



**Maryland
TEDCO
&
MSCR
Commission**

Calendar Year

2008

**MSCRF
[Awarded
Research]
Annual
Report**

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- Fred Bunz, Johns Hopkins University
- Chi V. Dang, Johns Hopkins University
- Jeniffer H. Elisseeff, Johns Hopkins University
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- Nicholas Gaiano, Johns Hopkins University
- Yoon Young Jang, Johns Hopkins University
- Carol L. Keefer, University of Maryland, College Park
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- David M. Loeb, Johns Hopkins University
- Stuart Martin, University of Maryland, Baltimore
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- Sarah Netzle-Arnett, University of Maryland, Baltimore
- Martin G. Pomper, Johns Hopkins University
- Yun Qui, University of Maryland, Baltimore
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- Venu Raman, Johns Hopkins University
- Linda Resar, Johns Hopkins University
- Charles M. Rudin, Johns Hopkins University
- Nitish Thakor, Johns Hopkins University
- Leslie Tung, Johns Hopkins University
- Piotr Walczak, Johns Hopkins University
- Katherine Whartenby, Johns Hopkins University
- Huakun Xu, University of Maryland, Baltimore
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- Jonathan Alder, Johns Hopkins University
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- Jessica Carmen, Johns Hopkins University
- Jon Gerber, Johns Hopkins University
- Hugo Guerrero, Johns Hopkins University
- Maged Harraz, Johns Hopkins University
- Biju Joseph, Johns Hopkins University
- Tarja Juopperi, Johns Hopkins University
- Vasiliki Machairaki, Johns Hopkins University
- Celine Plachez, Johns Hopkins University
- Marina Pryzhkova, Johns Hopkins University
- Kara Scheibner, Johns Hopkins University
- Farhad Vesuna, Johns Hopkins University



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MSCR Commission 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 National 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)

Two individuals who work as scientists for the University System of Maryland (appointed by USM) and do not engage in stem cell research

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), Dean and Marjorie Cook Professor of Law at the University of Maryland School of Law (appointed by USM)

Dr. Jeremy Sugarman, Harvey M. Meyerhoff Professor of Bioethics and Medicine, Berman Institute of Bioethics 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

Dr. Joseph Capizzi, Professor at Catholic University in Washington, D.C.

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

OVERVIEW

This is the second year 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 and FY2008 appropriation. This report also provides an abstract of the research to be performed (FY2008) or an abstract of the research activity (FY2008). These abstracts were provided by the respective Principal Investigator to the MSCRF.

Maryland Stem Cell Research Commission (Commission) Meetings

In calendar year 2008 the Commission had five scheduled meetings, four of the meetings were open to the general public and one meeting (April) was a closed session that complied with the Maryland Open Meetings Act . Meeting minutes can be found on the appropriate section on the website.

MSCRF New Website

The Commission and TEDCO launched a new and informative website, www.mscref.org, to provide 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.

Funding Programs

Overall, Maryland's stem cell funding program has gotten off to a rapid and effective start. In just two years, \$38 million has been granted to 82 different research projects. The MSCRF has funded a variety of Programs: Investigator-Initiated, Exploratory and new Post Doctoral Research Grants. It has funded a variety of human stem cells: adult, embryonic, cancer stem cells, iPS or combinations of them. 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 a variety of institutions in the State, some connecting private sector collaborators with academic institutions.

Investigator-Initiated research grants are soliciting applications for Investigator-Initiated Research, which are designed for investigators with preliminary data supporting the grant application with maximum amount of \$300,000 a year, for up to five years (updated from the first two years at the request of Investigators and approval of the Commission).

Exploratory research grants are soliciting applications for Exploratory Research, which are designed for investigators who are new to the stem cell field and for exploratory projects without preliminary data with maximum amount of \$100,000 a year, for up to two years.

Post-Doctoral research grants are soliciting applications 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 with maximum support of \$55,000 a year, for up to two years.

Fiscal Year 2007

The MSCRF has continued supporting awards from FY07. All Principal Investigators from the 24 projects submitted their progress reports and funding has been continued. Seven Investigator-Initiated awards have moved to year two of the three year funding and 17 Exploratory awards have also moved to their second and final year. All awardees presented their research results at the Maryland Stem Cell Research Symposium on December 3, 2008, and all 24 detailed research descriptions may be found in this report.

Fiscal Year 2008

For FY2008 the Commission and TEDCO received 122 applications for funding. From this pool of applications, the Commission recommended for funding and the TEDCO Board of Directors approved 11 Investigator-Initiated research grants, 32 Exploratory research grants, and 15 Post-Doctoral Fellowship research grants. These grant agreements have been executed and the research has begun. All 58 detailed research descriptions may be found in this report.

Fiscal Year 2009

For FY2009, the Commission and TEDCO received 187 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 and final research grants will be in place no later than June 30, 2009. The Commission has also issued a Request for Information (RFI) to seek ideas and suggestions for possible new grants for shared resources to be funded by the MSCRF. Information from the RFI will be discussed at future Commission meetings.

Future Activities

Maryland will host the 2009 World Stem Cell Summit, which will bring together nearly 1,500 stem cell stakeholders from across the nation and around the globe to discuss critical issues in stem cell research and the future of regenerative medicine. Maryland is and will continue to be recognized as a leader in stem cell research, as well as a leader in economic development through investments in life sciences.



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: Evaluation of transplanted human embryonic stem cell-derived oligodendroglial progenitors in a rat contusion model of spinal cord injury

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

Spinal cord injury (SCI) currently affects 300,000 people in the United States and ranks second after mental retardation among neurological disorders in terms of cost to society. Physiologically, SCI is characterized by the rapid development of necrosis in the damaged tissues, followed by a delayed secondary degeneration of surrounding neural tissue, specifically, oligodendrocytes surrounding the motor neurons leading to paralysis. Although the regenerative capacity of the adult CNS is limited to restore this damage, embryonic stem cells (ESCs) provide novel prospects for cellular replacement strategies because of 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 these cells for SCI, it will be essential to understand their pattern of migration in damaged tissues, their survivability, and whether they can reestablish a neural architecture with therapeutic effect.

For this purpose, we utilized a contusion model of SCI in rats with the NYU impactor for controlling and measuring the extent of injury. Human ESC-derived oligodendrocyte progenitors (OP's) were transplanted into the spinal cord to study their survival and migration route towards injury. As one critical aspect of successful cell-based SCI therapy is the time of injection following injury, OP's were injected at a clinically relevant time of 3 hours as well as 1 day following injury when most of the damage to these cells occurs. Migration and survivability was measured postmortem. In vitro immunofluorescence revealed that most hESC-derived OPs expressed oligodendrocyte markers, including CNPase, A2B5, 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. The extent of injury and recovery after transplant were measured using Basso-Beattie-Bresnahan motor behavioral scores (BBB) and Somatosensory Evoked Potential (SEP) monitoring. Preliminary work to validate the use of both approaches with this contusion model was demonstrated by consistent, positive correlations between the intensity of the injury and intensity of the contusion between both models. In addition, without contusion, rats injected with live 5×10^5 hESC-derived OPs or saline demonstrated no further decrease in BBB and SEP scores indicating that the injection or the presence of cells does not induce further injury.

Together these results show the integration of hESC-derived OP's into the spinal cord with or without contusion injury and importantly without disruption of the parenchyma. Cells survive for a minimum of 8 days after injury, with no formation of tumors. The BBB and SEP analyses demonstrate that our injections of OP's do not cause further injury or destruction of neuropathways. In fact, limited, short-term improvement in recovery after injection detected by these analyses is consistent with possible remyelination.

Name of Principle Investigator: Jeffrey W.M. Bulte

Project Budget: \$ 1,190,276.00

Grantee: Johns Hopkins University

Title: Evaluation of transplanted human embryonic stem cell-derived oligodendroglial progenitors in a rat contusion model of spinal cord injury

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

In neurodegenerative diseases, the ability of endogenous adult stem cells to self-renew, replace lost cells, and restore function are limited. Thus, the development and validation of exogenous cell therapy is crucial. Previous studies of experimental therapeutic cell transplantation in neurodegenerative models have proved that stem cells have potential as a therapeutic application to repair or ameliorate these diseases. However more investigation is required to realize this potential in future clinical cell therapy. In particular, it is critically important to determine, noninvasively, whether transplanted stem cells migrate towards lesions, how well these cells can repair the lesions, and how long these cells survive for therapeutic effect. We have transplanted oligodendroglial progenitors (OP) derived from human embryonic stem cells (hESC) into the brain of mice with experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS), and performed serial magnetic resonance imaging (MRI) to monitor the primary migration route of transplanted cells in the brain.

EAE was induced in 8 week-old female C57Bl/6 mice by immunization with MOG35-55 peptide in Freund's adjuvant containing H37RA. In vitro immunofluorescence revealed that most hESC-derived OPs expressed oligodendrocyte markers, including CNPase, A2B5, O4, and Olig1, but occasionally cells immunostained with Tuj1, a neuronal marker. Prior to transplantation, the hESC-derived OPs were incubated with a mixture of Feridex and PLL for magnetic labeling. At seven (before disease onset) or 14 (acute phase of disease) days post EAE induction, mice from the live-labeled group were injected with live 5×10^5 hESC-derived OPs that were magnetically labeled with Feridex-PLL complex into the right cerebral ventricle. Mice from the dead-labeled group received dead-labeled hESC-derived OPs as a vehicle, and mice from the live-unlabeled group received live-unlabeled hESC-derived OPs as a control. After transplantation, the clinical scores of both live groups were lower than the dead-labeled group. There was no significant difference in the disease attenuation between the live-labeled group and the live-unlabeled group, indicating that the magnetic labeling does not impair the therapeutic benefit induced by transplanted cells. Serial in vivo MRI of EAE mice that were transplanted with hESC-derived OPs showed that, on day 1 post-transplant (PT), hypointense MRI signals were observed mainly in the ventricle and subventricular zones, and occasionally detected in the subarachnoid space and cerebral cortex of the EAE mice of the live-labeled group. This was also observed on days 5, 15, and 30 PT, when the hypointense MRI signal in the ventricle gradually decreased, with a gradually increased signal in the cerebral cortex. After serial in vivo MR imaging, we confirmed the hypointense MRI signal observed on in vivo imaging by ex vivo MRI. Hypointense signals were also detected in the thalamus, hypothalamus, and optic tract at day 30 PT.

Based on the above results, we postulate that cell migration mainly occurs in the peri-ventricular white matter, and occasionally in the gray matter. These migration patterns are similar to T-cell infiltration and microglial activation in the periventricular zone in pathological events in the acute and peak stages of EAE suggesting that the signals generated from inflammatory lesions modulate the migration patterns of transplanted cells.

Name of Principle Investigator: Curt I. Civin

Project Budget: \$ 1,725,000.00

Grantee: Johns Hopkins University

Title: Different MicroRNAs Can Positively or Negatively Regulate Differentiation, Proliferation, Apoptosis, and Drug Resistance of Human Leukemia Cells and Normal Hematopoietic Cells

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

Based on expression of microRNAs (miRs) and their predicted target messenger RNAs (mRNAs) in human CD34⁺ hematopoietic stem-progenitor cells (HSPCs), we hypothesized that certain HSPC-expressed microRNAs (HE-miRs) can down-regulate key hematopoietic proteins and thereby regulate hematopoiesis. As a first example, we found that miR-155 was expressed in human CD34⁺ HSPCs and in mouse Kit⁺Sca1⁺Lin⁻ HSPCs (a subset enriched in early HSPCs). MiR-155 was known already to be overexpressed in several types of cancer, including many lymphomas and leukemias. To study the functional role of miR-155 in hematopoiesis and leukemias, we developed molecular tools to efficiently up- and down-regulate miR-155 in hematopoietic cells. To supplement enforced expression experiments using a miR-155/GFP dual promoter lentivector, we lipofected a synthetic 22-mer miR-155 sense oligonucleotide into cells. In functional studies, enforced miR-155 expression increased hematopoietic cell proliferation, in addition to inhibiting hematopoietic differentiation. For loss-of-function experiments, we designed an antisense locked nucleic acid (LNA)-containing anti-miR-155 that potently bound to the complementary miR-155. Upon transfection into hematopoietic cells, this LNA anti-miR-155 blocked miR-155-mediated inhibition of target mRNAs. Thus, modulation of miR-155 and the pathways it regulates may be useful both in ex vivo expansion of HSPCs and in leukemia treatment.

As a second example, we investigated the action of miR-27a, which appeared to have 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 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. Certain predicted miR-27a targets may explain this effect on cell cycling. 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.

Name of Principle Investigator: W.J. Lederer

Project Budget: \$ 1,724,988.00

Grantee: University of Maryland Biotechnology Institute

Title: Mesenchymal Stem Cells Provide Benefit to Injured Cardiac Ventricular Myocytes

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

Diverse investigations suggest that irreversible cellular damage occurs following myocardial injury. Thus injection of human mesenchymal stem cells (hMSCs) into damaged heart tissue following myocardial infarction has been exploited as a new therapeutic intervention. Despite the growing enthusiasm for this intramyocardial injection stem cell approach, the understanding of how MSCs evoke cardiac benefit is ever more controversial. New evidence by our team reveal that hMSCs can provide specific benefit to damaged ventricular myocytes and to injured heart tissue. In fact these beneficial effects are manifest in genetic reprogramming of the cardiac myocytes themselves. In experiments cultures of mouse or rat ventricular myocytes were subjected to a stress stimulus, exposure to endotoxin, lipopolysaccharide (LPS).

There was profound myocyte injury as manifest by altered calcium signaling and abnormal contractile function in the beating cardiac myocytes. Intriguingly, myocytes that were co-cultured with hMSCs were resistant to LPS-evoked damage even up to 5 hrs after exposure. In search of a molecular explanation, we have found that the cardiac cell proinflammatory cytokine expression profile, including TNF α and IL-6, normally seen in response to LPS is markedly inhibited when hMSCs are present. This reprogramming of cardiac cytokine production is accompanied by fundamental changes in myocardial intracellular signaling cascades in the hMSC co-cultures. Flow cytometry experiments showed that the LPS-dependent activation of the protein kinase, Akt, and a transcription factor, NF- κ B, which have been linked to stress responses and hypertrophy in heart, are depressed by the presence of hMSCs. Taken together, our results support a remarkable and unexpected notion of the mechanisms that underlie the benefits of hMSCs. The hMSCs do not become new heart tissue and do not take on the function of the damaged heart cells as had been argued over the last five years. Instead, hMSCs provide protection from damage or enable cellular repair through a provocative paracrine stem cell signaling mechanism. 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.

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):

The long-term objective of this project is to enable genetically enhanced embryonic or adult stem cells to retain their ability to function as stem cells. The ability of stem cells to persist while repopulating tissues is a property that motivates the field of stem cell research. Many therapeutic strategies hope to achieve long-term persistence 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 which permits the correction of genetic defects in patient derived stem cells while simultaneously minimizing ectopic expression of the transgene (making protein at the wrong time). Autologous cells should be compatible and may avoid rejection following transplantation.

The treatment of genetic diseases using patient derived stem cells requires that they are genetically 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 an endogenous gene 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 while linking protein expression to 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 many other 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 developing 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 been working on all three types of stem cells focusing on their neuronal potentials. First, 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. Our previous studies have identified specific factors and signaling pathways that instruct ANSCs to adapt a specific fate and are currently applying this knowledge to adult human stem cells. Second, 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, a cell type lost in patients with Parkinson's Diseases. In parallel, we have been able to transplant human stem cells 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. Third, we have generated iPSCs from normal human skin fibroblasts and those with genetic defects. We showed that these stem cells express all known markers as hESCs and can differentiate into cell types in three germ layers. We have also identified specific neuronal differentiation defects from human iPSCs with genetic defects. Previously, we have characterized the neuronal potentials of both ANSCs in culture and endogenous ANSCs in vivo using immunocytochemistry, electrophysiology, imaging and electron microscopy. We are currently using these established approaches in our laboratory to characterize the functions and properties of neurons derived from hANSCs, hESCs, and iPSCs. 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: Efficient Erythroid Differentiation of a PGD-Derived Human Pluripotent Stem Cell Line Affected with Sickle Cell Hemoglobinopathy

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

The technology of preimplantation genetic diagnosis (PGD) screens IVF-derived cleavage-stage embryos or oocytes from parents with inherited disorders, and is routinely used to avoid births with severe genetic disorders. More than one hundred testable genetic conditions, including severe hematologic diseases such as beta thalassemia, sickle cell anemia, and Fanconi anemia can be PCR-screened from either a micro-manipulated blastomere, or a pre-fertilization ovarian polar body. Derivation of human embryonic stem cell (hESC) lines from diseased IVF blastocysts has recently been reported, and these PGD-hESC are untested yet potentially valuable tools for investigating cellular and molecular events of human embryogenesis in diseased states. For example, a great deal of interest has recently been generated in treating hematologic diseases with genetically-corrected hematopoietic stem cells (HSC) derived from patient-specific pluripotent stem cells. Generation of hematopoietic progenitors from PGD-hESC affected with genetic syndromes may thus provide novel opportunities for testing cell-based and gene therapeutic strategies. An important candidate for such cell-based therapy includes sickle cell disease (SSD) hemoglobinopathy, a classic inherited single gene disorder resulting from the substitution of glutamic acid by valine at position 6 of the beta hemoglobin chain. Human pluripotent stem cells derived from PGD-selected blastocysts or induced pluripotency (iPS; e.g., using patient's somatic cells), will serve as critical reagents for testing such therapeutic strategies.

In these studies, we report the characterization and erythropoietic differentiation of a novel PGD-hESC line affected with SSD hemoglobinopathy. The sickle point mutation was confirmed in this PGD-hESC line with direct genomic sequencing of the beta globin locus. This hESC line possesses typical pluripotency characteristics and forms multilineage teratomas in vivo. SSD-hESC can be efficiently differentiated to the hematopoietic lineage under serum-free conditions, and gave rise to robust primitive and definitive erythropoieses. The expression of embryonic, fetal and adult globin genes in SSD PGD hESC-derived erythroid cells was confirmed by qRT-PCR, intracytoplasmic FACS, and in situ immunostaining of PGD-hESC teratoma sections. Moreover, we defined culture conditions for massive, long-term liquid culture expansion of sickle affected erythroid progenitors that remained in an undifferentiated erythroblastic phenotype for at least two months. These sickle erythroblasts were continuously maintained as a primary cell line that could be frozen and thawed without loss of viability. In vitro-expanded sickle erythroblasts expressed CD71⁺CD36⁺ and CD71⁺CD235a⁺ phenotypes, and underwent developmentally appropriate embryonic, fetal, and adult hemoglobin switching over a period of several months. Moreover, hESC-derived erythroblasts were readily infected with Plasmodium falciparum malaria parasites, thus demonstrating their potential utility in studying the effects of this important pathogen in normal and diseased erythropoiesis. These data demonstrate the utility of using patient-specific, hemoglobinopathic hESC for generating significant numbers of erythroid progenitors for molecular, developmental, gene therapeutic, pharmacologic, and microbiological studies.



FY 2007

Exploratory

Awarded

Research Abstracts

Name of Principle Investigator: **Shyam Biswal**

Project Budget: \$ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **Elevated Nrf2 in cancer stem cells upregulates chemoresistance genes in Leukemia**

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

Cancer stem cells (CSC) are critical to the pathogenesis of hematologic malignancies. These rare cancer cells are phenotypically indistinguishable from normal hematopoietic stem cells (HSC) (CD34⁺ and lack surface markers of lineage commitment) and undergo self-renewal as evidenced by their ability to serially engraft immunodeficient mice. CSCs are extremely drug resistant and radioresistant. Our previous studies have shown that Nuclear factor erythroid-2 related factor-2 (Nrf2) is a redox-sensitive transcription factor that regulates the expression of electrophile and xenobiotic detoxification enzymes and efflux proteins, which confer cytoprotection against oxidative stress and apoptosis in normal cells. Recently we have reported that increased Nrf2 activity in cancer cells promotes tumorigenicity and contributes to chemoresistance by upregulation of glutathione, thioredoxin and the drug efflux pathways involved in detoxification of electrophiles and broad spectrum of drugs. To determine the status of Nrf2 pathway in normal hematopoietic stem cells, we isolated CD34⁺ and CD38⁺ cells from 8 normal healthy volunteers and measured the relative expression of Nrf2 and its downstream target genes. Normal HSCs with CD34⁺CD38^{neg} phenotype demonstrated significantly higher expression of Nrf2 and its target cytoprotective genes like γ -glutamyl cysteine synthetase, glutathione reductase, Heme oxygenase-1, thioredoxin, thioredoxin reductase. To further investigate the expression of NRF2, and its downstream target genes (drug detoxification and efflux pumps) in CSC cells in acute myeloid leukemia (AML), and correlate increased Nrf2 activity with chemoresistant phenotype of CSCs, we isolated CSCs from 5 patients with AML and measured the activity of Nrf2 pathway. Human AML CSC with CD34⁺CD38^{neg} phenotype displayed elevated expression of NRF2 and its downstream target genes than mature CD34⁺ and CD38⁺ tumor cells. Enhanced Nrf2 activity in CSC plays a critical role in protection against genotoxic stress and thus confers chemoresistance. Efforts are underway to disrupt the Nrf2 pathway in leukemic cells to intervene chemoresistance.

Name of Principle Investigator: Srinivasan Chandrasegaran

Project Budget: \$ 230,000.00

Grantee: Johns Hopkins University

Title: Targeted Engineering of the Human Genome in Stem Cells

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

Zinc finger nucleases (ZFNs) are rapidly emerging as powerful tools for targeted and permanent genome modification of a variety of cells and cell types. ZFN-mediated gene targeting yields high gene modification efficiencies (>10%) by delivering a recombinogenic double-strand break (DSB) to the targeted chromosomal locus of the genome. The main focus over the past year has been on Specific Aim 1 of our proposal, which is to create highly sequence-specific ZFNs that will greatly increase the efficiency and efficacy of ZFN-mediated gene targeting in human cells. We reason that increased specificity will also result in reduced non-specific DSBs and thus lower cellular toxicity. We have generated several 3- and 4-finger ZFNs for targeted disruption of the human CCR5 gene. We have shown using cell-free assays that these engineered ZFNs bind to their target sites encoded in a plasmid substrate and cleave them in a sequence-specific manner.

We have also attempted to reduce the toxicity of the engineered ZFNs as follows: The original ZFN architecture requires dimerization of the cleavage domain to induce a DSB, which necessitates the design and engineering of two different ZFNs for binding to the adjacent inverted half sites. Since the protein-protein interaction for the cleavage domain dimer formation is weak, the ZFNs are likely to remain monomeric at concentrations of at least 15 μM ; and dimerize only when bound to their specific targets. Although this requirement for dimerization of the cleavage domain restricts cleavage by a pair of ZFNs to long sequences, it could also introduce a potential problem, since this dimer interaction does not select for the heterodimer species; homodimer species which form could lead to unwanted off-target cleavage elsewhere in the genome of cells. Two different homodimers could result from the two individual ZFNs with different sequence specificities, and more often than not, they do occur. The homodimers, although are not relevant for gene modification, but potentially could and often do, affect the safety and efficacy of ZFN-mediated gene targeting. Two recent articles (Miller et al., 2007; Szczepek et al., 2007) have addressed this issue of ZFN cytotoxicity by redesigning the ZFN dimer interface to inhibit homodimerization, thereby, greatly reducing off-target cleavage by ZFNs, and hence their cytotoxicity. Similarly, we have re-engineered the Fok I cleavage domains of our CCR5 ZFNs to eliminate the off-target cleavage by the individual ZFNs.

We have also generated the Flp-In HEK293 cell line with a GFP gene targeting reporter system to test the efficiency and efficacy of the engineered ZFNs. Our preliminary results are very encouraging. Some ZFNs are effective in their ability to selectively modify a mutant GFP gene encoding the CCR5 target site in the HEK293 Flp-In cells via homology-directed repair. The next step is to initiate experiments to study targeted modification of the CCR5 chromosomal locus in human stem cell lines using standard transfection protocols. Our plan is also to explore the use of integration deficient lentiviral vectors (IDLVs) encoding the CCR5 ZFNs for efficient delivery into the human stem cells. rf2 pathway in leukemic cells to intervene chemoresistance.

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.

A powerful method for the introduction of new DNA into cells is via transposons, discrete pieces of mobile DNA that can translocate from position to position within a genome. One class of such elements are DNA cut & paste transposons: an element-encoded transposase recognizes and binds to specific sites at the ends of the transposon, excises the transposon from that donor site and inserts it into a new target site. Upon integration, the element becomes stably associated with the host genome and can serve as a long-term source of an element-encoded therapeutic product. We are probing the use of such DNA cut & paste elements 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.

A general feature of transposable elements is that they insert in many different target sites. While this is a valuable property when such elements are used as insertional mutagens, such widespread insertion is a hazard in gene therapy. Insertion of even a therapeutic gene may inactivate a host gene, leading to a deleterious mutation. Indeed this consequence has already been unfortunately realized: in a gene therapy trial for a SCID defect in France using retroviral vectors, although cured of the SCID disease, several subjects acquired leukemia because of a retroviral integration next to a cellular oncogene¹. Thus avoiding random mutation, 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.

Name of Principle Investigator: Shengyun Fang

Project Budget: \$ 219,420.00

Grantee: University of Maryland Biotechnology Institute

Title: Ubiquitination-dependent regulation of Oct4 activity

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

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. 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. We hypothesize that E3s regulate 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.

E3 requires two additional enzymes, namely ubiquitin-activating enzyme (or E1) and ubiquitin-conjugating enzyme (or E2) to facilitate ubiquitination. Particular E2-E3 pair often dictates the linkage of polyubiquitination. By screening of a panel of 10 E2s (UbcH2, UbcH3, UbcH5a, UbcH5b, UbcH5c, UbcH6, UbcH7, UbcH8, UbcH10, UbcH13) in an in vitro ubiquitination assay, we identified UbcH5a as the potential cognate E2 for RNF2. This may provide a novel target to modulate RNF2 E3 activity through modulating UbcH5a expression or activity. Surprisingly, none of above E2s supports DPF2's E3 activity. We will further screen all 33 human E2s for both RNF2 and DPF2. To follow the fate of Oct4 protein during ESC differentiation, we used retinoic acid to induce H9 cell differentiation. We confirmed previous reports that Oct4 is downregulated during H9 differentiation. To the contrary, the expression of DPF2 is gradually upregulated. RNF2 exhibits an initial downregulation followed by upregulation. These changes support a role of DPF2 and RNF2 as candidate E3 for Oct4. For fast analysis of Oct4 ubiquitination by RNF2 and DPF2, we found that 293 and Hela cells express both RNF2 and DPF2. Oct4 will be expressed in these cells and the effects of DPF2 and RNF2 on Oct4 ubiquitination, localization and activity will be initially analyzed in these model cells and will be confirmed in H9 cells. My lab is specialized in protein degradation mediated by the endoplasmic reticulum membrane-spanning ubiquitin ligases gp78 and Hrd1. Hrd1 has been shown to regulate p53 activity by targeting p53 for proteasomal degradation. We found that both gp78 and Hrd1 are highly expressed in H9 cells. Gp78 expression is changed during H9 cell differentiation in the way similar to RNF2. Therefore, the potential roles of gp78 and Hrd1 in H9 cell differentiation will also be studied. In summary, our preliminary data support the hypothesis that DPF2 and RNF2 are E3s for Oct4 and regulate Oct4 activity by ubiquitination (Supported by a Maryland TEDCO grant 2007-MSCRE-0139-01 to SF).

Name of Principle Investigator: Ricardo Feldman

Project Budget: \$ 230,000.00

Grantee: University of Maryland Biotechnology Institute

Title: A model for generation of Gaucher-specific human embryonic stem cells, and for reconstitution of glucocerebrosidase expression in hES cell-derived macrophages

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

In order to find effective therapies for patients harboring inherited genetic diseases, it would be very desirable to have disease-specific human embryonic stem (hES) cells that can recapitulate the corresponding genetic defects. While disease-specific embryos from IVF clinics can provide disease-specific hES cells after preimplantation genetic diagnosis (PGD), only a handful of diseases are amenable to this approach. A second potential method of obtaining disease-specific hES cells is nuclear transfer (NT), using somatic cells from patients. Although NT has been used to create animals, in humans this approach is fraught with many technical hurdles that have not been solved. A more recent approach is to derive induced pluripotent (iPS) cells from fibroblasts of affected individuals. Another alternative is genetic manipulation of hES cells, which provides the opportunity to model many diseases for which a genetic alteration is known. Our primary goal is to establish a flexible experimental system for the creation of disease-specific hES cells and our initial focus is on type I Gaucher's disease. This is an autosomal recessive lipid-storage disease that is caused by the accumulation of glucosylceramide in cells of the reticuloendothelial system. This accumulation is due to mutations in the glucocerebrosidase (GC) gene, which result in insufficient enzyme levels of GC. At the present time the most effective therapy for the 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, at an annual cost of hundreds of thousands of dollars per patient. The goals of our research are to simulate loss of endogenous GC activity in hES-derived macrophages using shRNA specific for GC to knock down GC expression. The next step is to determine whether GC expression can be restored in cells expressing the silencing shRNA. 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. Our ultimate goal is to test whether hES cells can be used to treat Gaucher patients.

In order 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 by up to 90%. 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. We are now optimizing the conditions for efficient differentiation of Gaucher-specific hES cells to macrophages. The GC-low macrophages will be used to determine if they exhibit the cytologic abnormalities seen in macrophages from Gaucher patients and for drug discovery. We will also examine the properties of iPS cells that were derived from fibroblasts of a patient with Gaucher's disease. These cells were generated at Harvard Medical School and will be provided by George Daley. The GC gene in this patient harbors the common N370S mutation. Our newest results will be presented at the meeting.

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):

This work investigates the utility of human mesenchymal stem cells (hMSCs) loaded on a cyclic acetal hydrogel construct for orbital floor regeneration. 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 hMSCs within EH-PEG hydrogels. This study investigates the effect of porosity and pore size on osteogenic cell signaling of hMSCs, and as a result, cell function in EH-PEG hydrogels. Human MSCs were loaded in porous EH-PEG hydrogels with a pore size and porosity of 100 μ m / 65%, 100 μ m / 70%, 250 μ m / 70%, and 250 μ m / 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 100 μ m / 65% EH-PEG hydrogels with fibronectin concentrations of 0, 0.5, 2.5, and 10 μ g/mL gel. At days 1, 4, 8, and 12 total RNA was isolated and reverse transcribed. Quantitative real-time PCR was completed on BMP-2 and BMP-2 receptors to analyze osteogenic signal expression, as well as alkaline phosphatase and osteocalcin to describe differentiation. Results demonstrate that pore size and porosity does impact differentiation, with increased alkaline phosphatase levels observed for hMSCs in 100 μ m 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. Compression testing and three point bending testing were then performed on these composite, porous EH-PEG scaffolds using an Instron 5565 mechanical tester. 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 develop a strategy to enhance the neuronal differentiation of human neural progenitor cells (hNPCs) through the introduction of transcription factors known to be vital to 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 where the proteins were linked to a small reporter peptide (FLAG). Commercially obtained neural progenitor cells were maintained as aggregate cultures known as neurospheres for this work, are 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 were evaluated for both expression of the delivered factors as well as changes in neuronal or glial differentiation.

Transfection with either of the vectors led to sustained expression of either of the factors in 40-50% of cells. Immuno-histochemical evaluation showed that both transcription factors were localized to cellular nuclei, an observation that is consistent with our previous studies and 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. The largest increases were seen in hNPCs that had been transfected with both factors simultaneously. These changes appeared to be somewhat dependent on culture or transfection conditions, occurring most consistently with transfection prior to plating and normal differentiation. An alternative strategy to viral vector delivery of the appropriate gene to enhance intra cellular concentrations of these transcription factors is direct delivery of the purified proteins. Plasmid constructs were created to drive over expression in mammalian cells of either Pitx3 or ND2 which had been linked to the protein transduction peptide Tat. Currently ND2-Tat has been purified from transient transfection of the HEK293 cell line, with purification of Pitx3-Tat in progress. Incubation of hNPCs with 1ug/ml amounts of this purified protein shows predicted enhancement of cellular internalization of the factor. Incubation also enhances expression of beta tubulin in a manner similar to transfection with the gene for ND2. The major obstacle of this strategy is the strong preference of hNPCs to differentiate along a glial (astrocytic) lineage. A significant fraction of cells expressing neuronal beta tubulin continued to express the astrocytic 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: Engineering Functional Substrates to Regulate Neural Stem Cell Fate

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

Neural stem cells (NSCs) offer tremendous potential for treating degenerative diseases and traumatic injuries of the central nervous system. However, their clinical applications continue to face the challenges of insufficient cell number and poor control over NSC state and fate during expansion and differentiation, which is partly due to our limited understanding of signaling regulation of NSCs by the extracellular matrix (ECM) microenvironment cues. We have developed functionalized electrospun fibers as a platform to study the effect of nanotopographical cues and surface-tethered biochemical signals on expansion and differentiation of stem cells.

Contact guidance, as one of the functions of the ECM microenvironment, is exemplified by the highly directional organization of the central nervous system during normal embryonic development. We have demonstrated that the differentiation of rat adult NSCs (rANSCs) cultured on functional fiber matrixes was dependent on fiber diameter and alignment, and surface ligand. Laminin (LN)-coated fibers were most effective in mediating rNSC adhesion. Under the differentiation condition (in the presence of 1 μ M retinoic acid and 1% fetal bovine serum), rNSCs showed a 40% increase in oligodendrocyte differentiation on 283-nm fibers and 20% increase in neuronal differentiation on 749-nm fibers, in comparison to LN-coated tissue culture plates. In addition, aligned LN-coated nanofibers guided rNSC processes conforming to the axis of the aligned fibers, and directed higher efficiency of neuronal commitment. Similar results were observed in human ESC-derived neural progenitor cells.

Many important signaling molecules for stem cell regulation in vivo are naturally restricted to either the extracellular matrix or anchored to cell plasma membrane. The impact of such local presentation of signaling molecules on stem cell signaling and behavior remains elusive, largely due to the lack of well-defined model systems. We have developed a quantitative platform that presents fibroblast growth factor-2 (FGF-2) locally as either monomers or clusters when tethered to a polymeric substrate. We have demonstrated that the molecular presentation of tethered FGF-2 enables switch-like signaling and cellular responses in neural stem cells, including MAPK activation, cell migration, proliferation and differentiation. This switch-like phenomenon induced by matrix-restricted molecular presentation underlines a bimodal signaling mechanism recapitulating niche regulation of signaling molecule presentation locally to stem cells and how stem cells sense a gradually changed ligand concentration to make a robust cellular decision in vivo.

This study demonstrated the potential of biomaterials engineering as a power tool to study stem cell-substrate interactions for understanding the mechanisms of niche presentation of key cellular cues in stem cell microenvironment.

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, TGF β /activin/nodal, Wnt/b-catenin and Akt/Pk β 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 TGF β /Activin/Nodal pathway including TGF β R1 (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: **Illuminating human cardiac development and disease through transcriptional analysis in differentiating human embryonic stem cells**

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

Cardiac disease accounts for the largest proportion of adult mortality and morbidity in the industrialized world. However, an incomplete understanding of the genetic programs controlling early cardiogenesis hampers progress toward effective clinical treatment. The advent of embryonic stem cell technologies, and their recent application in the propagation and analyses of human ES cells (hESCs), has opened the door to the generation of in vitro models of human developmental processes, the development of novel drug discovery strategies and the promise of cell-based therapies. In an effort to better understand this process, we set out to build upon our success in profiling early cardiac progenitors in developing mouse embryonic stem cells by identifying genes whose expression is enriched within early human cardiac-fated populations. Currently, all of our attempts to establish a transgenic hESC line harboring an Nkx2.5 cardiac-specific regulatory sequence driving GFP have met with limited success. As an alternative method to identify novel human cardiac genes, we have set out to evaluate human transcript levels using quantitative real-time PCR (qRT-PCT) on RNA isolated from whole embryoid bodies differentiated using a cardiac enrichment protocol. RNA isolated on day zero (undifferentiated hESCs) through day ten (putative mature cardiomyocytes) will be queried using probes for known cardiac genes to establish their legitimacy as a model for cardiogenesis. Thereafter we will systematically assay candidate cardiac genes identified in our recent mouse ESC-based studies. Information gathered from these experiments will enable us to draw parallels between the transcriptional profiles of mouse and human cardiac progenitors. This, in conjunction with our ongoing efforts to isolate and profile a pure population of human cardiac progenitors will further elucidate the genetic programs underlying cardiogenesis.

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 proliferates. Our current understanding of the cellular and molecular mechanisms regulating the self-renewal of hESCs, however, is very limited. Recently, neurotransmitter γ -aminobutyric acid (GABA) has been shown to be effective in promoting hESC cultures. We have confirmed that GABA promotes hESC expansion. We have further characterized the effects of GABA on proliferation and survival of the pluripotent state of hESCs with pharmacological approaches. In addition, we have obtained mechanistic insight of GABA action on hESCs with Ca^{2+} imaging.

Our study addresses a fundamental question in human stem cell biology and may lead to the development of highly effective and reliable protocols to derive and expand hESCs with high quality and in large quantities.

Name of Principle Investigator: **Akhilesh Pandey**

Project Budget: \$ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **Temporal analysis of neural differentiation using quantitative proteomics**

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

The ability to derive neural progenitors, differentiated neurons and glial cells from human embryonic stem cells (hESCs) with high efficiency holds promise for a number of clinical applications. However, investigating the temporal events is crucial for defining the underlying mechanisms that drive this process of differentiation along different lineages. We carried out quantitative proteomic profiling using a multiplexed approach capable of analyzing eight different samples simultaneously to monitor the temporal dynamics of protein abundance as human embryonic stem cells differentiate into motor neurons or astrocytes. Using this approach, a catalog of ~1,200 proteins along with their relative quantitative expression patterns was generated. The differential expression of the large majority of these proteins has not previously been reported or studied in the context of neural differentiation. As expected, two of the widely used markers of pluripotency - alkaline phosphatase and Lin28 - were found to be downregulated during differentiation while S-100, DCLK1 and tenascin C were upregulated in astrocytes and neurofilament 3 protein and nestin upregulated during motor neuron differentiation. We also identified a number of proteins whose expression was largely confined to specific cell types – embryonic stem cells, embryoid bodies and differentiating motor neurons. For example, PYGL FABP5 were enriched in ESCs while NARF & SPTBN5 were highly expressed in Embryoid bodies. Karyopherin, heat shock 27kda protein 1 (HSPB1) and CRABP2 were upregulated in differentiating motor neurons but showed down regulation in mature motor neurons. We validated some of the novel markers of the differentiation process using immunoblotting and immunocytochemical labeling. To our knowledge, this is the first large scale temporal proteomic profiling of human stem cell differentiation into neural cell types highlighting proteins with limited or undefined roles in neural fate.

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. Our recent studies on the migration of stem cells in the normal embryonic/postnatal mouse brain suggest a role for the family of matrix metalloproteinases (MMPs) in this process. These enzymes are also highly associated with the ability of cancer cells to metastasize throughout the body. We are studying whether modulating expressing/activity of MMPs could be a tool to control transplanted stem cell migration. Our preliminary data show that an ES stem cell line can be efficiently transfected with MMPs (MMP2, MMP9 and MT5-MMP to date). Transfected cell cultures increased expression of MMP9 protein 100-fold compared to non-transfected control with commitment increases of enzymatic activity. Injecting these cells into the adult mouse brain demonstrated they survived, and migrated. These preliminary transplants suggest transfected cells may migrate with higher efficiency than non-transfected. MMPs thus 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):

Most of the focus on stem cell research has been on the therapeutic potential for bone marrow derived or embryonic stem cells. More recently, a number of groups have identified kidney progenitor cells, primary in experimental models. We built on this data to test the hypothesis that stem cells could also be isolated and expanded from normal adult human kidney. We used a kidney progenitor cell isolation technique previously described in heart (Circulation 2007) and mouse kidney (J Am Soc Nephrol 2007 abstract), and applied this to humans. Human kidney biopsy samples were taken from 11 kidney transplant donors immediately prior to implanting the kidney in the recipient (both live and deceased donor samples). Biopsies were collected and digested with collagenase solution, sliced into small fragments or explants and grown on fibronectin coated plates. Cell produced from the explants were harvested and transferred to special poly-D-lysine coated cell culture flask. These cells developed into spheres similar to previously described spheres from rodent-derived stem cells. The kidney spheres were collected and grown in normal tissue culture flask until confluence. The presence of kidney stem cells were demonstrated by flow cytometry with expression of stem cell marker (CD133) as well embryonic kidney marker (CD24), and lack of expression of hematopoietic stem cell markers (CD45 and CD34). Approximately 20% of the cell population expressed both CD133⁺CD24⁺, indicating renal progenitor cell status. These data demonstrate that 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, severe acute rejection, and chronic allograft nephropathy.

Name of Principle Investigator: **Karen Zeller**

Project Budget: \$ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **The Role of HMGA1a in Normal & Leukemic Stem cells**

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

Human embryonic stem cells (hESCs) are characterized by 2 unique properties: 1) Self-renewal or the ability to form new hESCs, and, 2) Pluripotency or the potential to differentiate into any mature cell. Because of these 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 characteristics should enable us to harness these cells for use in regenerative medicine and cancer therapy. Our work is directed at understanding the molecular pathways that regulate stem cell properties. Our focus is the HMGA1 gene because preliminary studies suggest that it plays an important role in this process. HMGA1 is expressed at high levels during embryologic development. We showed that HMGA1 is highly expressed in hESCs and its expression falls dramatically with differentiation. In fact, the expression pattern parallels that of Nanog and SOX2, two genes important in hESC renewal. These findings indicate that HMGA1 may function in stem cell survival or proliferation. 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. This is consistent with the idea that cancer cells with stem-like properties (cancer stem cells) lead to aggressive cancers that are refractory to therapy because of their inherent hESC properties, namely, the ability to self-renew indefinitely, and low baseline proliferative rates. This latter characteristic is thought to confer resistance to therapy because most chemotherapeutic agents target cells that are actively dividing. Thus, we hypothesize that HMGA1 functions in the survival or proliferation of stem cells, both in normal development and in cancer. We hypothesize further that this property contributes to the refractory nature of cancers overexpressing HMGA1. To study HMGA1 in cancer, we engineered transgenic mice overexpressing HMGA1 and all mice succumb to aggressive leukemia that closely models refractory human leukemia. The tumors can be serially transplanted into recipient mice, indicating that at least some HMGA1-induced tumor cells have long-term self-renewal capabilities. In studies with primary human leukemic blasts, we found that HMGA1 mRNA expression is highest in the stem cell-like fraction compared to the bulk tumor cells in preliminary studies. Taken together, these results suggest that HMGA1 is important in maintaining "stemness" and our studies are directed at elucidating its role in this process. In our recently funded MSCRF application, we propose the following Specific Aims: 1.) Define the role of HMGA1 in hESC survival and proliferation, and, 2.) Elucidate the role of HMGA1 in human leukemic stem cells. Results from these studies will advance our knowledge of "normal" and "cancer" stem cells. A better understanding of the molecular circuitry that underlies stem cell properties should lead to the discovery of novel therapies directed at cancer stem cells and the capability to harness stem cells for use in regenerative medicine for diseases affected by abnormalities or damage to normal stem cells.



FY 2008

Investigator Initiated

Awarded

Research Descriptions

Name of Principle Investigator: Laure Aurelian

Project Budget: \$ 827,171.00

Grantee: University of Maryland, Baltimore

Title: Stem cell transplantation is associated with HSV-induced severe skin eruptions

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

Stem cells transplants are a promising new therapeutic approach for various diseases, including leukemia, lymphoma and myeloma, but the associated risks are still poorly understood. Understanding the potential risks with which it is associated is of paramount importance, as it will enable the development of risk-reducing modalities that will enhance its potential and maximize its therapeutic advantage. We have recently shown that a high proportion of such transplant patients (80%) develop severe skin eruptions, which we called Stem Cell Transplant Erythema (SCTE). Our Preliminary data suggested, but did not definitively prove, that these eruptions are associated with reactivation of latent infection with Herpes simplex virus (HSV), which is commonly seen in over 80% of adult subjects. These data suggested that reactivated HSV preferentially infects the transplanted stem cells, and they carry the viral genes to the skin, where they cause the eruption. The proposed studies are designed to test the validity of this interpretation and determine whether the frequency/severity of SCTE can be reduced by treatment with an antiviral drug that better prevents virus reactivation. These studies are of utmost significance and will directly contribute to the accomplishment of the goals of the Maryland Stem Cell Research Fund.

Name of Principle Investigator: **Stephen Baylin**

Project Budget: \$ **1,725,000.00**

Grantee: **University of Maryland, Baltimore**

Title: **Genomic and Epigenetic Instability of Human Stem Cells Used In Regenerative Medicine**

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

One of the biggest potential problems for the use of embryonic stem cells to treat, via transplant delivery, human chronic severe diseases, such as traumatic neurological situations, degenerative nerve diseases, cancer, etc is that all experimental evidence shows they have a potential to generate tumors over time. Until this property of such cells is controlled, the future use of the cells in humans is problematical. In this proposal, we bring together scientists expert in studies of molecular abnormalities underlying the formation of cancer with those expert in the cultivation of stem cells and their transplantation to try and uncover some of the major steps in tumor potential and formation from human embryonic and adult stem cells. Importantly, this includes adult stem cells which have been engineered to adopt embryonic stem cell properties by inserting key genes. During the cultivation of all of the above cells, and in transplant settings for the cells in mice, we will study aspects of cell stress that cause genetic changes known to potentiate tumor development. These stresses, like chronic injury processes and inflammation, are key aspects of the settings where transplantation of stem cells will be the most valuable. We will also study a change in DNA that causes dysfunction of genes in adult cancers without causing mutations – a process known as abnormal epigenetic changes. Both of the abnormalities under study are experimentally reversible such that their discovery as a factor in tumor potential of embryonic and other stem cells will suggest defined approaches to minimizing this potential. This could help, markedly, in speeding up the time in which the stem cells of all types can actually be used to treat human disease.

Name of Principle Investigator: **Ted M. Dawson**

Project Budget: \$ **1,725,000.00**

Grantee: **Johns Hopkins University**

Title: **Derivation and Characterization of Human Dopamine Neurons Derived from Inducible Pluripotent Stem Cells**

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

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. Parkinson's disease (PD) is the most common movement disorder that is due, in part, to the preferential loss of dopamine (DA) neurons. The relative selective degeneration of DA neurons makes PD a particularly attractive human neurodegenerative disease to establish patient specific cells in culture. There are well established protocols for differentiating stem cells into DA neurons and there are robust cellular and animal models that can be used to study the function of these stem cell derived DA neurons. Using standard protocols and technology, the co-investigators of this grant will establish inducible Pluripotent Stem (iPS) cells from patients with PD, correct mutations in disease causing familial PD genes and produce DA neurons from iPS cells for studies in cellular and animal models. To accomplish these goals human iPS cells will be generated and characterized from sporadic and genetic PD and control patients. Methods to correct genetic defects and genetic manipulation of patient-specific iPS cells will be developed and validated. Neuronal precursors and DA Neurons will be differentiated and characterized electrophysiologically, biochemically and genomically from human patient-specific iPS cells. In summary, we propose an innovative set of specific aims 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):

This application meets the stated RFA program “to broaden and advance basic knowledge of human stem cell biology that will be relevant for eventual development of clinical applications.” We propose to study Notch and Botch signaling in hNPCs and understand how these pathways lead to differentiation and maturation into specific cells of the nervous system.

There are approximately 250 neurological – brain and nervous system – disorders. Combined, neurologic disorders are the leading cause of death, disability and loss of quality of life worldwide according to the World Health Organization. Unfortunately there are few therapies for individuals who suffer from neurologic disease. Drug therapy has lagged behind drug development for other human diseases, in part because drugs cannot be developed and tested on human neural cells. Since most neurologic diseases are due to loss of neural cells. Replacement therapy through the generation of the needed cell population derived from differentiation stem cells into neurons or oligodendrocytes is an attractive possibility. It is likely that both approaches, drug therapy and replacement therapy, will be required to effectively treat neurologic disease.

However, under current protocols for differentiation and maturation of hESCs 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. Cell fate specification is a highly ordered process in the nervous system that balances the maintenance and proliferation of neural stem/progenitor cells, with the generation of functional neurons and glia. The Notch pathway is of fundamental importance during this development. Recently we have found that manipulating the Notch/Botch signaling pathway in rodent neural precursor cells results in a reversal of this ratio so that the majority of the cells differentiate and mature into neurons. However, all of this work has been done non-human mammalian systems. In this application we propose to understand these signaling events in human cells and determine if these cells develop into mature functional neurons and to find small molecules that can produce the same effect so that hESCs/hNPCs can be driven towards a neuronal and perhaps oligodendrocyte cell fate by a defined chemical cocktail. This would allow for the relatively straightforward and consistent generation of large numbers of neurons or oligodendrocytes for drug-screening and drug development as well as eventually for human cell replacement therapy.

Name of Principle Investigator: **Richard L. Eckert**

Project Budget: \$ **941,315.00**

Grantee: **University of Maryland, Baltimore**

Title: **Epidermis-Derived Multipotent Cells for Cell Therapy**

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

The skin surface (epidermis) is comprised of a large reservoir of readily-accessible cells that can be easily tapped for cell therapy applications. Using the epidermis as a source of cells is an attractive plan because the cells are abundant (the skin is the largest organ of the body) and because the epidermis is readily accessible (it is the outside layer of the skin). Our strategy is to change the epidermal cells into multipotent cells that can then be converted into other cell types for cell-based therapy. It has been previously shown by us that epidermal stem cells from the skin are multipotent, and that upon de-differentiation these cells can populate several tissue types. If the technology required to convert these cells to the multipotent state can be perfected, epidermal cells could provide an abundant and accessible substitute for embryonic stem (ES) cells. The goal of this project is to demonstrate that this approach is feasible. The concept that a single manipulation could induce genetic reprogramming an adult skin cell has tremendous medical importance for the large scale generation of therapeutically useful cells. Given the number of cells in the skin and the relative ease with which these cells can be harvested, they should provide a potentially huge reservoir of reprogrammable cells for use in cell-based disease therapy.

Name of Principle Investigator: John T. Iaascs

Project Budget: \$ 910,522.00

Grantee: University of Maryland, Baltimore

Title: Developing Methods for the Identification and Isolation of Prostate Cancer Stem Cells

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

Presently there is no effective treatment for either preventing the initial development of prostate cancer or curing a man once he develops prostate cancer throughout the body (i.e. metastatic disease). Despite intensive efforts to develop new treatments, the standard therapy for prostate cancer once it has spread throughout the body has remained the same for the last 50 years. The standard approach is to block the body's ability to produce androgens. This is termed androgen ablation or more commonly hormonal therapy. In men with prostate cancer spread throughout the body, such androgen ablation does produce an initial positive relief from clinical symptoms. Unfortunately, however, after a variable period of symptom relief, symptoms return eventually killing the patient. Thus, prostate cancer will kill 30,000 US males this year alone and the death rate is twice as high in African Americans with Maryland having one of the highest incidence rates in the nation. The killing ability of prostate cancer is due to the presence of Cancer Stem Cells (CSC) within the patient. Presently, there are no reproducible methods for growing these CSC cells outside of the patient's body. This is a major barrier for drug development since these CSC cells must be used to identify new drugs which for their elimination from the patient's body. Therefore, methods urgently need to be developed for identifying, isolating, and establishing prostate cancer stem cell lines from a series (n=10-15) of prostate cancers patients so that these can be used a panel of test cells for drug development. Such a goal is realistic due to the unique tissue (i.e. at least one radical prostatectomy specimen available 5 day a week), equipment (i.e. P.A.L.M /Zeiss Laser Microdissection and Pressure Catapulting microscope for isolating live cells from culture), and personnel (i.e. a multi-disciplinary prostate cancer stem cell working group) resources available within the Sidney Kimmel Comprehensive Cancer Center at Hopkins. Once established, this series of CSC lines will be placed in American Tissue Culture Collection, a non-profit national repository, so that any individual or organization can obtain these CSC for mechanistic studies needed to accelerate rational develop of effective therapies for both the prevention and treatment of prostate cancer.

Name of Principle Investigator: Dara L. Kraitchman

Project Budget: \$ 1,378,731.00

Grantee: Johns Hopkins University

Title: Magnetic Resonance Imaging (MRI)-Visible Microencapsulation of Allogeneic Mesenchymal Stem Cells for Arteriogenic Therapy in Peripheral Arterial Disease

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

Peripheral arterial disease (PAD) is a problem with blood flow in the arteries, most commonly due to narrowing of the arteries, that affects ~8-12 million Americans. The most common complaint from PAD is pain in the calf or thigh with walking. However, if severe enough, PAD may lead to pain at rest, distal limb ulceration and, ultimately, the need for amputation. Quality of life scores of PAD patients with such critical blood flow loss have often been compared to terminal cancer patients. Due to the extent of disease, many patients may not gain relief from conservative medical therapy or invasive surgical therapy. Moreover, it is thought that PAD may result from an inherent problem with a person's own stem cells such that the normal repair of blood vessels is impaired. Giving stem cell back to PAD patients may offer a way to provide these building blocks for regenerating vessels or "angiogenesis." Because of this potential for a PAD patient's own stem cells to be poor quality, stem cells from healthy donors may be the best choice for a readily available supply to treat patients. However, destruction of these mismatched stem cells can severely limit the dose of stem cells that survive and thus effectiveness of the treatment. In fact, current cellular therapies all suffer from extremely low survival of stem cells at 24 hours after delivery. In addition, clinical cellular therapy trials will require methods to monitor delivery, engraftment, and therapeutic benefit in a non-invasive manner. Therefore, methods to protect stem cells from early destruction and also prevent patient rejection of donor cellular therapies that could be monitored non-invasively would be of tremendous benefit.

We propose to develop a novel method to immuno-isolate donor stem cells by microencapsulation ("MCaps") for enhanced survival without immune suppressing drugs and also make them visible by Magnetic Resonance Imaging (MRI) for guiding delivery and Positron Emission Tomographic (PET) imaging for tracking cell fate. Thus, the specific aims of this proposal are to demonstrate that: 1. MRI-visible alginate-based microcapsules (MCaps) for encapsulation of donor human mesenchymal stem cells (hMSCs) do not adversely effect cell viability while providing sufficient contrast for stem cell delivery and tracking using MRI AND PET; 2. MCaps containing hMSCs increase MSC engraftment in a rabbit model of hindlimb ischemia compared to non-encapsulated hMSCs; 3. MCaps with hMSCs enhance arteriogenesis, compared to MCaps with human iliac artery endothelial cells, non-encapsulated MSCs, or empty microcapsules sham injections; and 4. MCaps with hMSCs from fat tissue are as effective as hMSCs from bone marrow. Because MCaps are composed of clinical grade products, we anticipate that these studies will provide a pathway for translation of MCaps to therapeutic arteriogenesis clinical trials in PAD.

Name of Principle Investigator: John Laterra

Project Budget: \$ 751,699.00

Grantee: Hugo W. Moser Research Institute at Kennedy Krieger

Title: Molecular Mechanisms of Tumor-Propagation by Human Glioblastoma Stem Cells

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

Glioblastoma multiforme (GBM) is the most common and most aggressive brain cancer. Survival for patients with GBM remains dismal. Half of the patients survival for less than 14 months and few live beyond 5 years. Understanding how GBM resists our most aggressive surgery, radiation, and chemotherapies is critical to improving patient care, quality of life and survival. Recent findings have found that a small subset of cancer cells within human GBM have the features of cancer stem cells. 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 (GBM-SCs) are required to maintain GBM growth and it is possible that a single surviving GBM-SC can cause the cancer to recur. These findings predict that curing glioblastoma will require therapy that either kills the malignant stem- cells or permanently prevents them from growing. Since GBM-SCs are relatively resistant to current therapies, identifying the molecular processes that allow them to form tumors is needed to target and treat the GBM-SCs effectively.

We have recently found that the ability of GBM-SCs to grow in the laboratory and generate tumors in experimental animals is blocked by a class of chemicals called histone deacetylase inhibitors (HDACIs). HDACIs alter cellular gene expression. We have identified a number of genes and their protein products that are affected by HDACIs in GBM-SCs. Two of these genes are particularly novel and exciting within the context of GBM and cancer stem cell biology. We have already found that one of these genes, called DNER, can directly block the ability of GBM-SCs to grow in the laboratory and form tumors in animals. We now plan to determine how HDACIs, DNER, and a second promising HDACI-responsive gene, ID4, alter human GBM-SC growth and tumor formation. Our research will involve the production of multiple new GBM-SCs from consenting Johns Hopkins Hospital neurosurgery patients. These cells will become a valuable resource for other Maryland scientists interested in studying GBM-SCs. Our successful results will identify how GBM-SC grow and maintain themselves, identify new ways to target GBM-SC and thereby treat patients with GBM.

Name of Principle Investigator: **Nicholas J. Maragakis**

Project Budget: \$ **976,243.00**

Grantee: **Johns Hopkins University**

Title: **Human Astrocyte Progenitor Transplantation for Neuroprotection in a Model of Amyotrophic Lateral Sclerosis (ALS)**

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

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, is a disorder that affects the motor neurons of the brain and spinal cord. Approximately 7 out of every 100,000 people have the disease making it the most common form of adult motor neuron disease. This disease results in progressive weakness and eventual paralysis of the muscles in the arms, legs, and muscles of breathing. There is no known cure for this disease and patients typically die from 2-5 years after the disease starts.

While many cell transplantation strategies have focused on the replacement of neurons, we will use stem cells called glial restricted precursors (GRPs). These cell types may be able to protect the body's own motor neurons from dying by preventing the buildup of the neurotransmitter glutamate or by other mechanisms.

We will use a rat model of ALS to study how well these cells work after being transplanted. We will target our cells to the neck region of the spinal cord—a region where nerves that control the muscles for breathing are located. In doing so, we will be able to assess whether the transplantation of GRPs can result in an improvement in limb strength, breathing, and a prolongation of life in these animals. We believe that targeting these regions with the cells in this study will provide us with a strategy for an eventual trial of these cell types in humans with ALS.

Our preliminary studies in the rat model of ALS (using rat GRPs) show that transplanted GRPs are already capable of integrating into the spinal cord next to motor neurons and surviving for the life of the animal. It also appears that motor neurons are protected by the transplanted GRPs. This is reflected in that the ALS rats live longer, remain stronger, and have preserved breathing function. In the first part of the study we will determine that the GRPs become our target cell type (astrocyte). We will also look to see if the GRPs migrate up and down the spinal cord and make sure that they do not form tumors. In the second part of the study we will establish whether the transplanted GRPs protect the ALS rat's motor neurons from dying. We will also establish the mechanism behind how this neuroprotection occurs. In the final part of our study we will establish whether there is any difference in the strength of the ALS rat's limbs. We will also establish whether the ALS rats are able to maintain their breathing, and determine if the animals that are transplanted with GRPs live longer. These aims of this study parallel the experimental design we have already carried out using the rat GRPs. Demonstrating whether the human GRPs have the same properties and promises for neuroprotection are important in proceeding to human ALS clinical trials.

Name of Principle Investigator: **Jeffrey D. Rothstein**

Project Budget: \$ **1,010,173.00**

Grantee: **Johns Hopkins University**

Title: **Human Astroglial Stem Cells: Combinatorial Drug Discovery Program**

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

Motor neuron diseases- amyotrophic lateral sclerosis (Lou Gehrig's disease) and spinal muscular atrophy are chronic progressive, neuromuscular diseases that are caused by death of brain cells-motor neurons in brain and in spinal cord. Loss of motor neurons causes muscle weakness, atrophy, and ultimately inability to breath followed by death. In the USA, ALS is the cause of death in 1 out of every 800 men that die, and spinal muscular atrophy is the second most common autosomal recessive disease of newborn babies. There is no therapy capable of substantially slowing disease or reversing symptoms and both diseases are invariably fatal. There has been little interest in pharmaceutical companies to develop drugs for these relatively rare diseases. The disease occurs because motor neurons in the spinal cord and brain die which leads to loss of the ability to move muscles. The death of these cells is due, in part, to the dysfunction of neighboring cells, known as astrocytes. These surrounding cells contribute to the loss of motor neurons, in part, because they cannot remove a toxic chemical glutamate. There have been no methods to effectively discover new drugs for the diseases- because of a lack of human-relevant tools.

In this proposal we will use human astrocytes, derived from embryonic and fetal stem cells as tools to discover new drugs. The human stem cells will be tested with a large collection of FDA approved drugs in order to find therapeutics capable of enhancing the normal protective properties of brain astrocytes. This is an ideal use of these unique human cells. New drug discovery can take 10-15 years at a cost of more than billion dollars per drug. Most drugs fail because of safety and toxicity properties. However we intend on screening our ALS-relevant cells with FDA approved drugs in a novel combination paradigm- looking to discover drug combinations that synergistically act together to slow cellular degeneration. This approach has proven highly effective in speeding up drug discovery for cancer, but has heretofore not been applied to fatal neurological disease. In this three year proposal we will use recently generated human stem cell lines to perform a large drug screen, looking at up to a million different drug combinations, make sure the best drugs can get into the brain, and finally test our best candidates in an animal model of ALS. We believe this approach will lead to rapid discovery of powerful, FDA approved drugs for ALS and SMA patients. This novel expedited drug discovery program will capitalize on the strength Maryland academic scientists and clinicians working together with Maryland Biotechnology companies. At the conclusion of this program we hope to have several new candidate drugs that will be available for Maryland ALS and SMA patients.

Name of Principle Investigator: **Elias Zambidis**

Project Budget: \$ **1,725,000.00**

Grantee: **Johns Hopkins University**

Title: **Efficient Generation of Patient Specific Human Embryonic Hemangioblasts**

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

Although pluripotent human embryonic stem cells (hESC) promise the potential for generating unlimited amounts of clinically transplantable hematopoietic stem cells (HSC), this goal has thus far been elusive. In addition to overcoming the technical hurdles of generating HSC from hESC, another technical obstacle is the generation of histocompatible hESC that can generate autologous HSC for those patients that do not have cord blood or bone marrow-derived transplant antigen matches.

Our group first elucidated the step-wise differentiation of hESC to embryonic erythro-myelopoiesis, and demonstrated that these hematopoietic progenitors are similar to those found in human yolk sacs, and therefore too immature to provide adult-type hematopoietic reconstitution. We are now focused on a developmental approach for maturing primitive hematopoietic progenitors (hemangioblasts) from hESC into the adult-type transplantable HSC that are normally found in adult bone marrow niches. This project proposal now extends these efforts toward the generation of patient-specific human hemangioblasts for therapy.

We will evaluate, in detail, the potential for deriving hemangioblasts following the derivation of patient-specific pluripotent human stem cells. Generation of human hemangioblasts from autologous hESC promises great therapeutic opportunities for a variety of hematologic diseases. We will utilize normal (IVF-derived), diseased (preimplantation genetic diagnosis (PGD)-screened), parthenogenetic, and induced pluripotent (iPS) hESC to directly address the major obstacles faced in deriving transplantable HSC from hESC, by employing a rational, developmental approach.

Finally, we will initiate a discovery-based analysis on understanding a new class of regulatory molecules called microRNAs that promise great potential for efficiently guiding human pluripotent stem cells toward HSC differentiation. To accomplish this goal, we will characterize the comparative microRNA profiles of hESC, human hemangioblasts, and neonatal cord blood HSC. This series of studies will serve as a roadmap for ultimately elucidating how to efficiently direct hESC to the hematopoietic lineage using these small RNA master regulators of development.

The ultimate goal of our proposal is the optimized, large-scale production of autologous human hemangioblasts for patients. These versatile, newly-discovered human stem-progenitors are expected to ultimately provide transplantable HSC for treating hematologic disorders, for facilitating endothelial regeneration in myocardial and retinal diseases, and perhaps also for the induction of immune tolerance in regenerative medicine.



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):

Applying fresh ideas of stem cell biology to cancer is resulting in a significant change of our understanding of tumor growth and cancer progression. The emerging paradigm is that very few cancer initiating cells also known as cancer stem cells are at the origin of tumor development and that they play an important role in cancer progression and metastasis. Cancer stem cells have been originally identified in leukemias and subsequently been found in multiple solid tumors including breast cancer. These cells are inherently resistant to therapeutic intervention as they express specialized pumps in the cellular membrane that export drug molecules from the cell rendering the cell drug resistant. After successful treatment that removes the bulk of the tumor these few remaining stem cells can rapidly repopulate the tumor resulting in clinical tumor recurrence.

In this proposal we will develop a novel agent that can be used both for imaging of the breast cancer stem cells and for highly specific delivery of therapeutic drugs to these cells using a strategy that can circumvent drug resistance mechanisms described above. Molecular targets expressed at the surface of breast cancer stem cells will be used to guide delivery and uptake of the agent by the cancer stem cell. The agent will be tested in preclinical animal models of human breast cancer and if successful this strategy can be potentially moved to clinical trials. Since imaging modalities such as optical and MR imaging used to detect the agent delivery to the tumor are completely noninvasive this will facilitate clinical translation.

Name of Principle Investigator: Sergei Atamas

Project Budget: \$ 224,750.00

Grantee: University of Maryland, Baltimore

Title: Bone and Tendon Healing Mechanisms of Mesenchymal Stem Cells and Hematopoietic Progenitors Derived From Human Adult Bone Marrow

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

Tendons, tough bands of fibrous tissue that connect muscles to bones, may become involved in a disease process called tendinopathy, especially in association with sports or military training, workplace activities, and in patients with chronic diseases (diabetes, kidney diseases, and arthritis). Many tendinopathy cases can be successfully treated, but often tendinopathies become chronic (long lasting), and require surgery that is usually performed by orthopedists - surgeons specializing in musculoskeletal diseases. The recovery from such surgeries is slow and often incomplete; patients are left with significant limitations of joint mobility; and in some cases surgery does not improve the disease course. Orthopedists often deal with another serious and long-lasting disease process not only in tendons but also in their patients' bones. In this process called bone nonunion, the bone fragments after fracture or surgery fail to fuse and restore the integrity of the bone. We have developed novel experimental treatment for these two groups of patients with tendinopathies or bone nonunions. During the surgery, we take stem cells from the bone marrow of a patient and insert them into the surgery site in the tendon or bone in the same patient. We have treated more than 120 patients already, and such treated patients heal significantly quicker, with less swelling and pain, and with more complete functional restoration following the surgery than do similar patients after surgery alone. In addition to these observations in patients, preliminary studies in the diseased tendon tissues from patients showed disturbances in molecules that regulate swelling and degradation of the tissue, whereas stem cells produced molecules that have potential to facilitate tendon healing. The objective of this study is to characterize the molecules produced by two types of bone marrow stem cells (so-called mesenchymal stem cells and so-called hematopoietic progenitors), focusing on specific molecules that are known to facilitate healing of tissues. Furthermore, the study will determine whether stem cells from the bone marrow of otherwise healthy patients with traumatic tendon injuries have higher healing capacity than similar cells from patients with long-term non-healing tendon diseases. Most importantly, the cells that compose tendon tissue will be placed together with stem cells in a Petri dish, and activation of molecular healing processes within tendon cells in the presence of stem cells will be investigated. The results of this study may identify specific molecular mechanisms by which bone marrow-derived stem cells facilitate tendon healing, and thus form basis for novel treatments based on adult stem cell.

Name of Principle Investigator: Ilia V. Baskakov

Project Budget: \$ 230,000.00

Grantee: University of Maryland Biotechnology Institute

Title: Design of self-assembling peptide scaffold for controlling neural stem cell state and fate

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

Stem cell therapy holds great promises in medical treatment. Neural stem cells (NSC) can potentially be used to replace tissue loss due to neurodegenerative diseases or brain trauma. Because the extracellular environment determines the outcome of cell transplantation, the design of biomaterials for replacing natural extracellular matrix is essential for implementing successful therapeutic strategies. The currently used biomaterials have limited potentials in delivery and in controlling stem cell characteristics. Engineering of 'smart' self-assembling biopolymers with physical and functional properties reminiscent to those of the natural extracellular matrix is a key in achieving desired outcomes for stem cell therapy.

In the first part of the proposed study, we will assess the role of the prion protein in controlling neuronal fate. The prion protein has been previously shown to provide positive effects on neuronal development. We hypothesize that the prion protein is important for the early stages of neurodifferentiation. We will test the effect of the prion protein on differentiation of human NSCs, and evaluate whether the prion protein can be utilized in engineering a peptide-based extracellular scaffold.

In the second part, we propose to design new class of self-assembling, peptide-based scaffolds with an intrinsic ability to control NSCs fate and to serve as delivery media in the course of regenerative therapy. Using human neural stem cells, we will examine the biological properties of the new class of scaffold. The physical properties will be investigated using a broad range of biophysical techniques.

The current studies promise to develop novel peptide-based scaffold with intrinsic ability to control NSCs fate. This new material holds a high promise to be utilized for 3D cultivation of ESCs and for cell delivery in regeneration medicine. In addition, the current work will provide new important insight into functional role of the prion protein and examine its utility for engineering of peptide-based scaffolds.

Name of Principle Investigator: **Fred Bunz**

Project Budget: \$ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **Developing alternative methods for iPS cell generation**

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

Among the most promising developments in biomedical science has been the recent demonstration that normal cells can be reprogrammed into stem cells, ie. cells that can develop into any tissue in the body. The derivation of stem cells from adult cells - rather than from embryos - would circumvent both technical and ethical obstacles to developing stem cell-based therapies. In seminal experiments published in the past several months, several groups have shown that the addition of a minimum of four genes is sufficient to give rise to type of stem cell, known as induced pluripotent stem cells (iPS cells). Current methods to generate iPS cells involve the use of potentially carcinogenic viruses to introduce these genes and are highly inefficient. The safe translation of this exciting technology into the clinic will require alternative methods for the generation and isolation of iPS cells.

In this exploratory project, we propose to develop a system that uses cancer cells to promote the generation of iPS cells without the use of dangerous viruses. This system is based on a recently described gene, known as UTF1, that is only active in stem cells. A novel form of UTF1 will be carried as a genetic element that does not integrate into the genome, known as an episome. The delivery of the modified UTF1 gene as an episome will decrease the potential that a cell that carries this gene will develop into a cancer cell. Our scheme is to block the growth of that carry the UTF1 episome at various stages of their growth cycle. Then, we will release these synchronized target cells into culture conditions that support the growth of stem cells. The presence of the modified UTF1 gene will allow us to separate and propagate the population of cells that have undergone some of the changes seen in reprogrammed cells. Our results will be assessed quantitatively, by counting the number of cells that have turned on UTF1, and qualitatively, by detection of known iPS markers. In a relatively short period of time we hope to determine whether an episomal selection scheme can isolate reprogrammed cells, and whether we can induce cells to reprogram a discernable rate. The goal of these studies is to begin to establish conditions by which normal human cells can be selectively reprogrammed for use in the clinic.

Name of Principle Investigator: Chi V. Dang

Project Budget: \$ 230,000.00

Grantee: Johns Hopkins University

Title: A novel, robust strategy to map human stem cellness transcriptional network

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

Self-renewal capacity and the potential to become all mature cells (or pluripotency) are two hallmarks of human embryonic and pluripotent stem cells. These hallmarks require that stem cells maintain a highly flexible set of genetic instructions capable of being translated for stem cells to become a specific cell type. There are several master regulators of these hallmarks. These regulatory proteins, called transcription factors, switch on or off other genes that in turn affect the ability of a cell to produce components of the cell or to divide into equal daughter cells. The OCT4 and SOX2 genes are master switches that are not only known to maintain the pluripotency of human embryonic stem cells to , but they are also capable of converting an adult skin cell to a pluripotent stem cell that has virtually all known characteristics of a human embryonic stem cell. Despite there powerful activities, the ways by which Oct4 and Sox2 regulate the genetic information readout are not very well understood. What genes Oct4 or Sox2 control to maintain pluripotency are very poorly understood. In this application, we propose to use our newly develop set of tools that will reveal an unprecedented view of how these stem cell regulators function to alter the reading of the human genome. These studies are of fundamental importance as well as being critical for the basic understanding of human embryonic stem cells for therapy. The success of Maryland biotechnology sector will depend on the deeper understanding stem cell therapy, which is in part addressed by our proposed studies.

Name of Principle Investigator: Jennifer Elisseeff

Project Budget: \$ 227,601.00

Grantee: Johns Hopkins University

Title: Adult and embryonic stem cells for cartilage repair and OA

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

Osteoarthritis (OA) is a degenerative disease that affects the cartilage lining the surface of bones in the joints. This loss of cartilage causes significant pain, often leading to reduced physical activity, increasing risk factors for other life threatening ailments such as cardiovascular disease. OA can arise from small cartilage defects, which cannot repair and for which there is no efficacious treatment. The cartilage defects gradually increase in size and there is progressive degeneration of cartilage over the joint surface. OA is also associated with aging, resulting from general wear and tear on the joints. Current clinical strategies to treat cartilage today are generally end stage (joint replacement) or palliative in nature (hyaluronic acid injections to lubricate joint and anti-inflammatories). However, new technologies aim to provide a biological repair which promotes new cartilage growth. Treatments aimed towards cartilage tissue regeneration currently available include surgical microfracture or the cell therapy autologous chondrocyte implantation (ACI). The first clinical trial utilizing mesenchymal stem cell (MSC) injections to treat joint injuries has also provided a glimpse of potential beneficial effects of stem cells. Other new technologies for cartilage repair in research or clinical development phases include degradable materials designed to induce tissue repair and stem cells. These new therapies aim to repair cartilage more efficiently than the current surgical gold standard microfracture (which generally forms a scar-like cartilage with short term durability) and in a simpler, more cost effective manner compared to ACI which is surgically challenging and costly. However, these biological cartilage repair strategies have rarely been tested in an arthritic environment.

Our research addresses cartilage repair by generating new technologies (biosynthetic hydrogels and adhesives) that can be used with exogenous or host cells to generate new cartilage. One of our biomaterial technologies is currently in clinical trials in conjunction with microfracture. However, severe (large) lesions or lesions in a diseased (OA) environment will likely need additional help such as a unique biomaterial and/or exogenous cells to fully repair. We have produced cartilage tissue from chondrocytes, adult (bone marrow-derived), and embryonic stem cells in conjunction with our biomaterials. The purpose of this exploratory stem cell grant is to compare the tissue forming capabilities of human adult MSCs with a similar cell population we have derived from human embryonic stem cells and evaluate their application to cartilage repair in the challenging arthritic environment. After in vitro comparison, we will then apply these human cell populations to cartilage repair in a normal and osteoarthritic rat joint.

Name of Principle Investigator: **Alan D. Friedman**

Project Budget: \$ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **Regulation of Human Hematopoietic Stem Cell Proliferation by RUNX1, Notch and Wnt**

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

The focus of this application is developing an improved understanding of the intrinsic regulation of human hematopoietic stem cell (HSC) proliferation, with the ultimate goal of expanding human adult or ES-derived HSC to benefit patients. Expanding HSC will benefit expand our ability to offer stem cell transplantation or autologous transfusions to patients with cancer or marrow 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 those with aplastic anemia, will serve as a vehicle for improving gene therapies for genetic diseases such as sickle cell disease, and will provide a stem cell population that might be induced to trans-differentiate to non-hematopoietic cell types to treat a range of diseases. We have found that a protein termed RUNX1 directly stimulates hematopoietic cell proliferation and that a modified form of RUNX1 that occurs naturally cells is even more effective. Aim 1 of this proposal is to determine whether RUNX1 or its modified form is capable of expanding human HSC obtained from cord blood or adult bone marrow or peripheral blood. Notch is protein that cooperates with RUNX1 to expand HSC in mice and in zebrafish. Aim 2 of this proposal will determine whether Notch1 induces the proliferation of human HSC in cooperation with RUNX1. Wnt is a third protein that induces HSC proliferation. Aim 2 will also determine whether Wnt and RUNX1 cooperate to induce human HSC expansion. These studies with adult HSC have the potential for direct clinical application. In the long-term we expect to expand our findings to the human ES system, which may offer additional translational opportunities.

Name of Principle Investigator: Nicholas Gaiano

Project Budget: \$ 230,000.00

Grantee: Johns Hopkins University

Title: Exploring the role of NF- κ B signaling in human neural stem cells

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

How the brain develops from neural stem cells (also sometimes called “progenitors”) into the most intricate structure in the human body is a central question facing modern biology. The proposed work will investigate this question in human neural progenitor cells (hNPCs) derived from human embryonic stem cells (hESCs). In particular, we will examine how the NF- κ B signaling pathway controls the behavior of hNPCs. The NF- κ B pathway is a molecular cascade that activates a biological signal after a number of potential stimuli. We are interested in determining how this pathway and its signals influence the decision of hNPCs to remain progenitors, or to become neurons and supporting glial cells. The function of the NF- κ B pathway has been intensively studied primarily in the context of the immune system where it plays an important role. While numerous studies have examined the role of NF- κ B in mature neurons, until recently little was known about the role of this pathway in neural progenitors. Our recent studies, using mice as the experimental mammalian model, have found that the NF- κ B pathway is highly active in neural progenitors, where it regulates the balance between cell division and neuronal or glial development. Much remains to be understood about the role of NF- κ B in mammalian neural progenitors, and currently nothing is known about the functioning of this pathway in human NPCs. In the proposed work we will pursue three primary objectives: (1) We will characterize NF- κ B pathway usage in hESCs and hNPCs. This will be done by examining gene expression in these cells (the proteins in the NF- κ B molecular cascade are encoded by numerous different genes), and by using what are called “reporter” strategies, which produce easily visualized fluorescent protein when the signaling pathway is stimulated, or the characteristics of the cells have changed in certain ways. (2) We will examine the effects of activating or blocking NF- κ B signaling on the balance between hNPC maintenance and cell division, and differentiation into neurons and glial cells. (3) We will use cell transplantation into the embryonic mouse brain to determine what types of cells (i.e. among the many different kinds of neurons and glia) hNPCs can generate under ordinary circumstances, or with the NF- κ B pathway activated or inhibited. By placing cells into the highly dynamic and permissive embryonic environment, we will learn about their intrinsic character. Subsequent studies (seeded by this work) will consider how NF- κ B modulation could contribute to the generation of cell types that can integrate into the mature brain after transplantation. 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.

Name of Principle Investigator: Yoon Young Jang

Project Budget: \$ 230,000.00

Grantee: Johns Hopkins University

Title: Human Embryonic Stem Cell Transplantation For Liver Regeneration

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

Liver transplantation has been the most successful treatment for many liver diseases. However, the efficacy is limited by the shortage of appropriate donors. Human embryonic stem cells (hESCs) may serve as an ideal source for liver cell transplantation. Although hESCs have recently been shown to efficiently differentiate into liver cells in vitro, the engraftment level of these liver cells is low thus whether these cells have normal liver cell function in vivo (i.e. regeneration of damaged liver) is not clear. Stem cell derived cells in vitro expressing several liver markers are not always functional in vivo. Therefore, it is necessary to investigate their in vivo function with liver disease models, which has been successfully performed in our laboratory using mouse stem cells. Further, to clinically utilize hESC derived cells, we should prevent the hESC related tumor formation (i.e. teratoma).

Based upon our strength in stem cell transplantation into animal models of liver diseases, we propose a new project to investigate the potential of hESC derived liver cells for the repair of injured liver tissues without producing tumors. Thus, we will utilize multiple strategies to enhance the engraftment level and control tumor formation.

(1) We will determine the in vivo function of the hESC derived liver cells to restore the experimental liver failure in multiple liver disease models. Mouse models of acute and chronic liver injury which can induce high levels of engraftment and proliferation of donor cells will be utilized. Stage specific cell selection approaches will be carried out to examine whether the early stage liver cells have distinct capabilities for liver repair from the mature liver cells obtained at late stages of hESC differentiation. Time-course analyses of human liver specific markers and functional activities for the recipient livers will allow us to determine the therapeutic potential of hESC derived liver cells.

(2) We will thoroughly investigate tumorigenic potentials of these cells by long-term analyses and test several strategies against tumor formation: comparing the donor cells of different maturation stages, cell selection methods, graft sites, delivery methods, or an in vivo cell ablation system, etc. Performing these strategies, individually or in combination, will help develop technologies for preventing tumor formation associated with transplantation of hESC derived liver cells.

Completion of this study will provide an advanced methodology to utilize hESC derived functional liver cells for both basic and clinical research. Ultimately, we anticipate that limitations of current cell therapy (i.e. the shortage of donors, the risk of tumor) will be improved by safely using hESC derived liver cells for a variety of liver diseases such as Liver cancer; 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 Aspects Of Human Embryonic Stem Cell Differentiation Using Atomic Force Microscopy

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

While human embryonic stem cells (hESC) have strong potential for therapeutic use in treatment of disease (e.g., heart disease, Parkinson's and spinal cord injuries), inducing targeted differentiation in a controlled manner has proved to be problematic as populations of ESC consist of mixed populations of cells. Limited numbers of endpoints, including changes in mRNA and protein expression, are available for identifying or monitoring changes in cell status as they occur. Usually such measurements involve either destroying the cells or altering the conditions of the cells (e.g., cell staining protocols involving antibody binding). Furthermore, most assays involve assessment of cell populations and do not have the ability to monitor individual cell responses. Atomic Force Microscopy (AFM) combined with haptic (sense of touch) feedback can provide a means of mechanical characterization and monitoring of live, individual cells over time. In the proposed studies, a live-cell reporter gene will be used to follow loss or gain of function in human stem cells, using an embryonic carcinoma cell (ECC) line. This ECC line is used as a standard for characterization of human embryonic stem cells. Mechanical measurements, such as cell surface stiffness will also be obtained by AFM through force curves at discrete locations on the cell surface. A pluripotency-determining factor promoter linked to the green fluorescent protein will provide information on pluripotency status while a promoter specific to a targeted lineage (e.g., a neuronal specific promoter) will provide information on differentiation status. Differentiation will be induced using appropriate stimuli and conditions. Cell membrane stiffness measured through a haptic feedback device and fluorescent microscopy will be used to monitor very early indicators of change in cell differentiation. Patterns associated with appropriate (towards the desired phenotype) and inappropriate differentiation will be identified. Haptics enabled atomic force microscopy will enable us to detect early changes and predict the direction of cell differentiation into different cell lineages (e.g., cardiac or neuronal). This system could be used to develop improved methods of targeted cellular differentiation for therapeutic purposes, to develop new diagnostic procedures, 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: **Control of Human Neural Stem Cell Survival, Proliferation and Differentiation using novel micro and nano fluidic platforms**

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

Studies of the early 1990's have provided first strong evidence of the birth of new neurons in restricted regions of the adult mammalian CNS, establishing the existence of adult neural stem cells (ANSCs). Recent, more in depth studies of the human brain by clinical experts from this and other universities have led to the identification of astrocytes in the subventricular zone (SVZ) as the putative adult neural stem cells. Through collaboration with Johns Hopkins clinicians, particularly Prof. Quinones-Hinojosa, we have embarked on an investigation of these stem cells, which are obtained directly from brain tissue removed from human patients undergoing invasive brain surgery. Our current understanding of the expansion, differentiation, and migration of these cells is extremely limited, and these topics are under intense investigation due to a very high clinical potential of these stem cells. The opportunity to use the human adult stem cells directly extracted from the patient tissue for facilitation of the brain tissue reconstruction in the same tissue is of considerable interest. First, the likelihood of stem cell graft rejection diminishes due to a reduced immune response. Second, the cells might have more appropriate differential potential specifically for the brain tissue repair. Third, the cell extraction is routinely performed during and following surgeries, allowing for robust collection and culturing of the cells. The ANSCs, when injected into the adult rat brain, undergo massive cell death and do not survive long enough to differentiate and integrate with the surrounding tissue. Numerous soluble growth factors and signaling molecules have been found to affect the behaviors of ANSCs; however, we are particularly interested in Epidermal Growth Factor (EGF), Vascular Endothelial Growth Factor (VEGF), and Platelet-Derived Growth Factor (PDGF).

In our application, we will propose to improve the control of cell survival, proliferation, differentiation and migration of ANSCs, using a set of novel microfluidic devices. Such devices provide one with an unprecedented capability of extremely precise control of the cell microenvironment, allowing performing thousands micro-experiments over the time needed to perform a few classical macro-experiments. Based on this analysis we will investigate, experimentally and computationally, the role of signal transduction pathways (including Erk, Akt, Notch) in the control of the a ANSCs function. We will explore specific pharmacological interventions to optimize the cells prior to potential implantation into the damaged tissue. We anticipate that we will be able to dramatically improve cell survival and tissue integration based on the insights from this research.

Name of Principle Investigator: **David Litwack**

Project Budget: \$ **230,000.00**

Grantee: **University of Maryland, Baltimore**

Title: **Regulation of neuronal differentiation by Nuclear Factor One transcription factors**

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

The brain is composed of many different types of neurons, as well as other types of cells. A key question is how during development these cells, all of which are required for the brain to function, are generated from precursor cells. This question is of particular importance, as the failure for particular neurons in the brain to develop, or the loss of those neurons, can have devastating physical and cognitive consequences. The process of generating the cells of the brain is regulated by transcription factors, proteins that bind to DNA and regulate the expression of other genes. An example of this is the family of Nuclear Factor One (NFI) transcription factors. Studies in mouse demonstrate that NFI proteins are critical for proper brain development, and mutations in the genes encoding NFI proteins lead to defects in the development of subsets of neural cells. Based on the results from mouse, this study will test the hypothesis that NFI proteins regulate the production of neurons from human embryonic stem cells (hESCs). To test this, NIH-approved hESCs will be cultured and used to generate neurons. In Aim 1, the expression of NFI factors during this process will be analyzed to determine if NFI proteins are found in hESCs, neurons, or other cell types, and how the levels of those factors change as neurons are being generated. In Aim 2, the levels of NFI factors will be manipulated in hESCs to determine how increasing or lowering those levels affects the ability of hESCs to generate neurons. In Aim 3, to determine which genes NFI proteins may regulate during the production of neurons, large-scale changes in gene expression in hESCs and hESC-derived neurons after manipulation of NFI levels will be determined. These experiments will determine how NFI proteins function to generate mature neurons from hESCs, and will lay the groundwork for future work into how NFI proteins function in the generation of the many different types of neurons found in the brain. Such knowledge can be applied in the manipulation of hESCs to form the different cell types required to treat specific neural diseases, such as Parkinson's Disease, and other disorders that affect specific populations of cells in the brain.

Name of Principle Investigator: **David M. Loeb**

Project Budget: \$ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **Regulation Identification and Characterization of the Ewing Sarcoma Stem Cell**

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

There has long been noted a discrepancy between the initial response of cancer patients to chemotherapy and their ultimate chance of survival. This is especially true of patients with metastatic Ewing sarcoma (the second most common bone tumor among adolescents and young adults). The majority of these patients achieve disease remission, but their long term survival rate is a dismal 10%. One proposed explanation for this observation is the cancer stem cell hypothesis, which predicts that tumors contain a very small population of chemotherapy-resistant cells that are capable of limitless growth, self-renewal, and the generation of new tumors. Because these are the traits that define a stem cell, these cells are referred to as cancer stem cells.

We propose a series of experiments aimed at proving the existence of Ewing sarcoma stem cells and at beginning to functionally characterize them. We will perform our initial experiments in Ewing sarcoma cells growing in culture, but rapidly progress to the use of human tumor cells grown in mice, and finally will use tumors taken directly from Ewing sarcoma patients. We will attempt to isolate Ewing sarcoma stem cells by a multi-step process that will include a step to isolate cells based on size (stem cells are smaller than other cells), a second step based on the presence of an enzyme known to be found in other types of stem cells, and a final step based on proteins found on the surface of other types of stem cells. We will confirm that we have isolated stem cells by injecting them into laboratory mice and demonstrating that they are capable of generating new tumors, as this is the hallmark of a cancer stem cell.

After we have identified and isolated Ewing sarcoma stem cells, we will determine how resistant they are to chemotherapy and investigate the mechanism for this resistance. We will also characterize their ability to “differentiate,” or form other, more mature cell types. This will help us design new treatments aimed specifically at eradicating these cells. If the hypothesis that stem cells are the cells that cause relapse, metastasis, and death, then targeting new treatments at this specific cell population has the potential to improve the outcome for patients with Ewing sarcoma in a way that decades of adjustments of chemotherapy regimens has failed to achieve.

Name of Principle Investigator: **Stuart S. Martin**

Project Budget: \$ **200,000.00**

Grantee: **University of Maryland, Baltimore**

Title: **Novel therapies to destroy circulating breast tumor stem cells**

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

A successful cure for breast cancer will require the destruction of tumor cells that spread throughout the body. Breast tumor stem cells can circulate in the bloodstream, invade distant tissues and lie dormant for long periods. The eventual reemergence of these disseminated cells as metastatic tumors is a major cause of patient death. Our research has shown that resistance to cell death promotes tumor dormancy by allowing tumor cells to survive the challenges of traveling through the bloodstream, but failing to initiate active tumor growth. Such dormant cells persist without active cell division and are therefore resistant to many traditional chemotherapies. Destroying these dormant tumor stem cells is critical to prevent metastatic tumor recurrence, since their presence predicts poor patient outcome.

Our lab has recently identified two novel characteristics of detached breast tumor cells that provide therapeutic opportunities to destroy circulating tumor stem cells, independent of cell division. Our first aim targets our discovery that detached breast tumor stem cells generate dynamic extensions of their surface, that we have termed microtentacles. Imaging circulating tumor cells indicates that microtentacles could allow tumor cells to attach to blood vessel walls. Inhibiting microtentacles would prevent attachment and promote fragmentation of large epithelial carcinoma cells in narrow capillaries. Our second aim focuses on how detached cells that avoid apoptotic cell death can survive dormant, but continue to accumulate proteins that tip them toward cell death, a process we call apoptotic pathway imbalance. This persistent stress could make tumor stem cells susceptible to inhibition of survival pathways. Since rounded cells have exceptionally high levels of the Bik cell death protein, it should be possible to promote tumor cell death and spare normal tissues by exploiting this imbalance in apoptotic signaling. Neither strategy requires active cell division, so each is a mechanistic alternative to traditional chemotherapies that target dividing cells.

Surgery and other localized treatments can actually increase circulating tumor cells, so our approaches aim to start the proposed therapies before local treatment of the primary tumor. Tumor stem cells escaping during surgery would be prevented from efficiently colonizing distant tissues. We have already identified six compounds that target microtentacles and five that target apoptotic pathway imbalance. In this Exploratory grant, we will use an innovative method to track tumor stem cells as they circulate in the bloodstream of living mice and test which compounds are the most effective at preventing metastasis. Since nearly 90% of human solid tumors arise as carcinomas from epithelial tissues, the principles and therapies we define in this project could be applicable to a wide range of human cancers.

Name of Principle Investigator: **Mervyn J. Monteiro**

Project Budget: \$ **230,000.00**

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

Title: **Role of ubiquilin in regulating proliferation and differentiation of human stem cells**

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

There is great hope and excitement that human stem cells will one day be used to treat and possibly cure many intractable human diseases. The therapeutic potential of stem cells, particularly embryonic stem cells, lies in their remarkable capacity to form all the different cells in the human body. The promise of stem cells lies in the hope that once methods are perfected to transplant and target stem cells to correct locations in the human body they could be used in regenerative medicine to replace and replenish cells that are either damaged or destroyed and whose loss is associated with causing human diseases. One important theme that has emerged from studies of the factors that drive stem cells to constantly divide is that the levels to which these proteins accumulate in cells appears to be strictly regulated. However, the mechanism(s) governing this regulation is unknown. Modern molecular studies have revealed that ubiquilin, a protein we first discovered, and which is known to function in regulating protein levels in cells, is highly expressed at both the RNA and protein level in rapidly dividing stem cells, but that during stem cell differentiation ubiquilin expression decreases. We hypothesize that ubiquilin proteins may be involved in regulating cell division of stem cells and that they also regulate the accumulation of the core stem cell factors involved in stem cell determination. Accordingly, we propose experiments that will directly test these hypotheses. We will measure expression of ubiquilin in human embryonic stem (hES) cells in conditions where they rapidly divide and when they differentiate to form other types of cells. We will determine if ubiquilin interacts and affects the degradation of the critical stem cell factors involved in conferring cell cycle division of the cells. We will examine what effects silencing and overexpression of ubiquilin has on hES cell proliferation and differentiation. The studies are likely to lead to novel insight into the hitherto unknown function of ubiquilin in stem cells and to the possible mechanisms by which ubiquilin regulates accumulation of the core factors involved controlling stem cell fates.

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. The use of adult mesenchymal stem cells for therapies to repair or regenerate damaged or injured tissues is limited by early death of the transplanted stem cells. In this proposal, we will exploit a natural host cell survival mechanism to develop a strategy to improve survival of post-transplantation stem cells, without affecting their growth and capability to regenerate injured myocardium. This strategy is based on delivery of naturally occurring pro-survival factors, known as Serpins. These Serpins block different death signaling pathways and promote cell survival in ischemic tissue microenvironments. In this exploratory grant, we will test the hypothesis that preloading of human mesenchymal stem cells with Serpins will prolong their survival during stem cell therapies for ischemic heart failure.

Name of Principle Investigator: **Martin G. Pomper**

Project Budget: \$ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **Stem Cell Probe Discovery via Bioluminescence**

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

An emerging concept in cancer biology is that tumors arise from a small population of renewable cells known as cancer stem cells (CSCs). Many believe that in order to eradicate the tumor, CSCs must be targeted and eliminated. The goal of our proposal is to find new small molecule probes that enable us to target and image CSCs in living subjects. One way to harvest CSCs from other types of cells is to take advantage of the fact that these cells do not concentrate certain fluorescent dyes – so they may be easily distinguished from other cells that do. That property of CSCs is referred to as the “side population,” or “SP” phenotype. The SP phenotype is due to expression of a pump on the cell surface that pumps dyes out of the cells. That pump is known as the ABCG2 multidrug resistance pump, since in addition to fluorescent dyes, it pumps out chemotherapeutic agents, rendering these cells resistant to treatment. We have developed the rudiments of a high-throughput assay that enables us to discover new inhibitors of the ABCG2 pump. We intend to use that assay to test thousands of compounds to uncover a very high affinity inhibitor that can be functionalized for imaging CSCs. Specifically, we will develop new radiolabeled probes that are readily translated to the clinic. The ultimate goal will be to be able to image CSCs, arguably representing the most malignant elements of the tumor, in patients. The purpose of doing this would be to see what fraction of the tumor is “renewable” and to monitor therapy that targets the CSCs. By using a therapeutic rather than an imaging radionuclide, we can extend this work to targeted radiotherapy of CSCs, however that is beyond the scope of this exploratory proposal. Furthermore, we will likely uncover strong ABCG2 inhibitors that could be used in conjunction with standard cancer chemotherapy – to keep the CSCs from pumping the chemotherapy back out of cells, enhancing its efficacy. That latter treatment strategy would not require any radionuclide. We believe that our approach will generate several new imaging agents for CSCs that we intend to pursue vigorously in the clinic.

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):

Stem cells are pluripotent cells that are capable of indefinite self-renewing expansion and differentiating into a variety of cell types in response to specific extracellular stimuli. The ability to steer stem cell differentiation into specific cell types holds great promise for regenerative medicine (e. g., tissue regeneration and repair as well as treatment of degenerative diseases). Oct4 is the key transcriptional factors required to maintain the pluripotency and self-renewal of embryonic stem (ES) cells. Our understanding of the control of growth and differentiation of human ES cells by these factors is quite limited despite of intensive studies in the past decade. The PI's lab has demonstrated that the stemness master regulator Oct4 is modified by phosphorylation in human ES cell lines. Based on the fact that more than 90% of proteins in human cells are subjected to regulation by phosphorylation, the PI proposes to identify phosphorylation sites of Oct4 and study how phosphorylation modulates its biological activity in stem cells. Successful completion of the proposed study on how Oct4 is regulated by phosphorylation will provide new insights into the basic mechanisms underlying stem cell maintenance and self-renewal. The antibodies developed in this study that specifically detect phosphorylated Oct4 could be used as a tool to monitor the behavior of the stem cells used in the therapy to make sure that they are under the proper control. 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: **Human Brain Cancer**

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

The identification of successful treatments for brain cancer remains elusive. Most brain tumors are lethal because of their highly aggressive and infiltrative nature. The infiltration of tumor may be towards eloquent brain matter, which makes this type of tumors impossible to completely resect since cancerous cells have very likely migrated to the contra lateral brain at the time of surgery. Efforts to improve patient outcomes have been hampered by a dearth in significant strides in developing innovative treatment strategies. There is a need to design better ways to treat not only primary brain cancer but potentially also metastatic brain cancer.

Mesenchymal Stem Cells have become a promising candidate for therapy in patients with malignant gliomas because of their ability to migrate substantial distances and selectively target areas of malignant cells by presumably following a cytokine gradient leading to the site of gliomas. The objective of our experiments for the Maryland Stem Cell grant would be to determine the interaction and fate of MSCs and brain tumors in vitro and in vivo. We hypothesize that MSCs are able to reach gliomas and decrease their tumor burden by modulating the host immune system. By understanding the underlying pathway employed by MSCs in attacking gliomas, we hope to modify MSCs in order to optimize their anti-glioma response in patients with malignant brain gliomas and potentially other tumors, such as metastatic disease.

Our long-term goals are to further develop our stem cell translational and clinical research program within the existing brain tumor program. This will enable us not only to broaden our understanding of brain tumors but also to bridge the gap between mesenchymal stem cell biology and adult neural stem cell biology. A deeper understanding of the mechanisms governing tumorigenesis will lead to more innovative and efficacious options for brain tumor therapies.

Our proposal will be unique in that, unlike previous groups who have examined the therapeutic potential of IL12-MSCs, we will use an animal brain tumor model derived from cancer stem cells in our laboratory that displays some of the important malignant properties of human GBMs, for example diffuse, aggressive micrometastasis. We will employ this simple, but first of its kind, entirely human glioma model within a mouse, which has the ability to engraft a completely functional human immune system with hematopoietic stem cell (Shultz 2000). These NOD/SCID/IL2rgammanull mice will allow us to see the interaction between IL12-MSCs, human brain cancer stem cells, and the hematopoietic stem. It will also allow us to optimally identify the inflammatory cells derived from the hematopoietic stem cell system and track the degree to which they are involved in mediating an anti-glioma response. The Maryland Stem Cell Research grant would undoubtedly allow us to be at the forefront of this ongoing battle against brain cancer.

Name of Principle Investigator: **Venu Raman**

Project Budget: \$ **229,609.00**

Grantee: **Johns Hopkins University**

Title: **Characterizing the Role of Twist in the Development of Breast Cancer Stem Cells**

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

The progression of a normal cell to a cancerous one involves the interaction of a number of genetic and biochemical factors that are modified by various insults such as genotoxic (mutagens and carcinogens that contribute to tumor development), environmental (tobacco smoke, exhaust fumes, pollutants) as well as genetic predisposition. The central dogma so far has been that following genetic alteration, a cell undergoes rapid proliferation to generate a clonal population that collectively evades the immune system, thus facilitating the growth of anomalous cells within a particular organ (tumor biogenesis). Tumor growth and progression, in many cases if not detected early and treated, can lead to the spread of these cells through the circulating blood and lymphatic system to other visceral organs (metastasis) eventually leading to organ failure and then death.

A new paradigm proposes the concept of cancer stem cells within the tumor that can continuously give rise to the bulk of the tumor and is primarily responsible for the providing the source of the metastatic cells. In addition, like embryonic stem cells, these cancer stem cells are radio and chemo-resistance, which could explain the recurrence of cancer following conventional therapy which is designed to specially kill the bulk of the tumors but not specifically cancer stem cells. Thus, understanding the genetic make-up of these cancer stem cells will help us design novel technologies to image and track them as well as provide the much needed clues to how they can be targeted to prevent cancer recurrence.

In this application, we propose to functionally characterize the role of a gene, referred to as Twist, in promoting the cancer stem cell phenotype. We have preliminary data to indicate that a very small inoculum (100 cells) of these transformed breast epithelial cells (by over-expressing Twist) can form tumors in the breasts of immunocompromised (SCID) mice, which is the pre-clinical breast cancer model we use in the laboratory. In addition, these cells can promote metastasis in both experimental and pre-clinical models of breast cancer using SCID mice. The functional characterization of these cells will provide much-needed information into the biogenesis of breast cancer stem cells. The importance of this research is that more than 60% of high-grade breast carcinoma samples from patients over-express Twist. Using the model we have generated to establish breast cancer stem cells, we will categorically identify novel markers of breast cancer stem cells and translate this information to patients samples obtained from the rapid autopsy program which is well-established at Johns Hopkins School of Medicine. Overall, the primary goal of this proposal is to functionally characterize these breast cancer stem cells with the intention of providing highly targeted patient care specially related to treatment as well as to increase the long-term survival rate.

Name of Principle Investigator: Linda Resar

Project Budget: \$ 230,000.00

Grantee: Johns Hopkins University

Title: The Role of HMGA1a in Normal and Leukemic Stem Cells

Description 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 hESC characteristics should enable us to ultimately harness these cells for use in regenerative medicine and cancer therapy. The proposed studies are directed at understanding the genes that regulate stem cell properties. Our focus is the HMGA1 gene because preliminary studies suggest that it plays an important role in this process. HMGA1 is expressed at high levels during development of the human embryo. We also showed that it is highly expressed in hESCs. Moreover, HMGA1 expression falls dramatically when hESCs are induced to differentiate or form mature tissues. These findings indicate that it may function 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. This is 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 hESC properties, namely, the ability to self-renew indefinitely, and the 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 HMGA1 functions in the survival or growth of hESCs and this property contributes to the refractory nature of cancers overexpressing HMGA1. 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 with serial transplant experiments, indicating that at least a subpopulation of leukemia cells possess the self-renewal properties of hESCs. Taken together, these results suggest that HMGA1 is important in the maintenance and renewal of hESCs and our proposed studies are directed at elucidating its role in this process. Our Specific Aims are: 1) Define the role of HMGA1 in the survival and growth of hESCs, and, 2) Elucidate the role of HMGA1 in leukemic 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 harness stem cells for use in regenerative medicine for diseases affected by abnormalities or damage to normal stem cells.

Name of Principle Investigator: Charles M. Rudin

Project Budget: \$ 230,000.00

Grantee: Johns Hopkins University

Title: Notch Signaling in Lung Cancer Stem Cells

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

Over 170,000 Americans die of lung cancer annually, more than from the 3 next most lethal cancers combined. New therapeutic approaches for lung cancer represent a critical medical need. We have become very interested in a biological pathway that normally regulates the functions of stem and progenitor cells in embryonic development, the Notch pathway. Experiments by several groups now suggest that the Notch pathway may similarly regulate the functions of tumor stem and progenitor cells. This project will use multiple models to test several key questions about the role of Notch signaling in lung cancer, with the ultimate goal of developing a new therapeutic strategy for lung cancer. These questions include: When and where does Notch activation first appear in the very earliest stages of lung cancer development? If Notch activity is shut off by genetic expression of a strong inhibitor of Notch, does that prevent the development of lung cancer? Do subpopulations of cells within human lung cancers that have high Notch pathway activity have stem cell features (i.e. the ability to form new tumors)? Do pharmacologic inhibitors of Notch inhibit the development of new tumors? These questions will be addressed in mice using 2 models of lung cancer. The first model is based on introduction of two genes, one of which selectively causes lung cancer, and the other which regulates Notch pathway activity. The second model is based on the transfer of human tumor cells directly from patients to mice without cell culture in the laboratory. We think this novel approach, and the use of multiple models to answer these key questions, will yield critical insights into a new therapeutic strategy for lung cancer based on targeting the tumor stem cells with selective Notch inhibitors, improving the long term outlook for patients with lung cancer

Name of Principle Investigator: **Nitish Thakor**

Project Budget: \$ **229,190.00**

Grantee: **Johns Hopkins University**

Title: **The formation of neuromuscular junctions in microfluidic chamber**

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

We propose to develop an in vitro microfluidic chamber to study the formation of neuromuscular junctions (NMJs) between human embryonic stem cell (hESC)-derived motor neuron and myoblasts, and roles of glial cell line derived neurotrophic factor (GDNF) in hESC-derived motor neuron differentiation and NMJ formation. Development of the microfluidic chamber technology of this proposal will provide for microtechnology in stem cell studies as well as improved motor neuron disease therapeutics using human embryonic stem cells. This microfluidic chamber can isolate sub-cellular components of neurons to study local environments. The development of a novel microfluidic device for the study of the formation of NMJs will be applicable to high throughput stem cell biology, cell-based microarray, tissue engineering, and the study of neural/neuro-muscular structures. In addition, currently, it has not yet been examined that hESC-derived motor neurons can form NMJs with muscles. This experiment will be the first study on NMJ formation using hESC-derived motor neurons and microfluidics. The success of the formation of NMJs between hESC derived motor neurons and muscles in vitro will show the potential of stem cell therapeutics. In particular, specific roles of GDNF in hESC differentiation and the formation of NMJs have not been demonstrated due to difficulties of segregation of soluble factors and extracellular matrixes. The identification of roles of GDNF in stem cell differentiation and the formation of NMJ will elucidate both processes. The integration of different facets of stem cell therapies and microtechnology will help bridge the gap between different scientific fields as well as provide exposure to, and possibly introduce, different techniques and methods for both areas. A routine examination of animal models of stem cell therapy will not isolate the specific pathways and phenomena associated with stem cells and their local microenvironment. The ability to isolate the local microenvironment where stem cells are transplanted using microfluidics and Bio-MEMS will help stem cell biologists and physicians plan strategically combined stem cell transplantation with other neurotrophic factor to enhance transplanted stem cell survival and axon attraction. The results of this proposal will hopefully lead to the eventual use of human hESC- derived motor neuron to treat motor neuron diseases and injuries. This research synthesizes the complementary capabilities of advanced stem cell application techniques with microfluidics to generate an integrative approach to stem research. Future collaborations with industry may also develop as the Johns Hopkins University has a technology transfer office, which assists members affiliated with the university in the patent process as well as the industrial facet of various projects.

Name of Principle Investigator: **Leslie Tung**

Project Budget: \$ **230,000.00**

Grantee: **Johns Hopkins University**

Title: **Antiarrhythmic Properties of Human Embryonic Stem Cell-Derived Cardiomyocytes in a Model of Dysfunctional Cardiac Tissue**

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

The discovery and isolation of human embryonic stem cells (hESCs) in 1998 revolutionized the field of tissue regeneration, and for the past decade researchers have sought to characterize and turn these cells into various cell types. Because of the high death rate associated with heart failure and the lack of sufficient donor organs, much attention has focused on turning hESCs into heart cells. So far, however, injection or engraftment of these cells into hearts with low cardiac output has resulted in only modest gains in cardiac function. What has been overlooked, however, is the possibility that these cells may have other therapeutic effects, namely inhibiting the formation of lethal cardiac arrhythmias (abnormal rhythms of the heart). Although many other cell types have been considered for cardiac regeneration, hESCs may have the greatest potential to attain the electrical properties of healthy heart cells and to actively restore electrical stability to damaged regions of the heart.

In this project, the electrical properties and anti-arrhythmogenic potential of de novo heart cells (hHCs) derived from hESCs will be investigated. We plan to employ a novel, as yet unutilized tactic of mechanical stretching the hESCs while they are turning into heart cells. We speculate that because this condition mimics how immature heart cells naturally develop when left in place, such an approach may significantly improve the yield of heart cells that can be obtained from each batch of hESCs. Moreover, unlike untreated cells, they may develop robust, stable, and consistent heart cell-like characteristics. Best of all, there is the potential that these cells may integrate into the heart and serve as a permanent preventive against certain types of arrhythmias.

Another novel aspect of our study is that the hHCs will be tested on a miniature sheet of animal heart cells that have been manipulated to mimic a key aspect of diseased hearts found in individuals with hypertension, myocardial infarction or heart failure, or even in the elderly. The hallmark of this condition is highly irregular electrical conduction, that may spur the onset of fatal arrhythmias. The application of hHCs may allow them to be effectively used as an electrical band-aid. By studying the behavior of hHCs in a benchtop, laboratory setting, we will have a high degree of control over how the hHCs are distributed among the cells in the sheet, and will be able to see and measure how the hHCs interact with the heart cell sheet to prevent arrhythmia. Although these kinds of studies are not a substitute for experiments conducted on whole animal hearts, they serve a useful purpose for initial studies by allowing relatively small numbers of hHCs to be tested in relatively large numbers of experiments.

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 bone marrow stem cells to generate anti-tumor responses**

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

Many cancer therapies fail because they are not able to completely eliminate tumor cells, leading to recurrence and ultimately disease relapse. The immune system is designed to recognize and kill tumor cells, but it sometimes becomes debilitated by either the tumor itself or the patient's own body. Immunotherapy, which is based on using the immune system's cells to treat disease, offers the potential for eliminating tumor cells using T cells to recognize tumor specific antigens and kill the tumor cells. Some approaches using immunotherapy have shown evidence of anti-tumor effects, particularly when a bone marrow transplant has been administered along with lymphocytes from a donor. However, achievement of such responses in the autologous setting (in which the transplanted cells are the patient's own cells rather than derived from a donor) has been difficult. Thus, development of immunotherapies that specifically target the tumor but are more effective as a result of more recently available technology would be a major advancement for this field of research.

The cells that are most often ultimately responsible for killing tumor cells are T cells. These cells are directed to kill specific cells as a result of their interaction with dendritic cells (DCs). The DCs instruct the T cells to be activated and to recognize tumor cells. Previous studies have tested DC-based vaccines, but these have met with limitations. Primarily, to generate an effective immune response against a tumor, DCs must express a tumor antigen in the secondary lymphoid organs. DCs can be removed from patients, grown in culture and loaded with antigen, but when these cells have been re-infused into patients, they have not traveled to the proper area in which they can stimulate potent, durable responses. Transfer of antigen to DCs that are already circulating in patients has also proven to be particularly difficult. The goals of the proposed studies are to generate new therapies that will utilize stem cells that have a greater potential to survive longer and traffic better to the proper locations as a replacement for mature DCs. This approach will depend on gene modifying stem cells so that they will become DCs that express a tumor antigen in the proper location in the body and stimulate a potent immune response that ultimately kills the tumor. The use of stem cells will allow a significant advantage in the development of this therapy in that they are longer lived cells with a greater potential for both expressing antigen for longer time periods and traveling to the proper location.

Name of Principle Investigator: **Huakun Xu**

Project Budget: \$ **200,000.00**

Grantee: **University of Maryland, Baltimore**

Title: **Stem Cell Delivery via Injectable, Nano-Apatite Scaffolds for Bone Engineering**

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

In the U.S., 7 million people suffer fractures each year. Musculoskeletal conditions cost \$215 billion/year. Osteoporosis alone results in 1.5 million fractures annually in the U.S. In addition, there is a need for mandibular, maxillary and craniofacial repairs. For example, US soldiers have body armor and solid armor plates to protect their abdomens and chests. However, there is no body armor to protect their faces, where most of the penetrating injuries and devastating traumas have occurred. Major reconstructions of the maxilla, mandible and cranium can greatly benefit from a moldable scaffold with improved fracture resistance, increased bone cell function and macropores for rapid osteogenesis. Another example is orbital fractures, which can significantly diminish the patient's quality of life. The orbital floor is opposed by the maxillary and ethmoid sinuses which are air-filled, without vascularized tissue to support regeneration of large defects by the body. All these repairs could greatly benefit from bone tissue engineering. The introduction of stem cells into the clinical setting opens new horizons. Despite its promise, stem cell-based bone engineering has been hindered by the lack of suitable carrier. Therefore, this project will: (1) develop human mesenchymal stem cell (hMSC) carriers that are injectable, load-bearing, biomimetic and resorbable, for the first time; (2) deliver hMSCs and use alginate hydrogel for cell encapsulation; (3) co-deliver bone morphogenetic protein and transforming growth factor to greatly enhance the osteogenic and bone-regenerating potential. The injectability of the new carrier is important because it can be used in minimally-invasive procedures such as in-situ fracture fixation and percutaneous vertebroplasty to fill and strengthen osteoporotic bone lesions, to shorten surgical time, reduce pain and scar size, achieve rapid recovery, and reduce cost. The load-bearing ability of the new carrier is also of crucial importance for the regeneration of load-bearing tissues such as bone, in order to avoid fracture and maintain spaces in the scaffold for cell growth and tissue production. Hence, this new class of hMSC/growth factor/nano apatite constructs is highly promising for a wide range of orthopedic and craniofacial repairs. It has the potential to greatly enhance bone healing and regeneration, to improve the health and quality of life for the citizens of Maryland and beyond. In addition, this project will provide an understanding of the effects of carrier composition and dual osteogenic growth factor stimulation on the osteogenic potential of hMSCs in the bone-mimicking nano-apatite carrier, for the first time. Furthermore, this project will establish the in-house capability at the University of Maryland Baltimore for developing human stem cell-based bone tissue engineering technology, with future research and development collaborations that can benefit the Maryland biotech industry.

Name of Principle Investigator: **Paul Yarowsky**

Project Budget: \$ **230,000.00**

Grantee: **University of Maryland, Baltimore**

Title: **Targeted Therapy for Neurodegenerative Diseases with Magnetically Labeled Stem Cells.**

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

Stem cell therapy in the nervous system is considered among the most promising strategies for the treatment of neurodegenerative disorders like Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS). 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. But a daunting problem exists in devising such a therapy, viz., how to deliver stem cells to treat numerous sites of neurodegeneration occurring at the same time in these diseases. One idea is to direct the placement of stem cells by multiple injections, but this is impractical; Our solution is by using directed magnetic fields and "magnetizing" the stem cells. We propose to develop a method of delivering iron oxide (ferumoxide, Feridex) labeled stem cells to specific areas of the cortex or to the entire spinal cord. In Aim 1, after injection of the stem cells, magnets will be placed either directly over the same side of the cortex (the stem cells are injected into the lateral ventricle for cortical and hippocampal targeting) or the magnets will be placed in a jacket along one side of the body nearest the ventral horn of the spinal cord, when stem cells are injected into the IVth ventricle. The stem cells will have also been infected with a virus which carries the human insulin growth factor-1 with green fluorescent protein gene as reporter. We will use production of these proteins as markers for the location and function of the stem cells in the magnetic field to verify our targeting approach. In Aim 2 we will try a less invasive method of injection of stem cells, intravenous injection, into control animals with the placement of the external magnets the same as in Aim 1. This will allow us to evaluate the ability to target the delivery of the cells to the spinal cord or cortex by transcytosis (transfer of stem cells across capillaries and into tissue) using external magnets. In Aim 3, since both the blood brain barrier and the blood spinal cord barrier becomes leaky in neurodegenerative diseases such as ALS and AD, we will also attempt in addition to intraventricular injection targeted delivery of stem cells by intravenous injection in the SOD1 ALS rat and using the same magnet placement on the skull on one side of the cortex or in a magnetic jacket nearest one side of the ventral horn of the spinal cord as described in Aim 1. We adopt these placements of magnets so that analysis of the tissue (cortex, hippocampus, or spinal cord), after two weeks of targeting will leave one side of the cortex or spinal cord with much fewer stem cells and the other side with a high concentration of stem cells. We hypothesize that this method of magnetic targeting of stem cells will allow for accurate spatial delivery of stem cells.

Name of Principle Investigator: Srinivasan Yegnasubramanian

Project Budget: \$ 230,000.00

Grantee: Johns Hopkins University

Title: Epigenetic Characterization of Normal Hematopoietic and Chronic Myeloid Leukemia Stem Cells

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

Normal blood cell production, or hematopoiesis, is an intricate process, initiated by hematopoietic stem cells (HSC). These rare cells have nearly unlimited ability to divide and ultimately generate ~500 billion blood cells/day. The HSC give rise to offspring which mature into cells that look and behave very differently both from their parents (HSC) and each other. While maturing, they lose the capacity to reproduce but gain specialized functions ranging from oxygen delivery to clotting to fighting infection.

Analogous to HSC, leukemia stem cells (LSC) sustain the growth of the leukemia. The LSC, typically < 1% of the leukemic cells, look and act much differently than their mature offspring which comprise the bulk of the leukemia. It is thought that chemotherapy often kills only the mature leukemia cells, sparing the more resistant LSC. The surviving LSC can then regenerate the leukemia. Because of this phenomenon, many leukemia patients achieve complete remission (meaning the absence of detectable disease) after initial therapy, only to later relapse. This suggests that only treatments which eliminate the LSC can achieve complete cures.

The HSC and LSC have unique characteristics compared to their mature offspring; e.g. their ability to renew themselves indefinitely. However, the HSC and LSC do not differ from their offspring in their genetic sequence. Instead, there is a level of coding beyond the genetic sequence that accounts for these differences. We refer to this level of coding as epigenetics, or “beyond genetics”. DNA methylation is a key epigenetic process whereby DNA is “marked” by “methyl groups”. These marks modulate the interpretation of the genetic code. DNA methylation at the start of a gene can cause suppression of its expression. HSC may place these methylation marks in such a way as to express a different set of genes compared to their offspring. Likewise, LSC seem to opportunistically misplace these DNA methylation marks, leading to inappropriate interpretation of the genetic code in such a way as to confer growth and survival advantages contributing to the development of cancer. The precise nature of these DNA methylation marks and how they regulate gene expression in these cells are largely unknown.

We have developed novel, innovative methods that harness the latest technological advancements in “DNA microchips” to characterize DNA methylation marks and the corresponding gene expression patterns on a genome-wide scale with unprecedented coverage and resolution. We will apply this methodology to HSC and their mature offspring from normal marrow donors and LSC and their mature offspring from leukemia patients. Such an analysis will enhance our understanding of normal and leukemia stem cells so that we can: (i) engineer normal HSC as therapeutic agents for blood diseases, and (ii) identify unique targets in the LSC that can be exploited to selectively kill them while sparing normal HSC.

Name of Principle Investigator: **Steven Zhan**

Project Budget: \$ **230,000.00**

Grantee: **University of Maryland, Baltimore**

Title: **The role of cortactin in EMT and differentiation of human embryonic stem cells**

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

Stem cell research has provided a great hope for treatment of tissue degenerated diseases because they are able to produce or differentiate into a variety of differentiated cells that have similar functions as those in normal tissues. In particular, embryonic stem (ES) cells, which are derived from embryos, can differentiate into virtually any types of cell lineages for all the adult tissues. Furthermore, these cells can be readily expanded to unlimited numbers, solving a major issue for the shortage of stem cells to be used in cell therapies. Indeed, recent studies have demonstrated that human ES cells can be induced into neurons and can dramatically improve the condition of rats with Parkinson's symptom. Also, the recent finding that somatic cells can be reverted to ES-like cells by simply introducing several ES specific genes makes a further leap in the potential to apply ES cells in clinical therapies because it reduces ethical concerns as well as potential immune rejection after implantation of ES cells not derived from the same individuals. In spite of these promising advances, using of embryonic or reprogrammed ES cells to treat patients remains a distance to the reality. One of the major concerns using ES cells is that these cells tend to form tumors as reported with studies involving transplantation of embryonic stem cells into animals. Also, it is believed that many cancer cells are developed from stem cells. Therefore, finding of a proper way to prevent them from formation of tumors will be critical for development of any ES cell based therapy. The growth and differentiation property of stem cells are often determined by their microenvironment or niche. For ES cells, they tend to tightly interact with each other and form distinct cell clumps. Upon differentiation the cells spread out, migrate around, and become less contact along with significant morphological changes. There is little known, however, about how these shape changes occur during the differentiation, neither about the significance of these changes for ES cell growth and differentiation. Recent studies have suggested that expression of certain molecules responsible for cell and cell interactions are changed during differentiation of ES cells. Notably, expression of E-cadherin, which is a major molecule on the cell surface that is responsible for epithelial cell-cell interaction, is often decreased in differentiated ES cells, while expression of other molecules representing mesenchymal cells is augmented. Interestingly, this transition from epithelial to mesenchymal-like cells is very similar to a well characterized process that is implicated in tumor cell invasion and leads to malignance. This finding raises a great concern that ES cells may not only produce local tumors at the implantation site but also spread ES cell derived tumors to other organs. Thus, it is important to understand how ES cells take such changes that may be potentially used for tumor formation. We have recently found that β -catenin, a molecule that is associated with and essential for E-cadherin mediated cell junction, binds to ES cell's cortactin, a protein that has been implicated in cell shape changes and tumor invasion. This finding suggests that cortactin and β -catenin may play a role in the morphological change of ES cells and ES cell derived tumor formation. This proposal is to characterize the interaction of cortactin with β -catenin and determine the role of the interaction in ES cell differentiation. Achievement of the proposed study should advance our understanding of the mechanics for ES cell differentiation and explore safer protocols in application of stem-cell based clinical therapies.



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):

Telomeres are DNA-protein structures which protect chromosome ends. Telomeres shorten after each round of replication and short telomeres lead to apoptosis or senescence. Telomerase is a specialized RNA-containing polymerase which maintains telomere ends (1, 2). Typically, somatic cells do not express telomerase and are therefore limited in their proliferative capacity. Stem cells, on the other hand, express telomerase which facilitates their extended proliferative capacity. Telomerase has 2 essential components: telomerase reverse transcriptase (TERT), the protein component responsible for its catalytic activity, and telomerase RNA (TR), which contains the template for de novo telomere synthesis. The levels of TERT and TR are tightly controlled and loss of function of a single allele causes autosomal dominant dyskeratosis congenita (DC) (3, 4). Affected individuals with DC display premature stem cell failure in tissues of high turnover; the skin, mucosal membranes and bone marrow (BM). This proposal seeks to expand our understanding of the relationship between short telomeres, telomerase dose and stem cell failure in a therapeutic context. It focuses on repairing the telomere defect first in a mouse model of bone marrow failure and then to extend these studies to a model that aims to repair the hematopoietic stem cell (HSC) defect in patients with DC. Thus the proposal seeks to build the platform for telomerase activating therapies in aplastic anemia and other marrow failure disorders through the following specific aims.

- 1. Rescue hematopoietic defects in mice with short telomeres by exogenous expression of mTert and mTR.**
 - a. To determine if exogenous expression of mTert and mTR can rescue the proliferative defect of HSCs with short telomeres.
 - b. To evaluate the efficacy of temporal telomerase expression through adenoviral vectors compared with lentiviral vectors at healing short telomeres in HSCs.
- 2. Rescue engraftment potential and short telomere defect in primary HSCs from patients with DC.**
 - a. To evaluate if exogenous expression of hTERT and hTR can rescue engraftment potential of hematopoietic stem-progenitor cells (HSPCs) from DC patients.

Name of Principle Investigator: Michael Bonaguidi

Project Budget: \$ 110,000.00

Grantee: Johns Hopkins University

Title: Extrinsic Regulation of Adult Human Neural and Cancer Stem Cells in Normal and Disease States

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. In the adult brain, accumulating evidence suggests essential roles of micro-environments, or niches, in supporting active neurogenesis from endogenous neural stem cells within the subventricular zone (SVZ) and hippocampal dentate gyrus (DG). These neurogenic niches also regulate different steps of adult neurogenesis in response to physiological and pathological stimulation. We and others have identified bone morphogenetic proteins (BMP) and excitation/depolarization stimuli as niche signals, which regulate different steps of adult neurogenesis in the DG. Specifically, BMP signaling acts as a negative regulator of DG neurogenesis by preventing hippocampal NSC self-renewal, proliferation, and neuronal fate commitment. Meanwhile, excitation stimuli such as KCl or Calcium channel agonists cause membrane depolarization and positively regulate DG neurogenesis by enhancing proliferation, neuronal fate commitment, and survival. Intriguingly, we have demonstrated that the BMP antagonist noggin or elevated KCl levels allow murine adult hippocampal NSC (mANSC) to be continually propagated as free-floating clonal clusters of cells called neurospheres. This novel system offers an easily manipulated cell culture tool to study the effect of niche components on mANSC development.

Despite the growing understanding of factors regulating adult murine neurogenesis, little is currently known about the mechanisms underlying human neurogenesis. This is due to ethical and technical limitations in working with human adult neural tissue. To circumvent ethical boundaries, tissue biopsies are prepared for tissue culture and xenotransplantation into murine models. Technically, adequate cell number and tissue availability remains the crux of basic science and therapeutic studies. Tissue removed from the medial temporal lobe region, during surgery to alleviate intractable epilepsy, is a good source of human tissue because it contains adult hippocampal and SVZ stem cells (hANSCs). In collaboration with Dr. Krauss and neurosurgeon Dr. Quiñones-Hinojosa, at Hopkins, we have been collecting fresh surgical tissues of human hippocampus and have developed effective 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 at present remain limited in their capacity for self-renewal and can only be passaged 4 or 5 times.

We propose, based on our studies with mANSC, that hANSC will continually self-renew and retain multipotentiality in response to noggin, elevated KCl, or a combination of both. This system will allow for adequate cell number for studies of human adult neurogenesis and neural stem cell therapies.

Name of Principle Investigator: Selen Cantania

Project Budget: \$ 110,000.00

Grantee: University of Maryland, Baltimore

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

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

Cardiovascular disease is the leading cause of death in the United States, and can lead to congestive heart failure as reported by American Heart Association. More than 870,000 Americans die annually due to congestive heart failure, usually within five years of being diagnosed, because of the limited self-repair capacity of cardiac muscle. Cellular therapies using stem cells, especially bone marrow (BM) mesenchymal stem cells (MSC), offer potential to regenerate heart muscle following a heart attack. However, despite the promising results from recent clinical trials, a major setback of MSC-based therapy remains unsolved -inconsistent therapeutic efficacies, most likely due to failure of MCS to efficiently differentiate into cardiomyocytes.

Our preliminary data revealed that genetic deletion of the low density lipoprotein receptor related protein (LRP1) in mouse MSC greatly enhanced their ability to differentiate towards osteoblast formation. LRP1 is a large multifunctional receptor that binds and endocytoses a variety of structurally and functionally distinct ligands¹. The role of LRP1 in MSC biology is not known, but it may play an important role due to its potential role in regulating the canonical Wnt signaling pathway by disrupting the Wnt ligand receptor (Fz/LRP5 or Fz/LRP6) complex. Recent work on stem cells revealed a developmental stage-specific role of Wnt signaling on cardiac development and regeneration. The distinct roles are determined both by the timing and the receptor context which influences multiple roles of Wnt signaling during development.

Our discovery that LRP1 modulates MSC differentiation raises the possibility of genetically engineering MSCs to enhance their cell fate choice towards cardiomyocyte formation. The central hypothesis of this proposal is that altering LRP1 function in human MSC facilitates cell fate choice towards cardiomyocyte differentiation. The specific hypotheses to be tested are: 1) that ablating LRP1 function either genetically or with receptor antagonists **in MSC will divert the cell fate choice towards cardiomyocyte differentiation.** 2) **that the mechanism by which LRP1 accomplishes this is by suppressing function of the canonical Wnt signaling pathway.** These hypotheses will be tested in the following two aims:

1. Define the role of LRP1 in human MSC (hMSC) differentiation and cell fate choice.
2. Use LRP1 silencing and overexpression to define its function in Wnt signaling pathway.

Name of Principle Investigator: **Jessica Carmen**

Project Budget: \$ **110,000.00**

Grantee: **Johns Hopkins University**

Title: **NG2 Human Precursor Dysfunction 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. We will transfect human embryonic stem cells (and the downstream neural derivatives) with mutant superoxide dismutase-1 (mSOD1), the molecule that has been shown to reproduce familial (genetic) ALS in the mouse model. We will transfect the pluripotent embryonic stem cells with mSOD1 and study the ability of these cells to differentiate into neural progenitors, glial progenitors, and finally astrocytes. We will also test 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 plan to determine the steps that result in the development of diseased astrocytes and to devise a therapeutic strategy in order to 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: Characterization and Expansion of Leukemia Stem Cells

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

Remission in myeloid leukemia often does not translate into cure. This phenomenon may reflect the failure of current therapies to adequately target leukemia stem cells (LSC). LSC are rare cells, typically comprising < 1% of the total tumor burden, which are thought to harbor all of the leukemia's self-renewal capacity. It is theorized that current therapies often kill only the differentiated bulk of the leukemia, sparing the inherently more resistant LSC, which subsequently regenerate the leukemia. The first aim of this project is to better characterize LSC as compared to their normal counterparts, with the goal of identifying potential therapeutic targets. Numerous candidates have been proposed as possible therapeutic targets; among the most promising are: Survivin, PRAME, Proteinase 3, WT1, and hTERT. However, it is largely unknown to what degree, if any, that these are expressed by LSC. It is expected that LSC would be resistant to any treatment directed against a target that they do not express. Treatments targeting antigens which are expressed at similar levels by both normal hematopoietic stem cells (HSC) and LSC would be expected to produce unacceptable toxicities (i.e., no therapeutic window). Alternatively, markers selectively expressed by LSC (versus normal HSC) may represent excellent therapeutic targets.

LSC will be isolated from patients with acute myeloid leukemia (AML) and chronic myeloid leukemia (CML); and their mRNA expression of each of the above candidates (quantified by real-time RT-PCR) will be compared against that in normal HSC. Our preliminary data demonstrated similar expression of most of these antigens in both LSC and HSC; however, WT1 was selectively expressed by LSC versus normal HSC. PRAME also was differentially expressed, with high expression levels confined to advanced disease (i.e., AML or blast crisis CML) and virtually no expression in normal HSC. These preliminary findings, if confirmed, would suggest that therapies targeting WT1 and/or PRAME may have selective activity against LSC.

The study of LSC has been limited by their rarity. Thus, the second aim is to expand, in vitro, the LSC fraction from primary AML and CML patient samples, through blockade of retinoic acid signaling. Our preliminary data suggest that inhibition of this signaling pathway can increase the LSC population. Expansion of LSC quantities would yield sufficient cells for further research, including the development and testing of new treatments.

Name of Principle Investigator: Hugo Guerrero

Project Budget: \$ 110,000.00

Grantee: Johns Hopkins University

Title: Role of Slit Proteins in the Migrator Behavior of Brain Tumor Stem Cells

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

High grade gliomas, specifically Glioblastoma Multiforme (GBM), despite advances in surgical therapy, chemotherapy, and radiotherapy, account for a significant amount of morbidity and mortality in the brain cancer patients. Recently, the discovery of brain tumor stem cells (BTSCs) within many brain tumors including GBM has provided a new potential target for brain tumor treatment. BTSCs are thought to arise from aberrant Neural Stem Cells (NSCs), and are believed to be responsible for the bulk of the tumor mass as well as its resistance to current treatments. It is thought that BTSCs, like NSCs, have a propensity to migrate into surrounding normal brain parenchyma, this property would prevent complete radical resection. Therefore, an understanding of the processes involved in BTSCs migration is vital to developing better treatment strategies for this devastating disease. The Slit-Robo system, which has been implicated in NSC migration, may play an important role in BTSCs migration. Slit proteins are a family of chemorepellant diffusible factors released in the Central Nervous System by midline structures and bind to the transmembrane Robo receptors. This system has also been found to participate in the invasion of other tumors including breast cancer, melanoma, small cell lung cancer, and recently gliomas where the expression of Slits is epigenetically inhibited by methylation. Furthermore it has been shown that the addition of Slits affects the invasion of tumor cell lines in-vitro. Our preliminary experiments show that BTSCs possess the ability to migrate and to express Robo receptors. Therefore, we **hypothesize** that the Slit-Robo plays a role in BTSCs migration. We propose to evaluate the effect of Slits on the migration and invasion of human BTSCs. We will use intraoperative tissue from GBM patients and cortical tissue from non-cancer patients as a control. We will isolate BTSCs from the samples. We plan to evaluate the expression of the Slit and Robo proteins by quantitative real time PCR, immunohistochemistry, and Western-blot. Furthermore, we will implement slit-releasing cells to evaluate their effect on the migration and invasion of human BTSCs. This will be done in-vitro, using matrigel assays and invasion chambers; ex-vivo in organotypic human cultures, and in-vivo in rodent tumor models. Finally, we will test the effect of 5'-Azadeoxycytidine on the migration and invasion of BTSCs. This hypomethylating drug is currently used in some myelodysplastic syndromes and has shown to re-induce the expression of Slits. These experiments will improve the understanding of BTSCs migration and potentially offer a novel therapeutic strategy for this devastating disease.

Name of Principle Investigator: Maged Harraz

Project Budget: \$ 110,000.00

Grantee: Johns Hopkins University

Title: The Role of Human Embryonic Stem Cells and Induced Pluripotent Stem Cells for Cellular Therapy of Parkinson's Disease

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

Adult neural stem cells (NSCs) can differentiate into mature glia or neurons that incorporate in the established neuronal circuitry, a process termed adult neurogenesis. Adult neurogenesis opens exciting possibilities for replacement therapy in neurodegenerative, neuro-traumatic and cerebrovascular diseases. Isolation of NSCs, expanding them in vitro then reintroducing them in an undifferentiated or a differentiated state to the affected nervous system region is a promising strategy for stem cell therapy. Interestingly, NSCs have been isolated from few regions of the adult human CNS including the olfactory mucosa, which is a safe and accessible source for adult human NSCs (hNSC). MicroRNAs (miRNAs) are conserved small non-coding regulatory RNAs that play major roles in cellular proliferation and differentiation. They regulate human embryonic and adult stem cell development. In addition, miRNAs are linked to cancer, heart and neurodegenerative diseases. Because of their small size, miRNAs delivery into cells is feasible in vitro and in vivo, which makes them a relatively safe and promising therapeutic intervention tool. We will investigate the role of miRNAs in hNSC biology through two specific aims. Specific aim one will investigate the effects of DiGeorge syndrome chromosomal region 8 (DGCR8) knock down on the proliferation, self-renewal and differentiation of human adult neural stem cells. DGCR8 is a dsRNA binding protein that is essential for miRNA biogenesis. While blocking miRNA biogenesis disrupts the mouse embryonic stem cells normal functions, the role of the miRNA pathway in human adult neurogenesis is unknown. Our preliminary data demonstrate efficient inhibition of DGCR8 protein expression in a mouse neuroblastoma cell line. We will knock down DGCR8 expression in hNSCs using a recombinant replication deficient lentiviral vector and investigate the effect of DGCR8 deficiency on hNSCs proliferation capacity, retaining their stem cell potential and differentiation into neurons or glia. Specific Aim two will identify the microRNA profiles in hNSCs versus mature neurons and glia. Previous studies emphasize that certain miRNAs play key roles in neurogenesis. However, the role of miRNAs in human adult neurogenesis is still unknown. Our preliminary results demonstrate that the expression profile of many miRNAs is different in adult mouse NSCs compared with the adult mouse hippocampus. This aim will induce the differentiation of hNSCs in vitro into neurons or glia and compare the miRNA profiles in each condition. The miRNAs that are different between the undifferentiated and differentiated states will be further validated with quantitative real time PCR. Results from these studies will identify key molecules that regulate hNSCs differentiation. This will serve as the basis for developing new strategies to direct hNSCs fate for therapeutic use in neuroregenerative medicine.

Name of Principle Investigator: **Biju Joseph**

Project Budget: \$ **110,000.00**

Grantee: **Johns Hopkins University**

Title: **HMGA1 in the Maintenance & Proliferation of Human Embryonic Stem Cells**

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

Human embryonic stem cells (hESCs) are characterized by 2 unique properties: 1) Self-renewal or the ability to form new hESCs, and, 2) Pluripotency or the potential to differentiate into any mature cell. Because of these 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 characteristics should enable us to harness these cells for use in regenerative medicine and cancer therapy. Our work is directed at understanding the molecular pathways that regulate stem cell properties. Our focus is the HMGA1 gene because preliminary studies suggest that it plays an important role in this process. HMGA1 is expressed at high levels during embryologic development. We showed that HMGA1 is highly expressed in hESCs and its expression falls dramatically with differentiation. In fact, the expression pattern parallels that of Nanog and SOX2, two genes important in hESC renewal. These findings indicate that HMGA1 may function in stem cell survival or proliferation. 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. This is consistent with the idea that cancer cells with stem-like properties (cancer stem cells) lead to aggressive cancers that are refractory to therapy because of their inherent hESC properties, namely, the ability to self-renew indefinitely, and low baseline proliferative rates. This latter characteristic is thought to confer resistance to therapy because most chemotherapeutic agents target cells that are actively dividing. Thus, we hypothesize that HMGA1 functions in the survival or proliferation of stem cells, both in normal development and in cancer. We hypothesize further that this property contributes to the refractory nature of cancers overexpressing HMGA1. To study HMGA1 in cancer, we engineered transgenic mice overexpressing HMGA1 and all mice succumb to aggressive leukemia that closely models refractory human leukemia. The tumors can be serially transplanted into recipient mice, indicating that at least some HMGA1-induced tumor cells have long-term self-renewal capabilities. In studies with primary human leukemic blasts, we found that HMGA1 mRNA expression is highest in the stem cell-like fraction compared to the bulk tumor cells in preliminary studies. Taken together, these results suggest that HMGA1 is important in maintaining "stemness" and our studies are directed at elucidating its role in this process. In our recently funded MSCRF application, we propose the following Specific Aims: 1.) Define the role of HMGA1 in hESC survival and proliferation, and, 2.) Elucidate the role of HMGA1 in human leukemic stem cells. Results from these studies will advance our knowledge of "normal" and "cancer" stem cells. A better understanding of the molecular circuitry that underlies stem cell properties should lead to the discovery of novel therapies directed at cancer stem cells and the capability to harness stem cells for use in regenerative medicine for diseases affected by abnormalities or damage to normal stem cells.

Name of Principle Investigator: Tarja Juopperi

Project Budget: \$ 110,000.00

Grantee: Johns Hopkins University

Title: A Comparison of Human Embryonic Stem Cells and Induced Pluripotent Stem Cells for Cellular Therapy of Parkinson's Disease

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 embryonic stem cell (ESC) has become an intensively studied target for cell-replacement therapy due to its unique ability to generate any cell in the body. Although human ESC (hESC) based treatment has remarkable therapeutic potential, there are concerns regarding the possibility of immunological rejection. Recently, investigators have been successful in reprogramming human skin fibroblasts to exhibit a pluripotent phenotype. Intriguingly, these induced pluripotent stem cells (iPSC) have similar properties to ESCs, including their developmental capabilities. The generation of human iPS cells (hiPSC) has opened up the possibility of custom designed cells derived from individual patients for specific disease treatment. Cell-based therapies are presently being studied for a variety of diseases including neurological disorders. Parkinson's disease is a neurodegenerative disorder that results from the loss of dopaminergic neurons in the substantia nigra of the brain causing motor dysfunction. There is no cure for this chronic disease affecting millions of individuals worldwide. Since current pharmacologic and surgical treatments are inadequate to prevent disease progression, optimization of cell replacement therapy may be the key to halt disease and restore function. Previous reports have provided evidence that dopaminergic (DA) neurons derived from ESCs can improve motor function in animal models. As the iPS field is in its formative stages, few studies have examined their therapeutic potential. In the proposed studies we provide a well-defined, experimental system to characterize the ability of hiPSCs to differentiate into a DA neuronal phenotype and to determine whether iPSCs are a feasible alternative to the use of ESCs in the treatment of Parkinson's disease in an animal model. The novel findings from these studies will further advance the iPS and neurodegenerative fields.

Name of Principle Investigator: Vasiliki Machairaki

Project Budget: \$ 110,000.00

Grantee: Johns Hopkins University

Title: A Comparison of Human Embryonic Stem Cells and Induced Pluripotent Stem Cells for Cellular Therapy of Parkinson's Disease

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

Considerable attention has recently been given to exploring the potential of HESCs (Human Embryonic Stem Cells) for neuroregenerative experimental strategies aimed at cell replacement. However, the challenge remains to design a reliable and efficient platform for the directed differentiation of HESC-derived NPs (Neural Precursors) into a diverse range of functional neural subtypes. We have used biofunctional polymeric scaffolds that can mimic the extracellular matrix microenvironment and support the desired cellular behaviors such as adhesion, proliferation and differentiation of NPs. Aligned and Non-aligned fibrous meshes of polycaprolactone (PCL) were prepared and further coated with Laminin to facilitate cell adhesion prior to cell culturing. Neural precursors derived from the NIH-approved hES line BGO1 were cultured on these substrates and their cellular responses characterized by immunostaining and gene expression analysis for both progenitor and differentiated neural cell markers. Preliminary studies of hNPs cultures on aligned fibrous meshes demonstrated that hNPs adopted an elongated and polarized cell morphology along the axis of fiber alignment, in contrast to hNPs on both 2-D and non-aligned fiber substrates that exhibited extensive but non-polarized neurite networks. hNPs cultured on aligned fibrous substrates showed a modest improvement (~15-20%) in neuronal differentiation over the other substrates, as quantified by expression of the early neuronal marker Tuj1. 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. Our recent studies on the migration of stem cells in the normal embryonic/postnatal mouse brain suggest a role for the family of matrix metalloproteinases (MMPs) in this process. These enzymes are also highly associated with the ability of cancer cells to metastasize throughout the body. We are studying whether modulating expressing/activity of MMPs could be a tool to control transplanted stem cell migration. Our preliminary data show that an ES stem cell line can be efficiently transfected with MMPs (MMP2, MMP9 and MT5-MMP to date). Transfected cell cultures increased expression of MMP9 protein 100-fold compared to non-transfected control with commitment increases of enzymatic activity. Injecting these cells into the adult mouse brain demonstrated they survived, and migrated. These preliminary transplants suggest transfected cells may migrate with higher efficiency than non-transfected. MMPs thus 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: Differentiation of Patient-Specific Human Pluripotent Stem Cells

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

The hematopoietic differentiation of human embryonic stem cells (hESC) has great potential for the generation of unlimited supplies of patient-specific adult-type, transplantable hematopoietic stem cells (HSC). Dr. Zambidis' group (ET Zambidis 2005, 2008) has developed efficient methods for generating hESC-derived hemangioblasts (primitive progenitors of both blood and endothelium) into adult-type hematopoietic progenitors. They have also discovered that angiotensin-converting enzyme (ACE) represents a novel marker for identifying (and purifying) hemangioblasts and HSC from differentiating hESC. This MSCRF research proposal expands upon these discoveries, by applying these novel hemangioblast differentiation protocols toward autologous, and disease-affected (preimplantation genetic diagnosis (PGD)-screened) hESC.

Since joining Dr. Zambidis' lab, I have successfully expanded several PGD-hESC lines derived by our collaborators at Reproductive Genetics Institute, Chicago. These PGD-hESC are affected with the two most important hemoglobin (Hb) mutations worldwide: SSD HbS (Line SC 233) and β -thalassemia (Line SC 164). We have confirmed pluripotency characteristics and genotyped these lines; genomic PCR amplification of hESC β -globin locus DNA, and direct sequencing confirms the homozygous β -6 SSD mutation for SC 233 line and heterozygous mutations on each of β -globin alleles for SC 164 line. I will first develop hematopoietic PGD-hESC differentiation models for two important human hemoglobinopathies. I will adapt established hESC erythropoietic differentiation methods developed in Dr. Zambidis' lab (e.g. using H1; H9 lines) to these PGD-hESC lines. We will characterize the primitive and definitive erythroid differentiations of these lines using EB and co-culture methods (e.g. on murine OP9 as well as human bone marrow stroma). hESC-derived hemangioblasts will be expanded and purified, matured in stromal culture, and then injected into immunodeficient mice (NOD/SCID IL2RG^{-/-}) to generate humanized hematopoietic models for these important hemoglobinopathies. The second aspect of my project aims to generate patient-specific HSC from pluripotent cells via methods that avoid the creation of viable human embryos (e.g., parthenogenesis and induced pluripotency (iPS)).

Prior to joining the Zambidis lab, I derived the first reported human parthenogenetic hESC (phESC) lines. Since phESCs are as much as 90% genetically identical (e.g. including at all HLA loci) to the oocyte donor, they can be used as a source of autologous transplantable cells for women, as well as their offspring. We previously demonstrated that these phESC lines can differentiate into progeny of all three embryonic germ layers, including mesodermal-derived beating cardiomyocytes, and spontaneous erythroblast differentiation. Their full hematopoietic potential, however, (as proposed herein) remains untested.

My overall plan for deriving patient-specific transplantable HSC from hESC derived by PGD screening, parthenogenesis, or iPS will include: 1) efficient differentiation of primitive hemangioblasts from these various pluripotent lines, followed by 2) further maturation of embryonic hemangioblasts into adult-type, transplantable HSC in a mesenchymal-derived stem cell niche. Our hope is that these experiments and methods will have great impact on the understanding and ultimate stem cell therapy of inherited hematologic disorders.

Name of Principle Investigator: Kara Scheibner

Project Budget: \$ 110,000.00

Grantee: Johns Hopkins University

Title: Regulation and Manipulation of Stem Cell Apoptosis by MicroRNA-27a

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

Based on expression of microRNAs (miRs) and their predicted target messenger RNAs (mRNAs) in human CD34⁺ hematopoietic stem-progenitor cells (HSPCs), we hypothesized that certain HSPC-expressed microRNAs (HE-miRs) can down-regulate key hematopoietic proteins and thereby regulate hematopoiesis. As a first example, we found that miR-155 was expressed in human CD34⁺ HSPCs and in mouse Kit⁺Sca1⁺Lin⁻ HSPCs (a subset enriched in early HSPCs). MiR-155 was known already to be overexpressed in several types of cancer, including many lymphomas and leukemias. To study the functional role of miR-155 in hematopoiesis and leukemias, we developed molecular tools to efficiently up- and down-regulate miR-155 in hematopoietic cells. To supplement enforced expression experiments using a miR-155/GFP dual promoter lentivector, we lipofected a synthetic 22-mer miR-155 sense oligonucleotide into cells. In functional studies, enforced miR-155 expression increased hematopoietic cell proliferation, 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 mRNAs. Thus, modulation of miR-155 and the pathways it regulates may be useful both in ex vivo expansion of HSPCs and in leukemia treatment.

As a second example, we investigated the action of miR-27a, which appeared to have 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 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. Certain predicted miR-27a targets may explain this effect on cell cycling. 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.

Name of Principle Investigator: Farhad Vesuna

Project Budget: \$ 110,000.00

Grantee: Johns Hopkins University

Title: Involvement of TWIST in Generating Breast Cancer Stem-Like Cells

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

Breast cancer is the primary cancer among women in the US in terms of incidence and first in terms of mortality. The largest single cause of death from all cancers including breast cancer is due to metastasis - a problem exacerbated by the emergence of therapy resistant cancer cells. A new paradigm that attempts to explain the biogenesis of breast tumor progression as well as the refractoriness of cancer cells is the identification of the breast cancer initiating/stem cell (CD44⁺/CD24^{-/low} phenotype). Twist (also called TWIST1) is an archetypical member of the bHLH class of transcription factors and binds to consensus hexanucleotide sequences (CANNTG) called E-boxes within promoter regions. Over-expression of Twist is observed in many cancers including gastric carcinoma, prostate cancer, and breast cancer. Moreover, over-expression of Twist induces an epithelial to mesenchymal-like transition (EMLT) both in mouse and human breast cancer cell lines.

The specific hypothesis in this proposed research is that Twist is a regulatory switch in the generation of breast cancer stem-like phenotypes in breast epithelium. The specific aims of this proposal are (1) To characterize the properties of Twist induced breast cancer stem-like cells, and (2) To decipher the functional role of Twist induced breast cancer stem-like cells.

We flow cytometrically sorted MCF-7/Twist cells based on CD44 and CD24 expression and verified by using immunoblotting protein extracts made from sorted cells. There was a high number of CD44⁺/CD24^{-/low} cells in Twist expressing cells versus parental MCF-7 cells (38% vs. 0.05%). Efficiency of drug efflux was estimated using Rhodamine-123 and Hoechst 33342 dye efflux experiments in which we found higher drug efflux in MCF-7/Twist cells compared to MCF-7 cells. In vivo functionality was determined by orthotopically xenografting 20, 50 and 100 CD44⁺/CD24^{-/low} sorted cells into female mice. We observed tumor growth in both animals starting at 6 weeks. Animals were sacrificed and both tumors were resected and sections were immunohistochemically stained.

In summary, characterizing the regulatory mechanisms controlling cancer stem cell biogenesis is crucial to both understanding the disease process as well as to design appropriate treatment regimens targeting resistant cells. We believe that deciphering the role of Twist in promoting breast cancer stem cell phenotypes will provide us with opportunities to design new drugs and appropriate treatment regimens for breast cancer patients leading to clinical translatability in the long term. The disruption of Twist has the potential to reverse the formation of breast cancer stem cells making tumors susceptible to other novel or existing chemotoxic or radiological agents.

Name of Principle Investigator: Jizhong Zou

Project Budget: \$ 110,000.00

Grantee: Johns Hopkins University

Title: Gene Targeting in Human Pluripotent Stem Cells

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

Human embryonic stem cells (hESCs) can self-renew indefinitely in culture and differentiate into any cell types in the human body. Recently human somatic cells have been successfully reprogrammed into induced pluripotent stem (iPS) cells that exhibit characteristics similar to hESCs. These pluripotent stem cells hold enormous promise for “customized” stem cell-based gene therapy to treat a variety of human diseases. Gene targeting, which is site- and sequence-specific modification of gene by homologous recombination (HR), has helped us to create knockout mouse models to investigate mammalian cell biology and mechanisms of human diseases. We and many others believe gene targeting in human pluripotent stem cells will not only provide unique insight of human genes’ function in human-cell based model systems, but also is essential to fulfill the promise of stem cell-based gene therapy. However, gene targeting efficiency in mammalian cells remains low ($<10^{-6}$) using the conventional system, which made it more difficult to achieve in hESCs due to its poor clonal propagation. The extremely low efficiency has limited the scientific application of this technology in the lab, as well as future clinical use. Built upon the expertise on hESCs in Dr. Cheng’s lab and my preliminary studies, I propose to further improve gene targeting in human pluripotent stem cells. In addition, I aim to target an endogenous gene in hESCs and create a Paroxysmal Nocturnal Hemoglobinuria (PNH) disease model.

Aim 1. Systematically improving gene targeting in hESCs and related iPS cells

Recently I have established a reliable GFP reporter platform for gene targeting with high-efficiency using zinc finger nucleases (ZFNs) in hESCs. It enhances HR rate up to 6% in human somatic cells and 0.24% in hESCs (>2000 folds higher than spontaneous event). I will optimize both gene-delivery technique and ZFNs design to further improve the efficiency and reduce the toxicity so that it can be easily and safely used in scientific and clinical applications. I will also test this GFP reporter system in the iPS cells. The comparison between iPS and hESCs gene targeting will illustrate their genetic and epigenetic properties and potentials.

Aim 2. Gene targeting in the PIG-A gene to establish a model for PNH disease

In PNH disease, mutation of human phosphatidylinositol glycan class A (PIG-A) gene results in the lack of all glycosyl-phosphatidyl-inositol anchored proteins in the hematopoietic lineages. However, many aspects of disease mechanisms are still not clear. The mouse models have not recapitulated the human disease phenotype. Establishment of true null PIG-A mutant in hESCs will illuminate the mechanisms of PNH disease. I have validated a set of gene targeting vectors with ZFNs designed to mutate PIG-A gene in human somatic cells. We will use the optimized system to target the PIG-A locus in hESCs and iPS cells. The similar approaches may be applied to other human endogenous genes in the future.