Maryland Technology Development Corporation
and
Maryland Stem Cell Research Commission

Annual Report

Calendar Year 2007

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OVERVIEW

This report identifies each grantee that received funding, as well as the amount of funding, from the Maryland Stem Cell Research Fund (MSCRF). These grants were made from the State's FY2007 appropriation. This report also provides an abstract in lay terms of the research to be performed. These abstracts were provided by the respective Principal Investigator in their application to the MSCRF.

Overall, Maryland's stem cell funding program has gotten off to a rapid and effective start. Within just a four-month period from August through November 2006, the Commission and TEDCO drafted and implemented program regulations implementing the Stem Cell Act, developed a conceptual framework for the grant programs to administer the funding under the Act, drafted detailed Requests For Applications (RFAs) for these grants and launched the first grant application cycle. Throughout 2006, the Commission dealt with key policy issues, such as requirements for grant recipients to make available to other parties any new cell lines created with State funds, and requirements for grants recipients to present the results of their State-funded work at annual in-State symposia open to the public. The Commission and TEDCO also devised a scientific peer review process for the evaluation and selection of grants, which is modeled on the National Institutes of Health process but tailored to the goals and requirements of the Maryland Stem Cell Research Act (for example, by expressly taking account of translation plans and the potential impact on biotechnology in Maryland in the grant applications), and put this peer review process into effect in February 2007.

The Commission and TEDCO received 85 applications for funding, requesting a total amount of almost $81 million. This number of applications clearly indicated a very strong turnout for the first year of a new program operating on a condensed timetable. From this pool of applications, the Commission recommended for funding and the TEDCO Board of Directors approved 7 Investigator-Initiated grants and 17 Exploratory grants. These grant agreements have been executed and the research has begun. All 24 detailed research descriptions may be found in this report.

Name of Principal Investigator: Curt Civin, M.D. **Project Budget: $1,725,000.00**

Grantee: Johns Hopkins University School of Medicine

Title: MicroRNA Regulation of Adult and Embryonic Human Hematopoietic Development

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

Short "microRNAs" bind specific messenger RNAs and block their translation to

proteins. Since a single microRNA can block protein translation of many messenger RNAs, microRNAs can serve as powerful switches to regulate cell differentiation. To extend our long-term studies on regulation of blood cell development (hematopoiesis) and stem cell biology, we recently measured microRNA expression in blood-forming stem cells. Based on the microRNAs and their predicted target messenger RNAs expressed in these stem cells, we proposed that many genes specifying hematopoietic differentiation are expressed by blood-forming stem cells, but held in check by microRNAs. The adult blood stem cell-enriched population that we studied is actually a complex mixture of rare stem cells and various stages of more mature "progenitor" cells. Therefore, we propose, first, to expand our microRNA profiles to highly purified populations of adult blood-forming stem cells and human embryonic stem cells to better understand which microRNAs regulate the development of different blood-forming cells. Second, we will determine if selected individual microRNAs actually inhibit adult blood cell development, as our model predicts. We will determine the proteins whose synthesis is blocked by each of these microRNAs and thereby the molecular mechanisms of their effects. At least 2 of these microRNAs, "mir-155" and "mir-16" are potential new major players in regulation of normal and cancer stem cells. Finally, we will study the functional effects of individual microRNAs on embryonic blood cell development generated by human embryonic stem cell lines. We anticipate that these fundamental insights into novel mechanisms of cellular differentiation will provide strategies to increase the quantities of hematopoietic and other types of stem cells that can be produced for research and potential clinical transplantation. Finding a method to expand blood-forming stem cells in tissue culture is a long-sought goal of stem cell research and would allow for major advances in medicine, such as making bone marrow transplantation and cancer and gene therapies safer and more effective. Further understanding of the effects of mir-155 and mir-16 in blood cell development may lead to specific methods to expand blood-forming stem cells for transplantation, and may provide new molecular targets for the treatment of blood cancers. In addition, our work may serve not only to elucidate the role of microRNAs in development of blood cells, but also to serve as a guide to regulation of other types of stem cells. Therefore, understanding how microRNAs regulate stem cells may provide general mechanisms of stem cell biology and cancer and have a broad impact on technology development for stem cell therapeutics, which is under active development by Maryland academic institutions and corporations.

Name of Principal Investigator: Jeff W.E. Bulte, Ph.D. **Project Budget: $1,190,276.00**

Grantee: Johns Hopkins University School of Medicine

Title: Human Embryonic Stem Cell-Derived Neurospheres for Treatment of Multiple Sclerosis

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

It is generally believed that multiple sclerosis (MS) is a result of an erroneous attack of white matter by our own immune defense. Most neurologists are now treating MS patients with medications that block or suppress this attack. To repair or make new white matter, a new approach is to transplant stem cells. This approach has been tested in animal models of MS and scientists have shown that animals that received neural stem cells did not get as sick as untreated mice. Until now, the researchers have studied the distribution of stem cells after transplantation by removing the brain from the animal and looking at the tissue under a microscope. When we are going to use these stem cells in patients, we cannot do this. We will use two novel methods to look at the survival and movement of transplanted cells in the brain. One is based upon the emittance of bright yellow light, and the other one is based on magnetic scans. Non-invasive imaging of cells is important to make sure that they are injected at the right place, if they survive, where they travel to, if they reach the problem areas in the brain, and whether they make new white matter. We will combine our experience in neurology and radiology to develop the best stem cell-therapy strategy in animals so that we can apply these protocols to MS patients.

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

Grantee: Johns Hopkins University School of Medicine

Title: Human Embryonic Stem Cell-Derived Oligodendrocytes and Electrophysiological Studies in a Contusion Model of Spinal Cord Injury in the Rat

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

Spinal cord injury (SCI) is a disease afflicting 300,000 people in the US. Consequences of this condition include loss of motor and sensory function. Trauma from incidents like falling or automobile accidents is the most common cause of SCI. Damage to the spinal cord leads to a difficult, time-consuming, and expensive treatment process that often has an unfavorable outcome for the patient. The difficulty lies in the nature of nervous tissue, which is incapable of regenerating as well as the other tissues of our body. Unlike the case of a broken bone, which simply requires time and fixation to heal, a damaged spinal cord is unable to heal itself leaving the brain disconnected from the body. This leads to conditions such as paralysis and quadriplegia. Research in SCI treatment has focused on methods to aid the regeneration of damaged nervous tissue, which could benefit greatly from stem cell therapy. Stem cells are unique in their capacity to change into the cells that make up the tissues of our bodies. Research has shown the clinical effectiveness of stem cells for cardiac injury as well as for diabetes. Furthermore, animal models have shown promise for embryonic stem cells as well as for neural stem cells in various injury models. In these studies neural stem cells derived from human embryonic stem cells have demonstrated the ability to replace damaged tissue in the spinal cord and restore lost function after injury. However, to bring stem cell therapy to clinical practice, important parameters such as time of application, exact location of injection, and the number of cells injected must be determined through rigorous studies on an animal model. Furthermore, a reliable and precise mechanism for measuring and quantifying the injury is very important in determining appropriate prognosis and therapy for each individual patient. Successful stem cell therapy will act as a scaffold, allowing spinal cord nerves to reconnect and establish healthy electrical connections between the brain and body. Hence, neuro-electrophysiological monitoring is a necessity to determine the integrity and function of these connections. In this proposal, this will be performed by measuring signals from the brain known as Somatosensory Evoked Potentials and Motor Evoked Potentials which can be used to assess SCI in a clinically relevant manner. These results will be followed up by traditional motor behavioral scoring, as well as postmortem histological analyses. Using these techniques, the main goal of this proposal is to apply advanced biomedical research to measure and quantify SCI and recovery following stem cell transplantation by applying novel instrumentation technologies and quantitative measurements for monitoring the extent of SCI and recovery produced by the stem cell therapy. Our research approach is unique in that the proposed cell therapy and diagnostic monitoring are both clinically applicable, thus setting the stage for clinical application as well.

Name of Principal Investigator: Hongjun Song, Ph.D. Project Budget: $

1,431,750.00

Grantee: Johns Hopkins University School of Medicine

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

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

Stem cells are special cell types with the capacity for unlimited self-amplification and for terminal differentiation into cell types with specific functions. Recent discovery and technical advance have led to the establishment of human stem cell lines from different origins, including embryonic stem cells (from human embryos) and adult neural stem cells (from adult human brains). Before the full potential of stem cells to be realized for cell replacement therapy for various diseases, we need to characterize functional properties of their differentiated progeny. The current proposal, building upon the expertise of investigators at the NeuroRegeneration and Repair Program of Institute for Cell Engineering and ongoing collaboration with other investigators from different disciplines at Johns Hopkins University School of Medicine, aims to develop novel tools and directly compare the physiological properties of neurons derived from these two different types of stem cells. We will use a battery of state-of-the-art technologies to examine human neurons in culture and after transplantation into animal models. In addition, we will evaluate their therapeutic potentials in established animal models of Parkinson's Disease. These studies may provide groundwork for future goals in treatment of degenerative neurological diseases using stem cells.

Name of Principal Investigator: Elias Zambidis, M.D., Ph.D. Project Budget: $ 1,110,826.00

Grantee: Johns Hopkins University School of Medicine

Title: Human Embryonic Stem Cell Models of Normal and Leukemic HSC

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

The original supply of human hematopoietic stem-progenitor cells (HSC) that serves to continuously regenerate the entire blood supply during adult life first arises in the four week-old fetus. The biologic events of human HSC genesis remain obscure, however, since human fetal tissue is difficult to study. A related problem is that the molecular events that convert a normal HSC into a leukemic "cancer stem cell" are impossible to study, since these genetic events elaborate in extremely rare stem cells, and occur long before patients are diagnosed with leukemic symptoms. Furthermore, few animal models faithfully mimic human leukemogenesis, especially for human diseases with complex genetic lesions that predispose to leukemia, e.g. Downs Syndrome, Fanconi anemia, or neurofibromatosis. Pluripotent human embryonic stem cells (hESC) derived from normal and disease-affected blastocysts can now provide solutions to these obstacles, since they can be readily differentiated into the earliest known hematopoietic progenitors, studied directly, and expanded in large numbers for treatment of various hematologic disorders curable by bone marrow transplantation. Our proposed studies will utilize hESC for understanding HSC genesis and self-renewal: the pivotal processes necessary for generating normal, transplantable HSC, and that conversely go awry in leukemia. An understanding of how HSC arise from hESC, therefore, has great importance since it will guide strategies for the unlimited generation of transplantable HSC for human blood diseases, and because it also provides a potent, new tool for understanding how leukemic stem cells are borne.

Name of Principal Investigator: Andrew S. McCallion, Ph.D. **Project Budget: $ 228,373.81**

Grantee: Johns Hopkins University School of Medicine

Title: Illuminating human cardiac development and disease through transcriptional analysis in differentiating human embryonic stem cells

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

The heart is the first organ to form during development; from that point forward all events in the life of the organism are dependent on its function. Cardiac defects are the most frequent of all birth defects and heart disease accounts for the largest proportion of adult mortality in the industrialized world. Consequently, science has a responsibility to strive to develop improved drugs and medical therapies. The advent of embryonic stem cell (ESC) technologies has opened the door to exciting possibilities including: the generation of methods to study human developmental processes in cells in a dish; the development of novel methods to find new drugs; and the promise of cell-based therapies. However, these endeavors remain hampered by a limited understanding of how cells "know" how to behave during the earliest stages of development, e.g. their progression from stem cells to heart muscle. We will illuminate the critical steps in human heart development using human embryonic stem cells (hESCs), and in doing so we will take the first steps towards each of the above goals. We will make a human embryonic stem line that will glow as it travels along a pathway from an undifferentiated hESC to become cardiac cells. This will allow us to purify these cardiac fated cells away from their neighbors, whose genetic programs differ. We will to compare the genetic program of these early heart cells with the programs of other stem cells so that we may gain a better understanding of the steps necessary to make heart muscle. This approach will also be useful in identifying new genes which may cause heart disease or may be targets for novel drug therapies e.g. after a heart attack.

Name of Principal Investigator: Guo-li Ming, M.D., Ph.D. Project Budget: $ 230,000.00

Grantee: Johns Hopkins University School of Medicine

Title: Mechanisms regulating self renewal of human embryonic stem cells

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

Stem cells are special cell types with the capacity to replicate and multiply itself (self-renewal) indefinitely and the capacity to give rise to specific cell types with specific functions (differenciation). Human embryonic stem cells (hESCs) are one type of stem cells that retain the potential to generate all cell types in a human body (pluoripotent) and thus have high clinical and therapeutic potentials. Recent advances in our ability to derive, maintain and differenciate hESCs into various cell types under controlled conditions offer us the opportunity to study the cell biology, to model diseases and to screen therapeutic drugs using human cells either directly with hESCs or the differenciated progeny of hESCs. For all these purposes, in addition to use these cells to replace specific lost cell types in degenerative diseases as cell replacement therapies, we will need to generate large quantity of homogeneous population of hESCs with stable genetic content and sustained pluoripotency. This is a daunting task since our knowledge about the self- renewal of hESCs is still very limited. We are interested in studying the mechanisms govern the unlimited self-renewal of hESCs. In this proposal, we will test one specific hypothesis: calcium signaling, an important second messenger in relaying stimuli from extracellular environment to influence the cell behavior, promotes hESC self-renewal. Specifically, we will examine mechanisms of two molecules, fibroblast growth factor (FGF-2) and gama-aminobutyric acid (GABA), both of which have been known to play important roles in proliferation of hESCs and to induce calcium signal inside the cells. The proposed study will broaden and advance our basic knowledge of human stem cell biology with the potential of developing clinical applicable stem cell source for degenerative disease and injuries.

Name of Principal Investigator: Nancy L. Craig, Ph.D. **Project Budget: $ 230,000.00**

Grantee: Johns Hopkins University School of Medicine

Title: Genome Engineering of Human Stem Cells for Gene Therapy

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

A fertilized human egg contains all the necessary information to make the many different cell types that carry out the variety of activities necessary for correct functioning of the human body. These different cell types arise because different sets of this genetic information are expressed in different cells. A feature, however, of many cell types is that once they reach their mature form, they can no longer divide to generate more cells despite the fact they still contain all the genetic information to do so. Thus if a particular cell is damaged from physical injury, disease or a genetic defect, the body cannot make new cells to restore the damaged cells. For example, a nerve cell may be cut and made non-functional by a physical injury such as a broken neck but the body is incapable of making new nerve cells to replace the damaged ones. Alternatively nerve cells may be non-functional when a necessary protein is lacking because of a genetic mutation. Stem cells offer great promise for the treatment of disease because of two of their fundamental properties: 1) they can divide without limit and 2) they can develop into many different cell types because they can express the many different sets of genetic information required for different cell types. For example, stem cells may be able to repair nerves damaged by physical injury by developing into new nerve cells that could replace the damaged cells. A key step to prompt the formation of a nerve cell from a stem cell may be the presence of a growth factor that prompts nerve cell gene expression in the stem cell. One strategy to provide this growth factor would be to introduce its gene in active form into a stem cell. Stem cells may also have the potential to cure a disease that results from a genetic defect. If nerve cells are not functioning correctly because a protein is lacking because of a mutation in a particular gene, addition of a new intact version of this gene into the stem cell could provide the necessary protein. Thus correctly functioning nerve cells could result from this introduction of a gene into a stem cell. We are proposing to develop new methods to safely and efficiently introduce new genes into stem cells. This sort of genome modification requires the stable introduction of the new gene in a way that does not damage the existing host genes. This sort of "gene therapy" of human cells including some types of stem cells has already been performed. However these experiments have always been accompanied by the risk that the newly introduced gene would be introduced into the host's genes in such a way that the host's genes would be damaged in a way deleterious to a patient. Unfortunately such damage has already been observed in some gene therapy experiments. We propose to develop new methods to introduce new genes into stem cells such that insertion occurs at specified " safe sites" where insertion will not damage the host's DNA.

Name of Principal Investigator: Hamid Rabb, Ph.D. **Project Budget: $ 230,000.00**

Grantee: Johns Hopkins University School of Medicine

Title: Isolation, Expansion and Regenerative Potential of Human Adult Kidney Derived Stem Cells

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

Acute renal failure (ARF) during transplantation occurs between 20-40% of deceased donor transplants. This adds over $ 20,000 in cost to each transplant at the time of admission, increases length of stay, risk of acute rejection, and decreases long term transplant function. In native kidney ARF associated with a 50% mortality rate. There is no specific therapy except for supportive care which includes dialysis. There is a tremendous opportunity to accelerate repair from ARF using stem cells. The applicant is an established investigator in ARF, and aims to shift into stem cell research to help his patients with transplant and native kidney ARF. The applicant has been learning the basics of stem cell biology and isolation techniques from experts at Johns Hopkins and nationally, and has learned to isolate and expand stem cells from adult mouse kidneys. Encouraged by this, the applicant proposes to isolate human kidney stem cells from donated kidneys that routinely undergo diagnostic biopsies prior to implantation in the recipient with kidney failure. The isolated adult human kidney stem cells will be expanded using techniques at Johns Hopkins that have been successfully for human cardiac stem cells. They will be extensively characterized for stem cell and kidney markers. They will be labeled, and then studied in mice with ischemic ARF. The survival of these stem cells, their location, and their effects on kidney structure and function will be studied. Efficacy and safety of these human adult kidney stem cells will be evaluated in animals in order to set the stage for studies in humans. In particular, the same patients who received the kidneys from which the stem cells were derived would be the perfect recipient for the stem cells when their kidney function deteriorated since these cells are from "safe" donors and the recipient is already immunosuppressed to accept them. These innovative studies could also be translated for use to patients with all types of chronic kidney disease and end stage kidney disease, which a huge burden in Maryland, particularly in the African American population who have a disproportionately high incidence of kidney failure.

Name of Principal Investigator: Akhilesh Pandey, M.D., Ph.D. Project Budget: $ 230,000.00

Grantee: Johns Hopkins University School of Medicine

Title: Proteomic Characterization of Neural Differentiation in human Embryonic Stem Cells

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

Human embryonic stem cells can be successfully differentiated into a variety of

more mature cell types in cell culture. Our proposal seeks to study the process of conversion of undifferentiated cells into differentiated cells using state-of-the-art proteomic methodologies. Specifically, we seek to identify markers that will help us identify and isolate cells that are specifically committed to form a certain type of differentiated cells. In addition, we hope to identify the specific signaling networks in cells that specify regulation of specific sets of genes that dictate differentiation. If one can direct the differentiation of hESCs into specific cell types, the differentiated cells can be used to treat diseases such as diabetes, heart disease and neurological afflictions.

Name of Principal Investigator: Candace L. Kerr, Ph.D. **Project Budget: $ 230,000.00**

Grantee: Johns Hopkins University School of Medicine

Title: Defining genes associated with human stem cell pluripotency and self-renewal

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

Pluripotent stem cells such as embryonic stem cells (ESCs), embryonal carcinoma cells (ECCs) and embryonic germ cells (EGCs) have the unique abilities to both self renew indefinitely as well as give rise to most, if not all cell types present in the human body. Consequently such pluripotent stem cells have enormous promise for the treatment of human disease. However, major

hurdles still need to be overcome to fully realize the potential of pluripotent stem cells. These include identifying the factors that control their growth as undifferentiated cells as well as the mechanisms that guide their differentiation into specialized cells. In either case, only a few factors have been identified despite several reports that have demonstrated the potential of these cells in models conducive to cell-based therapies. For this project we propose a series of genomic comparisons of the three known sources of embryonic stem cells, ESCs, EGCs and ECCs as well as the progenitor of EGCs and ECCs, primordial germ cells using microarray analyses. These comparisons will identify genes leading to the pluripotent nature and self-renewal of these cells and help fill gaps in our understanding of pluripotent stem cell derivation and maintenance.

Name of Principal Investigator: Karen Zeller, Ph.D. **Project Budget: $** 230,000.00

Grantee: Johns Hopkins University School of Medicine

Title: Myc's Role in maintenance and tumorigenicity of hESC

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

Stem cells have two remarkable properties of reproducing themselves (termed *self-renewal*) and the potential (termed *pluripotential*) to mature into any specific cell type such as brain or muscle cells. The ability for self-renewal of a stem cell is controlled by genes that produce proteins which are master switches. These proteins, called transcription factors, switch on or off other genes that in turn affect the ability of a cell to use energy, produce components of the cell or to divide into equal daughter cells. The MYC gene is such a master switch that not only regulates normal cell division but also contributes to cancer formation when MYC is itself out of control. In this application, we seek to understand the role of MYC in stem cell reproduction and the effect of MYC, when out of control, to contribute to the potential malignancy of human embryonic stem cells. These studies are clearly of fundamental importance as well as being critical for the safe application of human embryonic stem cells for therapy. The success of Maryland biotechnology sector will depend on the ultimate safety of stem cell therapy, which is in part addressed by our proposed studies.

Name of Principal Investigator: Shyam Biswal, Ph.D. **Project Budget: $** 230,000.00

Grantee: Johns Hopkins University School of Public Health

Title: Nrf2 as a target for cancer stem cell chemoresistance

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

Despite major advances in cancer surgery, radiotherapy and chemotherapy over the past 4 decades, majority of the cancer patients still die of their malignancies. Resistance to anticancer drugs is the major cause of poor survival among cancer patients. Recent discoveries have clearly indicated that a small proportion of cancer cells called cancer stem cells have self renewal capacity and survive chemotherapy because of resistance to anticancer drugs. Eradication of these cancer stem cells is likely a crucial component of any successful anticancer therapy. We propose to study a novel pathway in cancer stem cells that may determine the resistance to chemotherapy in blood cancer (leukemia and multiple myeloma). We have recently discovered a major factor responsible for cancer chemoresistance called nuclear erythroid-2 related factor 2 (NRF2). The NRF2 pathway turns on the main components of the anticancer drug resistance machinery in the cancer cells. This proposal aims at studies that will lead to greater understanding of a novel chemoresistance pathway in cancer stem cells. Furthermore, it strives to test the idea of overcoming chemoresistance by inhibiting/ suppressing this pathway. Successful completion of the proposed study will lead to major advancement in the area of cancer chemotherapy. This will also provide a novel strategy for targeting chemoresistance to save the lives of cancer patients in future.

Name of Principal Investigator: Srinivasan Chandrasegaran, Ph.D. Project Budget: $ 230,000.00

Grantee: Johns Hopkins University School of Public Health

Title: Targeted Engineering of the Human Genome in Stem Cells

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

A major challenge in the development of Genetic Medicine is to modify the human genome at specific sites in desired cells. Zinc finger nucleases (ZFNs) are proteins designed to cut at specific DNA sequences. Because the recognition specificities of the ZFNs can be easily manipulated experimentally, they offer a general way to deliver a targeted site-specific double-strand break (DSB) to the genome. Designed ZFNs have become powerful tools for enhancing gene targeting – the process of replacing a gene within a genome by homologous recombination (HR) - in cells. The development of ZFN-mediated gene targeting provides molecular biologists with the ability to site-specifically and permanently modify the human genome in cells via local homology-directed repair of a targeted genomic DSB. Site-specific engineering of the human genome so far has been hindered by the low frequency of HR in human cells. In ZFN-mediated gene targeting, this is circumvented by using designed ZFNs to cut at the desired chromosomal locus inside the cells. The DNA break is then patched using the new investigator-provided genetic information and the cells' own repair machinery. The high efficiency of this process combined with the ability to design ZFNs that target almost any DNA sequence makes ZFN technology a powerful research tool for targeted engineering of the human genome in cells. The current gene therapy protocols, which are based on gene addition approaches, try to compensate for defective genes by randomly inserting a new working copy into cells. These approaches are beset with complications arising from the random insertion of the therapeutic genes at undesired loci of the human genome in cells. The ZFN-based strategies could provide an efficient and effective means of directly and specifically editing "defective" bases in genes, making gene correction a viable option. It has the potential to dramatically change the field of gene therapy. Precisely targeted site-specific modification of primary human hematopoietic stem progenitor cells (HSPCs) and human embryonic stem cells (HESCs) cannot be done effectively by current routine technologies. This limits laboratory research in human cells and potential translation to stem cell based clinical therapies. The main focus of this proposal is to investigate the efficacy and specificity of ZFN-mediated gene targeting in these key cell types. If ZFN-based strategies are successfully applied for targeted engineering of the human genome in stem cells (HSPCs and HESCs), it will signify a major advance not only for laboratory research but also to translational clinical applications using stem cell based medical therapies. This research has the potential to contribute to the development and expansion of stem cell research in Biotechnology in Maryland and stem cell based medical therapies in general.

Name of Principal Investigator: Hai-quan Mao, Ph.D. **Project Budget: $ 230,000.00**

Grantee: Johns Hopkins University

Title: Engineering an Artificial Neural Stem Cell Niche

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

Stem cell therapy raises exciting hope for treating human neurodegenerative disorders. Before clinical trials are initiated, we need to develop an efficient strategy to obtain sufficient number of human neural stem cells (hNSCs) and to know how to control hNSC proliferation and differentiation into specific functional phenotypes. Currently available methods are neither efficient nor easy to scale up. In this study, we will develop a nanofiber scaffold platform with well-controlled nano-topographical cues and biochemical signals that will mimic several important functions of an in vivo NSC microenvironment. We will demonstrate the advantages of expanding hNSCs and inducing neuronal differentiation on these functional nanofibers, and understand the mechanisms involved in regulating hNSC through nanofiber cues. The artificial stem cell niche developed in this study will provide the next generation of scaffolds and methodologies for efficient hNSC expansion and neuronal differentiation. This study will contribute to shaping our understanding of how stem cell survival, proliferation and differentiation are regulated through factors in its extracellular microenvironment, and offer new insight into the design of functional scaffolds for tissue engineering and regenerative medicine

Name of Principal Investigator: Gary Fiskum, Ph.D. **Project Budget: $ 230,000.00**

Grantee: University of Maryland, Baltimore

Title: Stem Cell Antioxidant Gene Preconditioning for Improved Cell Survival and Neurologic Outcome after Traumatic Brain Injury

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

Approximately 1.5 million people sustain traumatic brain injury (TBI) each year in the U.S. alone. Of these, 50,000 die and 100,000 suffer long-term disability. TBI is also the leading cause of long-term disability in children and young adults. Each year it is estimated that 400,000 children visit the emergency department for TBI-related injuries, with 3,000 annual deaths and many experiencing long-term behavioral and emotional problems. While we investigators have made tremendous progress in understanding mechanisms of neuronal cell death and at developing neuroprotective interventions, a 50% reduction in trauma-induced neuronal death is probably the best that could be attained pharmacologically. There is therefore an urgent need for alternative therapeutic approaches directed at neuroregeneration. Transplantation of neuronal progenitor cells derived from human embryonic stem cells (NhES) into the brain after trauma may result in both neuroregeneration and neuroprotection and ultimately improve neurologic outcome. A major limitation in the effectiveness of this tactic is the survival of these cells after transfer from optimal cell culture conditions to the injured brain, where the anatomy and blood flow are grossly disturbed and inflammatory cells have created a hostile environment. One scheme for overcoming this limitation is preconditioning cells to become resistant to stress by first exposing them to a sublethal level of stress. Our preconditioning paradigm is exposure of NhES to sub-toxic concentrations of chemicals that activate elevate the expression of genes coding for proteins that detoxify reactive oxygen species. For instance, the chemical sulforaphane is found in broccoli and exerts its "antioxidant" activity by turning on genes that code for enzymes that have antioxidant activity. Our study will test the following hypotheses. 1. Compared to normal NhES, preconditioned cells will better survive cell death paradigms in culture that relate to the stressful conditions experienced during transplantation into injured tissue. 2. Preconditioned cells will exhibit improved post-transplantation survival in the brains of both mature and immature animals after traumatic brain injury. 3. Animals transplanted with preconditioned NhES will exhibit less neuronal death and better learning and memory than animals subjected to a sham-transplantation and animals that receive non-preconditioned cells. The significance of this exploratory research is that it could lead to methods of stem cell therapy for traumatic brain injury that will greatly improve the quality of life for those individuals that sustain neurologic injury after head trauma.

Name of Principal Investigator: Ricardo A. Feldman, Ph.D. Project Budget: $ 230,000.00

Grantee: University of Maryland, Baltimore

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

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

Human embryonic stem (hES) cells are capable of differentiating into virtually any cell type, making them very promising tools for use in basic research, and for tissue repair. There are many genetic diseases for which there is no cure. In these cases, it would be very desirable to have patient material to study the cause of the disease, and for testing novel therapies. However, cells from these patients are not always available. In these cases, having hES cells harboring the same inherited defects would be very useful. This would allow us to induce them to differentiate into the same types of cells that are affected in the patients, so that they can be studied. At the present time there are three possible sources of disease-specific hES cells. One is discarded embryos from IVF clinics, after they have been tested for known genetic defects. However, these embryos are not available for a large number of diseases. Another source of disease-specific hES cells is by nuclear transfer (NT) from adult cells of a patient into donated women's eggs. Although NT has been done in animals, there are many ethical issues with humans, including the discomfort and even danger to the female donors. In addition, there are many technical hurdles with NT in humans, which have not been solved. The third source of disease-specific embryos is genetic manipulation of hES cells. This is at the present time the option that raises fewer ethical questions. It is also the most flexible alternative because we can essentially recreate any known genetic defect. In this proposal we will use genetic engineering to generate hES cells that harbor a genetic defect similar to that seen in patients with Gaucher's disease, and we will establish the conditions for repairing the defect. In these patients, there is an enzyme for lipid metabolism (glucocerebrosidase) that is missing and as a result, the macrophages in various tissues become engorged with undigested lipids. As a consequence Gaucher patients have anemia, enlarged livers and spleens, and bone disease. At the present time the most effective therapy is enzyme replacement, using an enzyme produced in the laboratory. However, this therapy involves bi-weekly intravenous infusions for life, at an annual cost of hundreds of thousands of dollars per patient. We think that hES cells may one day help to provide a cure for these patients. Our approach will be to introduce into approved hES cells, small RNA molecules that are capable of lowering the glucocerebrosidase enzyme to the low levels seen in Gaucher patients. Then we will induce the modified hES cells to become macrophages, which will be studied to see if they exhibit similar defects to macrophages from Gaucher patients. Our second aim will be to assess if by restoring the missing enzyme levels in defective hES cells, we can restore the normal function of macrophages generated from repaired hES cells. Our last aim will be to test whether introducing genetically modified hES cells into mice can give rise to functionally capable macrophages in the intact animals. If the specific aims of this proposal are accomplished, the next step would be to do more elaborate work aimed at using the genetically engineered stem cells in clinical trials, to try to cure Gaucher patients. This disease was the first for which enzyme replacement therapy was used successfully and has served as a model for other inherited diseases. We think that the work we propose to do with hES cells to find a cure for Gaucher's disease will have applications for other genetic

diseases as well.

Name of Principal Investigator: Samir M. Jafri, Ph.D. Project Budget: $ 230,000.00

Grantee: University of Maryland, Baltimore

Title: A Delivery System for Stem Cells

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

A critical but frequently overlooked obstacle in developing therapies for neurological disorders is the method by which the therapy is delivered to the brain. The failed GDNF and fetal cell transplantation clinical trials provide important lessons in this respect. Many investigators in the field of neurodegenerative research believe that the failures of Amgen's two GDNF trials to treat Parkinson's disease was a reflection of the improper methods of GDNF delivery rather than the ineffectiveness of GDNF. Thus, after investments of hundreds of millions of dollars, countless man-years of work, and much hardship for the patients enrolled in the clinical trials, the scientific community still does not know whether Parkinson's patients can benefit from GNDF. The main motivation for this proposed project is to preempt a similar mistake for stem cells as they are brought to human clinical trials. Specifically, we seek to develop a better system for delivering stem cells to the brain. The technical challenges for delivering cells into the brain include spatial accuracy, reliability, and the ability to monitor the movement of minute volumes of solutions as they are being into the brain. Spatial accuracy is of particular importance for small targets deep in the brain. It is made more challenging by shifts in brain structures that can occur during surgery. Reliability is a problem that becomes apparent when research moves from the bench to the bedside. Whereas reliability is not serious problems in bench research because rats with misdirected injections can simply be discarded, even a small number of misdirected injections in humans could create unacceptable outcomes. Perhaps the more problematic challenge associated with cell delivery is monitoring the movement of cells in minute volumes of vehicle. When the volumes that are being delivered are ~1 µl, it is often hard to know how much if any of the therapeutic agents actually came out of the needle tip. Furthermore, it is not currently possible to know whether the therapeutic agent tracked up along the outside of the needle or dissect into a tissue plane or into the ventricle. Currently, there is no means to deal with the latter confounding challenge. We propose to solve these challenges by combining MRI guidance with an emerging clinical imaging technology, catheter-based optical coherence tomography (OCT).

Name of Principal Investigator: Adam Puche, Ph.D. **Project Budget: $ 230,000.00**

Grantee: University of Maryland, Baltimore

Title: Regulating stem cell migration in the adult brain

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

Stem cell transplantation therapy has the potential for replacing neurons lost in neural disorders/damage, as well as functioning as 'neurochemical factories' that endogenously produce growth factors or other neurochemicals to alleviate symptoms of neural disorders. While substantial effort is being directed towards understanding lineage specification and survival of stem cells used in transplantation, the problems presented by stem cell migration are poorly understood. Treatment of some disorders would benefit from restricting any transplanted stem cells to a particular brain region (e.g. transplanted dopaminergic cells should ideally remain restricted to the substantia nigra transplantation site to counteract the symptoms of Parkinson's disease). However, some nervous system disorders involve distributed conditions such as the progressive degeneration of motorneurons in ALS. In these conditions stem cell transplantation therapy hopes to replace these dispersed neurons, or to provide a companion cell generating survival/support factors. Both of these scenarios require the transplanted cells to distribute widely prior to differentiating and integrating into the brain. Treatment options for these sorts of neural disorders would benefit greatly from the ability to predetermine or 'tailor' the migratory ability of transplanted stem cells to each condition. Our lab has recently been studying the role of matrix metalloproteinases (MMPs) in regulating neural stem cell migration in adult. In particular, we have preliminary evidence that MT5-MMP and potentially MMP2 and MMP9 are involved in stem cell migration through mature brain tissue. This goal of this exploratory research application is to investigate how modulating expression and/or function of these MMP family members can modulate the migration behavior of transplanted stem cells. Specific aim 1 will examine how addition of MMP inhibitors or downregulation of MMP expression may restrict unwanted stem cell migration; Specific aim 2 investigates transiently upregulating MMP expression as a mechanism for improving transplanted stem cell migration through the intact brain; and Specific aim 3 takes the complementary approach of up-regulating naturally occurring MMP inhibitory molecules, the tissue inhibitors of matrix metalloproteinases (TIMPs), to reduce the migratory ability of transplanted stem cells. Understand the mechanisms and cues governing stem cell migration through the mature brain has important implications for the use of these cells as clinical therapy.

Name of Principal Investigator: Paul S. Fishman, M.D., Ph.D **Project Budget: $** 230,000.00

Grantee: University of Maryland, Baltimore

Title: Transcription Factor Driven Differentiation of Neural Stem Cells

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

A major goal of stem cell research is the creation of cells suitable for transplantation into humans to replace brain cells lost in diseases such as Parkinson's Disease, Alzheimer's Disease and ALS, and conditions such as stroke and traumatic brain injury. Current approaches to direct stem cells to become the appropriate forms of brain cells rely primarily on manipulation of cell culture conditions with growth factors and supporting cells. Recent progress in the understanding of brain development has identified a growing number of master genes. Many of these master genes code for regulatory molecules called transcription factors that are essential for activating a wide array of genes resulting in specific features of brain development. In a small number of studies, stem cells that have been genetically manipulated to produce a specific transcription factor have shown enhancement of brain cell properties. We propose to expand our preliminary studies in human cell lines and embryonic stem cells from mice on the role of these factors in transforming such immature cells into fully developed brain cells. One group of factors (bHLH type) is important in directing stem cells to become an functional brain cell (neuron) as opposed to a supporting type (glial) cell, while another group (including Nurr1 and Pitx3) are important to transform a stem cell into a dopamine producing brain cell, the type of cell that is lost in Parkinson's Disease. We will use neural stem cells for these experiments. The ancestors of these cells came from fetal human tissue, but they are capable of reproducing and maturing to some extent in a cell culture laboratory. These cells are already used to produce neurons but very inefficiently, and they usually do not develop into dopamine producing cells. In our first group of experiments, we will modify these cells by introducing artificial genes we have created to allow the neural stem cells to over produce the most effective factors from each group in other cell types (Pitx3 and Neuro D2). We will assess with both microscopic and biochemical methods how well these factors can change neural stem cells into dopamine producing neurons. For eventual transplantation in patients with neurological diseases such as Parkinson's Disease, it would be preferable for long term safety reasons that the cells not contain artificial genes. There is a new method where a transcription factor can be altered so that this large protein can pass through the cell membrane and directly activate genes within the cell nucleus. We are producing such modified transcription factors in pure form and have developed new ways of improving this delivery process. In the second group of experiments, we will apply these factors directly to the cells and determine if they can transform these stem cells into brain cells.

Name of Principal Investigator: William J. Lederer, Ph.D. Project Budget: $ 1,724,988.00

Grantee: University of Maryland, Biotechnology Institute

Title: Human Mesenchymal Stem Cell Treatment for Heart Damage

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

Many scientists believe that heart cells do not regenerate or re-grow following cellular loss and cannot be repaired following damage. Early work on stem cell treatment of other diseases has raised the possibility that stem cells could be made to become heart cells and thereby replace the dead or injured tissue. The investigators of this proposal did not find this to be true but discovered an unexpected important therapeutic behavior and a means by which stem cells may work in the heart. Preliminary findings show that damaged cells and damaged tissue can be effectively treated with human mesenchymal stem cells (hMSCs) if they are made to grow along side of the damaged cells. The hMSCs are taken from the bone marrow of adults and grown in the laboratory to provide a source of hMSCs. When the hMSCs are grown next to the damaged cells, the investigators have found that the hMSCs secrete substances that benefit the damaged cells and tissue. These substances have been identified and characterized at an initial level by the investigators. The proposed work seeks to carefully examine and extend the preliminary results, broaden our understanding of the mechanisms by which the hMSCs act and use this information to develop new treatments for human disease.

Name of Principal Investigator: Shengyun Fang, M.D., Ph.D. Project Budget: $ 219,420.00

Grantee: University of Maryland, Biotechnology Institute

Title: Ubiquitination-dependent regulation of oct-4 activity

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

Stem cells differ from other kinds of cells in the body. All stem cells—regardless of their source—have two general properties: 1) they are unspecialized and capable of dividing and renewing themselves for a long period of time; and 2) they can give rise to specialized cell types such as heart muscle, nerve cells, or the insulin-producing cells of the pancreas. The specialized cells derived from stem cells can be transplanted into patients for treating diseases such as heart disease, Alzheimer's disease, Huntington's disease, Parkinson's disease, and diabetes. To achieve this goal, it is essential to understand the fundamental properties of stem cells: 1) how stem cells maintain unspecialized status and self-renewal; and 2) how stem cells give rise to various specialized cell types. Previous studies have found Oct-4, a protein that plays a key role in keeping human embryonic stem cell in unspecialized status and self-renewal. Oct-4 is highly expressed in unspecialized embryonic stem cells. To become functionally specialized cells, Oct-4 protein has to be degraded and removed. However, how Oct-4 protein is degraded is unknown. Therefore, we hypothesize that Oct-4 is degraded by the proteasome, a large protease complex that is essential to degrade most proteins in the cell. To test this hypothesis, we will identify ubiquitin ligases that specifically catalyze attachment of the small protein ubiquitin to Oct-4. Once ubiquitin is attached to Oct-4, it creates a signal to deliver Oct-4 to the proteasome for degradation. The PI's lab is specialized in analyzing ubiquitin ligase-regulated function. We will use molecular, biochemical and cell biological techniques to approach the proposed study. The results of the proposed study will further our understanding as to how the fundamental properties of ESCs are regulated. Moreover, the ubiquitin ligases for Oct-4 may become a useful target for the development of reagents useful for biotechnology and medicine. Application of the reagents in ESC culture may provide a simple and economic way to produce large quantities of unspecialized ESCs for transplantation into patients to treat diseases. In addition, the reagents may become drugs to treat certain tumors.

Name of Principal Investigator: John P. Fisher, Ph.D. Project Budget: $ 229,792.00

Grantee: University of Maryland, College Park

Title: Human Mesenchymal Stem Cells for Craniofacial Bone Regeneration

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

Blunt head trauma often leads to extensive fractures of the bones that make up the face and skull. Current methods for the treatment of facial bone fractures include the grafting of autologous bone tissue and the implantation of plastics or metals. However, the development of a tissue engineering strategy for the treatment of facial fractures would greatly expand the options for the treating physician. The strategy we propose for the treatment of orbital fractures, as well as other fracture of the craniofacial bone, is bone tissue regeneration by autologous mesenchymal stem cell transplantation. Here, mesenchymal stem cells would be taken from the bone marrow of the patient, encapsulated in a synthetic biomaterial, and then transplanted into the defect site. After implantation, the biomaterial would slowly degrade away while the transplanted cells would first differentiation into bone forming osteoblasts and then synthesize new bone tissue in the defect site. While this approach has been proposed by many investigators, we hypothesize that the key factor that must be considered for successful transplantation of cells is the effect of the synthetic biomaterial on the ability of the cells to communicate with one another. Communication, or signaling, among cell populations is a process that has been widely investigated in scientific literature. However, little is known about how cells communicate with one another when they exist within a synthetic material. Clearly, the different environment that the transplanted cells experience must affect their typical processes, including the expression of signaling molecules and the subsequent function of these molecules. Therefore, we propose to examine these processes in biomaterials, so as to direct the development of constructs for successful cell transplantation. To this end, we propose to examine synthetic matrix properties that will promote the expression and function of osteogenic proteins, particularly bone morphogenetic protein-2 (BMP-2). Using a rational design, we first examine construct characteristics that augment the synthesis and function of BMP-2. We then examine whether enhanced BMP-2 function may be utilized to promote mesenchymal stem cell differentiation and thus bone formation. The successful completion of the proposed work will be an exciting, new approach for the engineering of orbital bone tissue as well as other mesenchymal tissues.

Name of Principal Investigator: Lloyd G. Mitchell, M.D. **Project Budget: $ 890,362.00**

Grantee: Retro Therapy, LLC

Title: Preservation of Potentiality in Genetically Altered Stem Cells

Description of the stem cell research (as submitted by Principal 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 capacity of stem cells to persist while repopulating tissues is the property that motivates the field of stem cell research. Many therapeutic strategies hope to achieve long-term persistence of cultured or transplanted stem cells. A potential problem for those applications which utilize genetically modified stem cells is premature expression of the transgene. Untimely expression of the transgene may disrupt the status of the stem cell, inhibiting it's ability to divide or differentiate appropriately. This proposal intends to enable a technique for correcting genetic defects in patient derived stem cells while preventing ectopic expression of the transgene (making protein at the wrong time). This proposal will utilize patient derived stem cells since autologous cells are the most compatible and may prevent rejection following transplantation. For many applications, stem cells will need to 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 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. Repairing or rewriting an endogenous gene is a facile means to regulate expression because expression of the repaired gene will be linked to the expression of the endogenous gene. Cystic fibrosis is one of the most prevalent diseases caused by mutation within a single gene, the cystic fibrosis transmembrane conductance regulator (CFTR). However, premature expression of the therapeutic gene interferes with the division and differentiation of pulmonary stem cells. We intend to develop a genetic construct that can specifically and efficiently repair most of the mutations which cause CF. Therefore, protein expression should recapitulate the natural regulation of the endogenous gene. Our specific Aims are: 1) To produce a highly efficient and specific pre-clinical lead candidate construct that can repair the greatest number of mutations underlying CF. 2) To demonstrate that therapeutic levels of correction of CFTR mutations 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.

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