Wts, or overexpression of Yki in Drosophila results in organ/tissue overgrowth characterized by excessive cell proliferation and diminished apoptosis. Mammalian homologs of the Hippo pathway: MST1/2 (Hpo), WW45 (Sav), LATS1 (Wts), YAP1 (Yki) and TEAD2 (Sd) constitute an analogous transduction cascade. Transgenic overexpression of YAP1 in mouse liver results in reversible increase of liver due to increased proliferation and decreased cell death, indicating that Hippo signaling pathway is an evolutionary conserved mechanism for organ-size control. Growing bodies of evidence suggests that Hippo signaling pathway may regulate stemness and the self-renewal properties of stem-progenitor cells: (1) transcriptional profiling of 3 types of murine stem cells (embryonic, neuronal and hematopoietic) identified both YAP1 and TEAD2 as 2 of the only 14 transcription factors commonly expressed in all 3 types of stem cell; (2) in many adult human tissues (e.g. lung, pancreas and prostate), YAP1 is preferentially expressed in stem-progenitor cell compartments; and (3) transgenic overexpression of YAP1 in murine intestine and chick neural tubes results in massive expansion of progenitor cells and concomitant loss of differentiated cells, a phenotype that is completely reversed upon cessation of YAP1 overexpression. Given the conserved role of the Hippo pathway in stem-progenitor cells of multiple lineages, we propose to employ loss-of-function and gain-of-function studies to investigate the role of Hippo signaling in controlling stemness in the hematopoietic system.

Name of Principle Investigator: **Tara Deans** Project Budget**: $ 110,000.00**

Grantee: **Johns Hopkins University**

Title: **Directing Mesenchymal Stem Cell Differentiation by Combining Principles of Synthestic Biology and Materials Science**

Abstract of stem cell research (as submitted by Principle Investigator):

Mesenchymal Stem Cells (MSCs) are found in the bone marrow and have the potential to differentiate into bone, cartilage, muscle, fat , and tendons. While methods for differentiating these cells in vitro are known, directing and controlling them in vivo remains a challenge. Having the ability to direct MSC differentiation using intelligent cell-biomaterial systems holds great promise for therapeutic applications for musculoskeletal diseases and tissue repair. The overall goal of this project is designed to provide a comprehensive assessment of the utility of engineering biomaterials to trigger genetic switching of gene circuits that produce key factors to be used for directing stem cell differentiation. Directing MSC differentiation is critical to therapeutically driving lineage choices in stem cells. The proposed system has the potential to provide precise and controlled amounts of growth factors in vivo and it offers a novel approach to facilitate healing and repair to damaged tissue. Biomaterials that can switch the production of growth factors on and off have significant advantages over current methods for delivering these factors. Although in vivo experiments are beyond the scope of this proposal, the next step to this study is to establish functionality in animals. Moreover, the genetic circuits can be engineered to function as sensors to detect injured tissue and/or the onset of disease and respond by driving stem cell differentiation to a specific lineage.

Name of Principle Investigator: **Tomas Garzon-Muvdi** Project Budget**: $ 110,000.00**

Grantee: **Johns Hopkins University**

Title: **ROLE OF THE INTERACTION BETWEEN CATION-CHLORIDE COTRANSPORTERS and EGF AND PDGF IN MIGRATION OF BRAIN TUMOR STEM CELLS.**

Abstract of stem cell research (as submitted by Principle Investigator):

Among brain tumors, glioblastoma multiforme (GBM) is the most frequent and destructive primary malignant brain tumor 1, 2. Its infiltrative nature limits the effectiveness of surgical excision and thus, is one of the main causes of its high recurrence of ~ 99% 3. Brain Tumor Stem Cells (BTSCs) are thought to be a part of the heterogeneous cell population in brain tumors. Like their normal counterpart, normal neural stem cells (NSCs), BTSCs have the ability to proliferate, self-renew and differentiate into multiple cell lineages, additionally, they are tumorigenic 4, 5. Migration of BTSCs is fundamental for their invasion into healthy brain parenchyma. Increasing evidence shows that regulation of cell volume and intracellular Cl- concentration ([Cl-]i) is crucial for the migratory ability of cells 6. The role of Cl- channels of the CLC family has been studied in GBM cell volume regulation and migration 7-9. These studies have concluded that outward Cl- fluxes through these channels are important for cell volume regulation in glioma cell lines, and suggest that their function has a significant role in glioma cell migration and proliferation. Further studies have evaluated the physiological role of other volume and chloride regulating mechanisms such as the Na+-K+-2Cl- cotransporter 1 (NKCC1, SLC12A2) and K+-Cl- cotransporters (KCC, SLC12A4-7) in a genetically modified glioma cell line 10. They found that these cells accumulate Cl- above electrochemical equilibrium. However, the role of NKCC (major Claccumulation and regulatory volume increase mechanism in mammalian cells) and KCC (major Cl- extruding and regulatory volume decrease mechanism) cotransport systems in BTSC migration still needs to be fully elucidated. Further, it has been demonstrated that PDGF increases KCC transport in vascular smooth muscle cells through the phosphatidylinositol-3,4,5-phosphate (PI3P) kinase pathway. An increase in the transcript expression of KCC1 and a decrease in transcript expression of KCC3 in response to the exposure to PDGF were observed, suggesting regulation of expression by this growth factor 11, 12. It has also been shown that the presence of EGF and PDGF 13, as well as a constitutively active mutant of the EGF receptor 14, leads to increased migration of GBM cells. Nonetheless, the effect of EGF and PDGF on NKCC1 and KCC1-4 expression has not been evaluated and correlated to the migratory ability of BTSCs. Further, in breast and ovarian cancer, where the presence of cancer stem cells has been demonstrated15-17, it has been shown that expression of KCC3 and 4 are necessary for tumor invasion and growth 18, 19, suggesting and important role of these transporters in tumor pathogenesis. With the use of intraoperative tissue, we propose to study the role of NKCC and KCC transport systems on the migration of BTSCs in vitro and in vivo. In addition, we will evaluate the migration of genetically manipulated BTSCs in human tissue by taking advantage of a human organotypic model reported previously by our group, as a more accurate model to study what occurs in the human brain. We will evaluate the migratory and proliferative properties of BTSCs with shRNA based knock-down of the transporters studied. The results obtained from this research will increase our understanding on the mechanisms involved in the invasion and migration of BTSCs, and potentially result in new strategies for currently suboptimal brain tumor treatments.

Name of Principle Investigator: **Nini Guo** Project Budget**: $ 110,000.00**

Grantee: **Johns Hopkins University**

Title: **Inhibiting Cyclin-dependent Kinase Inhibitors as Potential Therapy in Hematopoietic Stem Cell Failure Due to Short Telomeres**

Abstract of stem cell research (as submitted by Principle Investigator):

Telomeres are DNA-protein complexes that protect chromosome ends. Telomeres shorten after each round of cell division and short telomeres limit the replicative potential of cells in culture. The role of telomeres in human disease has risen to the forefront in the context of dyskeratosis congenita (DC), a rare disorder of stem cells that leads to bone marrow failure and idiopathic pulmonary fibrosis1. In DC patients, the primary cause of mortality is aplastic anemia due to a functional failure of hematopoietic stem cells (HSCs). Mutations in the essential components of telomerase, telomerase reverse transcriptase (hTERT) and telomerase RNA (hTR), cause autosomal dominant DC2, 3. In these families, genetic anticipation, an earlier and more severe onset of phenotypes in successive generations, is observed. The genetic anticipation correlates with telomere shortening and implies that the short telomeres, not telomerase mutations themselves, cause the functional decline in stem cells. Genetic anticipation of bone marrow failure also occurs in the telomerase knockout mouse where it appears as successive worsening of hematopoietic defects4. Thus although adult somatic stem cells are thought to replicate indefinitely, evidence from humans and mice with short telomeres indicates that telomeres limit this replicative potential. This proposal aims to explore the mechanisms by which short telomeres impair HSC function. To do so, we will examine levels of cyclin-dependent kinase inhibitors (CKIs) that are known to accumulate in aging stem cells and query whether short telomeres are the direct contributor to their accumulation. CKI accumulation in aging HSCs has been best described for p16 and p21. p16 accumulates and restrains aged HSC function7. p16 expression is also upregulated with age in adult neural stem cells and pancreatic islets, where this increase mediates an age-associated decline in stem cell function8,9. p21 expression levels have also been shown to increase by more than four-fold in aged mouse HSCs10. In Aim 2, we build on recent exciting evidence in the mouse linking the loss of p21 to an improved functional capacity of HSCs with short telomere defects in classic reconstitution assays6. This evidence is particularly compelling since in this animal model, loss of p21 did not lead to malignant transformation and suggests that p21 knockdown may be a viable target in ameliorating short telomere defects within and outside of the bone marrow.

Name of Principle Investigator: **Woon Ryong Kim** Project Budget**: $ 110,000.00**

Grantee: **Johns Hopkins University**

Title: **Application of Patient-Specific Induced Pluripotent Stem Cells for Understanding and Therapy of Huntington's Disease**

Abstract of stem cell research (as submitted by Principle Investigator):

Stem cells have the ability to renew themselves through continuous cell division and the potential to differentiate into a diverse range of cell types. Recent advances in stem cell biology has made it possible to derive pluripotent human stem cells from somatic cells by introducing specific transcription factors, named induced pluripotent stem cells (iPSCs). Such technology raises the possibility to patient specific iPSCs fo r the cell-replacement therapy for various neurodegenerative diseases and after injury as well as for undestanding human diseases. Huntington's disease (HD), a neurodegenerative disease, results from an abnormal expansion of trinucleotide (CAG) repeat encoding glutamine in the N terminus of huntingtin (htt). Loss and atrophy of certain types of neurons and receptors are responsible for the different symptoms and stages of HD. I will generate human iPSCs from skin fibroblasts of HD patients. I will these iPSCs to generate striatal spiny neurons to explore the signal cascade to undergoing apoptotic pathway in HD. Once the model established, I will use these system for screen small molecule compounds that may rescue the death of HD neurons. We will evaluate the functional recovery following transplantation of modified iPS cell using an HD animal model. These studies may not only lead to drug discoveries for HD, but also cellular therapy via iPS cells would be an important step for advanced clinical application in HD.

Name of Principle Investigator: **Ming Jung Kim** Project Budget**: $ 110,000.00**

Grantee: **University of Maryland, Baltimore**

Title: **Interplay of hypoxia-inducible factor-1 (HIF-1) and microRNAs in hematopoiesis**

Abstract of stem cell research (as submitted by Principle Investigator):

Hematopoietic stem cells (HSCs) are maintained in the quiescent state in the hypoxic endosteal niche of the bone marrow. Movement of HSCs to the vascular niche, with its higher oxygen concentrations, is associated with their proliferation and differentiation. Hypoxia-Inducible Factor-1 (HIF-1) is an important transcription factor that regulates gene expression in hypoxic conditions. HIF-1 is a heterodimer which consists of oxygen-regulated HIF-1 and constitutively-expressed HIF-1 subunits. In normoxic conditions, although HIF-1 subunits are transcribed and translated, levels of HIF-1 subunit proteins are very low. This is because hydroxylated HIF-1 subunits are rapidly degraded in the proteosomes. In hypoxic conditions, HIF-1 subunits are stabilized, and these stabilized HIF-1 subunits interact with HIF-1 subunits to form heterodimers in the nucleus. These heterodimers are then able to regulate gene expression by binding to hypoxia-response elements (HREs) in their target genes. HIF-1 was first identified as a regulator of erythropoietin (EPO) gene expression, and it has since been reported to also regulate additional genes involved in erythropoiesis. In several recent studies, HIF-1 has been shown to regulate not only conventional gene expression but also microRNA (miR) expression under hypoxic conditions. Since these studies have been done mainly in cancer cells, I propose to investigate a network of HIF-1 signaling and miRs that I hypothesize to be responsible for regulation of the response to hypoxia in human hematopoietic stem-progenitor cells (HSPCs). In preliminary data, culture of human CD34+ HSPCs under hypoxic conditions (1% O2, 5% CO2, and 94% N2) resulted in increased cell proliferation and increased formation of erythroid and granulocyte-macrophage colonies compared to the normoxia culture (5% CO2, and 95% air including 20% O2). After finding optimal hypoxia culture conditions of human CD34+ HSPCs, initial profiling studies will be done using both miR and mRNA microarrays. Based on these results, I will investigate if there are specific sets of transcription factors and miRs that are involved in HIF-1-mediated regulation of hematopoiesis. Subsequent functional (by overexpression and knockdown) and mechanistic studies of implicated miRs will help to understand how HIF-1 and hypoxia-related miRs are involved in the regulation of early HSPC proliferation, survival, and differentiation.

Name of Principle Investigator: **Ju Young Kim** Project Budget**: $ 110,000.00**

Grantee: **Johns Hopkins University**

Title: **DISC1 Interactome in Human Neurogenesis**

Abstract of stem cell research (as submitted by Principle Investigator):

Schizophrenia is a mental disorder commonly associated with hallucination, dilution and disorganized thinking and speech. DISC1 (Disrupted-In-Schizophrenia 1) gene was initially identified from genetic association studies in a larch Scottish family that was disrupted by a balanced chromosome translocation, and it is by far the best supported susceptibility gene for schizophrenia and other major mental disorders. DISC1 has been studied intensively in vitro and in animals to understand its biological roles. However, how DISC1 gene disruption contributes to the development of schizophrenia is still poorly understood. DISC1 is a scaffold protein with multiple coiled coil motifs potentially contributing to the association to many other proteins and facilitating the formation of protein complexes, termed DISC1 interactome. DISC1 interactome was first proposed based on a yeast two-hybrid and bioinformatics study, through which an intensive protein-protein interaction network was identified involving DISC1 and 127 other proteins. There are, however, substantial differences between rodent and human DISC1 at both the gene level and at the protein level. When, where, and how DISC1 interacts with its binding partners in regulating human neural development is almost completely unknown. Human embryonic stem cells (hESCs) provide a model system to study human embryonic neurogenesis and development. This study will identify direct interacting proteins of DISC1 using a non biased proteomics approach, and examine their functions during human embryonic neurogenesis using hESC derived neural progenitor cells. The goal of the current study is to identify the convergence in the biological function of DISC1 and its interactome in human neurodevelopment.

Name of Principle Investigator: **Desiree Krebs-Kraft** Project Budget**: $ 110,000.00**

Grantee: **University of Maryland, Baltimore**

Title: **Endocannabinoids and sex differences in human stem cells**

Abstract of stem cell research (as submitted by Principle Investigator):

All stem cells have an XX or XY genotype and sexually dimorphic expression of genes on sex chromosomes underlie some sex differences in the brain. Sex differences in stem cells can be important for a successful outcome during stem cell transplantation (Csete 2008). Female donor stem cells have a greater capacity to regenerate muscle tissue and are more likely to survive hypoxic and inflammatory insult than male donor stem cells (Csete 2008; Deasy et al. 2008). Endocannabinoids (eCBs) are developmentally regulated retrograde messengers implicated in brain development (Galve-Roperh et al. 2006) by affecting cell proliferation and differentiation of neural cell types (Aguado et al. 2005). Neural stem cells/progenitors have functional cannabinoid type 1 or type 2 receptors (CB1 or CB2) (Aguado et al. 2005; Molina-Holgado et al. 2007) and can synthesize their own eCBs (Aguado et al. 2005). We previously showed that cell proliferation occurs at a higher rate in regions of the female brain compared to males, and that females are more susceptible to the negative effects of CB receptor activation on brain cell proliferation than males. Thus, the goals of these experiments are to determine; 1) if there are sex differences in human neural stem cell (hNSC) proliferation and/or survival, and 2) if cannabinoids modulate hNSC proliferation and/or survival in a sex-dependent manner, and 3) if application of CBR ligands during hNSC transplantation will affect transplantation success in a sex-dependent manner. We hypothesize that activation of CB receptors will reduce proliferation and/or survival of female but not male hNSCs in vitro and after in vivo transplantation. In Experiment 1, male and female hNSCs will be incubated with vehicle (1% DMSO) or CB1 (ACEA; 10 μM) or CB2 (JWH-133; 10 μM) receptor ligands for 4 hrs. The cultures will be processed for immunocytochemistry of the mitotic marker Ki-67 following treatment to assess proliferation or for nestin at 14 days after treatment to assess survival. To explore the role of cannabinoids on in vivo hNSC transplantation, hNSCs will be transplanted into the neonatal rat brain and the recipient will be subcutaneously injected with vehicle (1% DMSO) or CB1 (ACEA; 3 mg/kg) or CB2 (JWH-133; 3 mg/kg) receptor ligands and will be given a single injection of the mitotic marker BrdU (300 mg/kg, i.p.). The pups will be euthanized 4 hrs later or on PN14 and their brains processed for BrdU and the stem cell marker SC121 double-label immunohistochemistry. Results will reveal if there is a sex difference in the proliferation and/or survival of hNSCs in vitro or of transplanted donor hNSCs. Furthermore, these findings will determine whether cannabinoids alter hNSC proliferation and/or survival in vitro or after hNSC transplantation in a sex-dependent manner.

Name of Principle Investigator: **Xingyu Liu** Project Budget**: $ 110,000.00**

Grantee: **Johns Hopkins University**

Title: **Development of ECM-based hydrogel maxtrix with controlled stability to improve the adhension of human induced pluripotent stem cells.**

Abstract of stem cell research (as submitted by Principle Investigator):

Human iPSCs offer tremendous promise to regenerative medicine. The use of hiPSCs allows customizing patient-specific therapies without ethical concern of using embryonic materials5-9. A recent report in generating hiPSCs without genome integration further demonstrated the potential of this technology in clinical applications.5, 6 To avoid the contamination of animal product, a feeder-free culture system is needed for clinical use of these cells. Despite extensive efforts made towards establishing the feeder-free culture system for human embryonic stem cells (hESCs) and hiPSCs,1-4 the current system remains poorly reproducible and inefficient.10, 11 The poor clonal efficiency and thus the high selection pressure for cell survival are believed to be the major factor to induce karyotype abnormality over an extended culture period.12 Representing the early pluripotent stem cell stage *in vivo*, hiPSCs are anchoragedependent and highly sensitive to anoikis—the ECM anchorage-dependant apoptosis.13,14 The survival of pluripotent stem cells, including hiPSCs and hESCs, can be regulated by the ECM properties.
A pilot study using an hydrogel matrix with 70% collagen IV, 27% laminin and 3% nidogen assembled in a slightly oxidative condition to allow intra- and inter-molecular disulfide bond formation among collagen IV molecules increased the clonal efficiency for more than 30 times compared with the "standard" Matrigel1, 2. In an extended culture of hESCs (H1, starting passage number is P29), about 107 and 105-fold higher expansion were achieved on this matrix with normal karyotype in comparison with "standard" feeder-free culture and MEF-feeder culture, respectively .This new ECM hydrogel matrix was also found to synergistically improve the clonal efficiency of H1 hESCs when supplemented with ROCK inhibitor, Y-27632.
The objectives of this proposed research are to develop an ECM-based hydrogel matrix with controlled structure and stability to improve the adhesion and clonal efficiency of human induced pluripotent stem cells (hiPSCs) in a feeder-free expansion culture, and to understand the mechanism of the self-assembly and stability of ECM hydrogel and their impact on hiPSC expansion efficiency. Therapeutic applications require efficiency clonal selection and expansion of a normal and functional therapeutic hiPSC line from the pool of generated hiPSCs. If successful, this study will provide a novel technology to for hiPSC derivation and expansion. This study will also strengthen our understanding of the role of ECM in the pluripotent stem cell niche and in the regulation of hiPSC survival, apoptosis and differentiation.

Name of Principle Investigator: **Ahmed Mohyeldin** Project Budget**: $ 110,000.00**

Grantee: **Johns Hopkins University**

Title: **The Effects of Hypoxic Adaptation and Signaling on Cancer Stem Cell Growth and Invasion of Glioblastoma Multiforme**

Abstract of stem cell research (as submitted by Principle Investigator):

Gliomas are the most common primary brain cancers in humans and glioblastoma multiforme (GBM) is the deadliest of these tumors . While some cancer survival rates have increased due to improvements in diagnostics and therapeutic applications, the prognosis of brain tumors with a median survival of 14.6 months continues to be dismal. GBMs either arise de novo or progress from lower-grade astrocytomas through multiple genetic alterations, yet to date the identification of the cellular origin of gliomas remains enigmatic. Furthermore, brain cancer is associated with high recurrence rates and local tumor hypoxia is associated with poor prognostic outcome and poor patient survival . Our lack of understanding of these two phenomena makes the behavior of this disease increasingly difficult to predict and treat. This poor insight is further exacerbated by the assumption that neoplastic transformation occurs from fully differentiated glial cells. However, evidence for this hypothesis is lacking and data from animal models and hematopoetic cancers suggests that germinal regions harboring tissue stem cells may be more susceptible to transformation . This has led our lab to propose that a defined ribbon of neurogenic astrocytes, that are now appreciated to be the stem cells of the brain, may undergo transformation to give rise to cancer stem cells . This hypothesis continues to accumulate evidence and has led several labs including our own to isolate brain tumor stem cells (BTSC) from primary gliomas . It has been proposed that the BTS cell is a CD133+ cell that shares many of the hallmarks of a normal stem cell including neurosphere formation, in vitro differentiation and capacity for self-renewal . This cell has recently become an attractive therapeutic target and may be the reason why brain cancers continually recur. It has recently been appreciated that nearly all cancer stem cells are known to reside in a hypoxic niche and hypoxia enhances the expansion of human CNS precursors . Publications from my dissertation work demonstrate that tumor hypoxia activates critical signaling pathways of which the transcription factor HIF (hypoxia inducible factor) and its downstream target gene the hormone erythropoietin (Epo) are a major component of the adaptive response that can promote cell survival, treatment resistance and cell invasion . However, we observed that this signaling pathway was also active in a small population of glioma cells under normoxia (Figure 2). Two recent observations make this proposal a compelling one to pursue. It has been shown that other factors beyond hypoxia are known to activate the pleiotropic transcription factor HIF and others have demonstrated that the HIF pathway was active in circulating hematopoietic stem cells under normoxic conditions. Despite this, the signaling pathways and local growth factors that maintain the malignant phenotype of the cancer stem cell population remain poorly understood. If HIF is implicated in the cell signaling cascade that maintains cancer stem cells, the existence of several HIF inhibitors can quickly become a therapeutic strategy in targeting these cells. Our long term goal is to identify and develop therapeutic strategies that target this unique population of brain tumor stem cells (BTSC) by characterizing and understanding their biology. The objective of this application is to identify if HIF-1α signaling is constitutively and exclusively active in cancer stem cells of gliomas and whether silencing this pathway can prevent *in vivo* xenograft growth and inhibition of the tumor renewing capacity of cancer stem cells. We hypothesize that the transcription factor HIF-1α will be stabilized under normoxic conditions in the cancer stem cell population of glioma cells and the silencing of this pathway and its gene products will diminish the malignant phenotype and self renewal capacity and undifferentiated state of cancer stem cells *in vitro* and reduce *in vivo* xenograft growth and animal survival.

Name of Principle Investigator: **Tea Soon Park** Project Budget**: $ 110,000.00**

Grantee: **Johns Hopkins University**

Title: **Development of Endothelial Progenitors from Human Induced Pluripotent Stem Cells for Vascular Regenerative Therapy**

Abstract of stem cell research (as submitted by Principle Investigator):

Vascular diseases have potentially serious consequences including loss of limbs, and deterioration of brain and heart function. Blood vessel regenerative therapy has great potential, but the possible sources of endothelial progenitor cells are limited. The recent discovery that patient-specific induced pluripotent stem cells (iPS) can be generated from somatic cells using defined factors has provided unprecedented opportunities for generating unlimited sources of autologous, transplantable lineage-specific progenitors. This project focuses on the development, characterization, and application of patient-specific endothelial progenitors derived from iPS for vascular regeneration and tissue engineering.
First, we will determine optimal culture conditions for promoting differentiation of human embryonic stem cells (hESC) and iPS cells in order to obtain large numbers of endothelial progenitor cells. The Laboratory of Dr. Zambidis has recently derived methods for generating hemangioblasts from hESC, and also generated several iPS cell lines from different cell types (fibroblasts, mesenchymal stem cells, and cord blood progenitors) that can readily be used for this project. We have also established culture conditions for the directed differentiation of hESC toward progenitors that express antigens of endothelial and perivascular cells (CD31 and CD146), angioblasts (KDR and CD133), and hemangioblasts (BB9/ACE). These preliminary culture conditions will provide a foundation for facilitating optimal differentiation of iPS cells toward endothelial cell lineages; putative endothelial progenitor cells will be purified with these selected markers, and ultimately tested for vascular regenerative potential. Our second aim will be to establish functional tests of hESC and iPS-derived endothelial progenitor cells. *In vitro* testing will include acetyl-LDL uptake and matrigel micro-vessel tubular formation. We will also carry out *in vivo* angiogenesis assays by injecting progenitors into immunodeficient mice (in collagen capsules). Furthermore, we will examine the regenerative capacity of these progenitors in a murine ocular angiogenic model (in combination with Dr. Gerald Lutty, Wilmer Eye Institute). Thus, this project will enable us to elaborate methodologies for generating endothelial progenitors that may ultimately lead to patient-specific treatment of vascular degenerative disorders.

Name of Principle Investigator: **Margaret Showel** Project Budget**: $ 110,000.00**

Grantee: **Johns Hopkins University**

Title: **Characterizing and Targeting Acute Lymphocytic Leukemia Stem Cells**

Abstract of stem cell research (as submitted by Principle Investigator):

Acute lymphocytic leukemia (ALL) possesses two unique and unexplained clinical properties: the first is that the excellent initial responses in both children and adults translate into outstanding long-term survivals only in children; the second is the success of maintenance therapy in children. We believe that the cells of origin of ALL, stem cells, could explain both of these clinical properties. The overall goals of this project are to study the biology of the ALL stem cell in order to identify therapeutic targets, and to evaluate the activity of differentiation therapy in ALL, with the intent to improve outcomes, particularly in the "adult-type" ALL. This data should serve as pre-clinical data for a pilot clinical trial of differentiation therapy for patients with relapsed or refractory ALL. We will study the differentiating effects of MS-275, bexarotene and 5-azacytidine first on the "adult-type" ALL cell lines RS4;11, SUP-B15 (t(9;22))and the "pediatric-type" cell line REH (t(12;21) and then on primary ALL patient specimens. We will determine the cytostatic dose of each agent: the dose that blocks proliferation, as evaluated by cell count and flow cytometry for cell cycle kinetics, without being cytotoxic3. Then the CD34+ cells isolated from all three cell lines will be incubated with each differentiating agent for 72 to 96 hours with the addition of GM-CSF, G-CSF or IL-7 at 24 to 48 hours. We will evaluate the expression of CD34, CD38 and CD19 by FACS before and after exposure to these differentiating agents, to assess for phenotypic evidence of differentiation. Clonogenic recovery will be evaluated by colony-forming assay in methylcellulose. Utilizing those agents that show activity in cell lines, we will repeat these studies with "adult-type" ALL stem cells, using the isolation strategy from aim #1. After exposure to these differentiating agents, these cells will be analyzed for phenotypic differentiation, loss of clonogenic recovery, and loss of expansion in NOD-scid mice and LTC. This data will serve as pre-clinical data for a clinical trial. We are in the preliminary stages of planning a pilot study of MS-275 with GM-CSF as therapy for patients with relapsed or refractory ALL and those with newly diagnosed ALL who are not eligible for traditional cytotoxic therapy due to age or performance status. We will evaluate biologic activity and clinical response by assessing differentiation, minimal residual disease and disease-free survival.

Name of Principle Investigator: **Guoming Sun** Project Budget**: $ 110,000.00**

Grantee: **Johns Hopkins University**

Title: **Biodegradable Hydrogel Scaffolds for Vascular Regeneration**

Abstract of stem cell research (as submitted by Principle Investigator):

The objective of this proposal is to develop dextran-based biodegradable hydrogel scaffold as an instructive environment for vascular differentiation and regeneration. To achieve this goal, we will (1) Synthesize and characterize dextran-derived hydrogels to generate polymeric instructive microenvironments for vasculogenesis; (2) Study in vitro vascularization using hEPCs and vascular derivatives of hESCs.; (3) Examine in vivo vascularization and integration with host tissue using subcutaneous murine model. We previously demonstrated that the modification of dextran backbone with differentfunctional groups affects both physical and biological properties of dextran-based hydrogels, and the incorporation of amine groups especially shows promising biological properties [5]. We propose to build on this experience to develop dextran-based hydrogel scaffolds specifically for vascular regeneration. Hydrogels will be chemically and physically modified to incorporate functional groups to promote cell differentiation and vascularization. We have categorized the design parameters into two groups: i. Space - organizing and orientation cues; and ii. Biologically active signal-eliciting components;We plan to address several parameters in each group, and to rationally design the instructive matrices using available and newly evolving technologies. We aim to provide a controllable milieu for the formation of 3D vascular networks, by mimicking some aspects of vasculogenesis, and induction of angiogenesis. This will begin by generating the initial space - organizing scaffold. We will develop dextran-based hydrogels and will chemically modify backbone and linker to vary properties of the hydrogel including spatial density (interior morphology, porosity and distribution), and mechanics (swelling and stiffness). This will follow by providing: an orientation cue, biologically active ECM signal eliciting features, and angiogenic growth factor enhancement. Initial modifications will include (a) incorporation of fibronectin and/or RGD domains to induce cell adhesion and tubular morphogenesis, (b) a tailored rate of growth factor release for in vitro and in vivo tubulogenesis and host angiogenesis. We hypothesize that dextran-based hydrogels can promote and direct vascular differentiation of human endothelial progenitor cells (hEPCs) and embryonic stem cells (hESCs), and can be further chemically or physically engineered to present functional and cell-favorable microenvironment for vascularization, which could be used in treating vascular diseases.

Name of Principle Investigator: **Jaylyn Waddell** Project Budget**: $ 110,000.00**

Grantee: **University of Maryland, Baltimore**

Title: **Androgens and sex differences in human neural stem cells**

Abstract of stem cell research (as submitted by Principle Investigator):

The influence of gonadal steroids on survival and differentiation of human neural stem cells is not well understood, despite the critical influence of steroids on neurogenesis, cell migration and brain organization. Stem cells, both neural as well as those comprising the inner cell mass of the blastocyst, express estrogen and androgen receptors (Branvall et al., 2002; Branvall et al., 2005; Chang et al., 2006). Further, cells constituting the neurogenic neuroepithelium and subventricular zone express androgen receptors and expression levels drastically decrease as the rat matures (Branvall et al., 2005). These changes in the magnitude of androgen receptor expression may point to a critical period in development during which androgens robustly modulate brain organization.Though estrogens appear to promote proliferation in all types of stem cells tested to date, androgens have a more complex action across tissues (Ray et al., 2008). Androgen administration attenuates the proliferative influence of epidermal growth factor (EGF) in rodent-derived neural stem cells (Branvall et al., 2005). Androgen treatment increases, however, the number of proliferating cells in an EGF-free culture (Branvall et al., 2005). Preliminary data from our lab suggest that male neonatal rats expressing dysfunctional androgen receptors, and thus androgen insensitivity, exhibit increased proliferation in the differentiating dentate gyrus, but decreased cell genesis in other regions of the hippocampus (i.e., CA1). Removal of endogenous androgens in adult male rats through gonadectomy results in decreased cell survival in the dentate gyrus, with no obvious effect on cell proliferation (Spritzer & Galea, 2007). Together, these results highlight the possible importance of the age of the animal as well as the area of the brain in determining a neural precursor cell's response to androgens. The experiments proposed here will assess the influence of androgens on division and survival of human neural stem cell cultures. Using androgen receptor agonists and antagonists, we will evaluate the role of androgens on cell division and survival. Androgens may have different effects, depending on the developmental stage of the cell. We will examine the response of human neural stem cells as they mature, focusing on critical stages of maturation. We will examine cell numbers as well as cell fate using immunocytochemistry. These experiments will contribute to our understanding of androgens and their potential role in stem cell therapies.

Name of Principle Investigator: **Karl Whalin** Project Budget**: $ 110,000.00**

Grantee: **Johns Hopkins University**

Title: **Induction of human iPS cells towards a photoreceptor-like fate**

Abstract of stem cell research (as submitted by Principle Investigator):

Retinal degenerative disease is a major cause of blindness throughout the world. Although many molecular causes to this degeneration exist, these diseases all lead to vision loss due to photoreceptor (PhR) cell death. This loss can be slowed in only a few rare forms of retinal degeneration, and once it has occurred the best hope to restore vision is through celltransplantation based strategies. Although not yet in practice, the hope is that a better understanding of stem cells, particularly in collecting, producing, cultivating and altering their cell fate to become retinal PhR's would allow transplantation of retinal progenitors to become an effective treatment approach. A pool of uncommitted progenitors that can be driven to a PhR fate is central to this strategy. Furthermore, 'inducible pluripotent' stem cell (iPS cell) technology offers a means to make custom tailored pools of plentiful transplantable retinal stem cells that could overcome graft-host rejection and improve transplant outcome. Mouse and human embryonic stem cells (ES cells) have been shown to proceed along a PhR pathway; however, this generally occurs at a low frequency. For stem cells to actually work we need to establish conditions capable of inducing a much higher frequency PhRs. A well choreographed sequence of differentiation and its highly laminar structure are additional advantages that makes the retina a good model for neural development in general, thus the proposed studies may have implications for other neurological diseases also. Towards the goal of understanding how to induce uncommitted stem cells to differentiate towards a retinal cell fate, one pursuit in our lab involves a novel high-content screen to identify molecules and factors capable of promoting PhR-like cells from mouse ES cells. This initial screen is funded through the Foundation Fighting Blindness. For my post-doctoral work, I would like to extend this line of investigation to include human stem cells, particularly iPS cells. The work proposed in this application will complement the above screen by applying knowledge of stem cell inducing factors to human stem cells. For the high content screen, which requires large numbers of cells, mouse ES cells were chosen as they are easier to work with, generate more cells, and have the advantage of many available natural and generated mutants. Fluorescent reporter mouse lines used to create ES cells are an experimental design element intended to speed up candidate gene identification and to monitor expression of PhR transcription factors and other genes. For my proposed human studies, I will take advantage of early successes in inducing human ES cells toward a PhR-like cell fate and will use what is learned from the mouse screen to try to develop more refined and efficient methods for human cells. I will also try to establish efficient methods for human iPS cells, and develop iPS lines from patients with retinitis pigmentosa or other retinal degenerations. These studies will benefit greatly from the expertise of our collaborator Hongjun Song (co-PI) who has expertise with both neuronal differentiation of human ES cells and the establishment of iPS cells from patients with human neurological diseases. We are in a good position to take advantage of human iPS cell's to study retinal degenerations because of the wide-range of stem cell expertise at Hopkins, availability of ophthalmic expertise and an extensive network of clinicians at Wilmer capable of providing us with appropriate human tissue samples.

Name of Principle Investigator: **Ying Yang** Project Budget**: $ 110,000.00**

Grantee: **Johns Hopkins University**

Title: **Identifying targets of miR-302 in human embryonic stem cells using a quantitative proteomic approach**

Abstract of stem cell research (as submitted by Principle Investigator):

PROBLEM: Although the miR-302 family represents an important stem cell-specific class of miRNAs, only one target of miR-302s was experimentally identified. BACKGROUND: Several lines of evidence have revealed crucial roles of miRNAs in self-renewal and differentiation of embryonic stem (ES) cells. For example, the miR-302 family is essential for maintenance of ES cell renewal and pluripotency. Thus far, only one target of miR-302s (Cyclin D1) has been experimentally verified. Based on the fact that miRNAs mainly regulate their targets at the translational level in animals, we propose to employ a quantitative proteomic strategy to systematically identify candidate targets of miR-302s in hESCs. HYPOTHESIS: We hypothesize that modulation of miR-302s levels in hESCs will lead to a significant alteration in the expression level of miR-302 target proteins. Further, this alteration in abundance of target proteins can be identified and quantitated using state-of-the-art high resolution mass spectrometry-based quantitative proteomic approaches. RESEARCH: We propose to identify targets of stem cell specific miRNA miR-302a using an iTRAQ based quantitative proteomic approach. A temporal proteome profile of hESCs transfected with miR-302a antagomir for various time points will be obtained. Relative changes in protein abundance between miR-302a antagomir and control oligonucleotides transfected cells will be calculated based on the intensity of reporter ions generated during tandem mass spectrometry analysis. Candidate miR-302a targets discovered by proteomic analysis will be validated by standard Western blot, immunocytochemical analysis and luciferase assay. OBSERVATIONS: We have successfully inhibited expression of miR-302a in the human embryonic stem cell line H1 (WA-01) cells by a sequence specific miRNA hairpin inhibitor (miR-302a antagomir). The expression of miR-302a in H1 cells was significantly decreased by miR-302a antagomir at 24h, 48h and 72h post-transfection. CONCLUSIONS: miR-302a is highly expressed in hESCs and can be significantly suppressed by miR-302a antagomir. We will carry out a quantitative proteomic approach to identify candidate targets of miR-302a.

Name of Principle Investigator: **Mingyao Ying** Project Budget**: $ 110,000.00**

Grantee: **Hugo W. Moser Research Institute at Kennedy Krieger**

Title: **Differentiation Therapy Induced by Kruppel-like Factor in Glioblastoma Stem cells**

Abstract of stem cell research (as submitted by Principle Investigator):

Glioblastoma (GBM) is the most prevalent brain tumor with high frequency of recurrence. During the past decade, the identification of cancer stem cells in diverse human cancers including GBM represents an important conceptual advance in cancer biology and provides an important therapeutic target. In human GBM-derived stem cells, retinoic acid (RA) can induce growth arrest and cell differentiation. We studied the gene expression changes during RA-induced differentiation and found that the expression of three kruppel-like factors (KLF 4, 6 and 9) was affected by RA treatment. KLF proteins are evolutionarily conserved zinc finger-containing transcription factors with diverse regulatory functions in cell growth, proliferation, and differentiation, especially in the context of cancer. Here, we will use lentivirus-based gene overexpression or knockdown methods to modulate the expression of specific KLF proteins in GBM stem cells. We will examine the effects of KLF expression modulation on GBM stem cell growth and differentiation in cell culture and on the ability of GBM stem cells to form tumor xenografts in vivo. Since cancer stem cells are particularly resistant to DNA-damaging agents, we will determine the effects of KLF protein modulation on GBM stem cell sensitivity to radiation and chemotherapy. This KLF-induced differentiation therapy will be combined with radiation therapy or chemotherapy in GBM stem cells, which are highly resistant to DNA-damaging radiation and cytotoxic drugs. These studies will provide novel information pertaining to the molecular regulation of the GBM stem cell phenotype and lead to innovative therapeutic strategies for targeting GBM stem cells.

Name of Principle Investigator: **Huimei Yu** Project Budget**: $ 110,000.00**

Grantee: **Johns Hopkins University**

Title: **Characterization of Neural Defects of iPSCs from Trisomy Patients**

Abstract of stem cell research (as submitted by Principle Investigator):

Patau Syndrome, caused by the presence of an extra copy of chromosome 13 (Trisomy 13), is the most severe autosomal trisomies. The frequency of this syndrome is 1:3,000 live births and the average survival of patients is only 7 days. Patau Syndrome is characterized by multiple malformations of brain, cardiac, and urogenital systems. The mechanisms underlying these malformations are not clear, partly because of lack of experimental model systems. We have generated the induced pluripotent stem cells (iPSCs) from the human skin fibroblasts of a Patau Syndrome patient as well as from the normal human skin fibroblasts. We will induce neural differentiation from these iPSCs and study molecular and cellular mechanisms of abnormal neural development in the Patau Syndrome. Using multiple approaches including electrophysiology, immunostaining, and imaging, we will compare the properties of neural differentiation (glia or neuron), axonal and dendritic development, receptor and ion channel expression, and synaptic transmission in cultured neural precursors derived from iPSCs induced from Patau Syndrome patient and normal human skin fibroblasts. We will also transplant neural precursors derived from the two types of iPSCs into mouse brains and analyze the cell differentiation, cell migration, dentritic and axonal development, synaptogenesis, synaptic transmission and plasticity, and neuronal circuit integration of the transplanted cells derived from the two types of iPSCs. These studies will provide novel insight into the etiology of Patau Syndrom and related trisomies and may provide a platform to develop therapeutic strategies for treatment of these genetic diseases.