Department of Health & Human Services
DHHS
NEBRASKA

Division of Public Health

State of Nebraska
Dave Heineman, Governor

October 2, 2014

Dear Senator:

In accordance with the Neb.Rev.Stat. §71-8801 et seq, the Stem Cell Research Advisory Committee met April 30, 2014, to determine which of the nine grant applications submitted for 2014 – 2015 should be funded. The following applications were approved for one year with a total amount of funding of $435,986.

| Project Title | Principal Investigator | Applicant Organization | Length of Grant | Total Amount |
|---|---|---|---|---|
| Reprogramming Human Somatic Cells without Transgenes | Dr. A. Angie Rizzino | University of Nebraska Medical Center | One Year | $110,000 |
| Reprogramming Airway Fibroblasts in Asthma | Dr. Stephen Rennard | University of Nebraska Medical Center | One Year | $105,986 |
| Calcium-Optimized Cartilage Formation from MSCs | Dr. Andrew Dudley (UNMC); Dr. Shadi Othman (UN-L) | University of Nebraska Medical Center/University of Nebraska-Lincoln | One Year | $62,260 (UNMC); $47,740 (UN-L) |
| mTOR Pathway and Glutamate Delta-1 in Neural Stem Cells | Dr. Shashank Dravid | Creighton University | One Year | $110,000 |

These research applications address the following medical areas: (1) reprogramming primary human somatic cells to induced pluripotent stem cells without the use of viral vectors or plasmid-based protocols (major goal in regenerative medicine); (2) the concept that the natural history of asthma can be improved by restoring normal function to airway fibroblasts by epigenetic reprogram; (3) can the regulation of calcium signaling increase the potential of mesenchymal stem cells to differentiate into mature cartilage to advance treatment of cartilage degeneration such as in osteoarthritis; and (4) the role of glutamate delta-1 receptor in the regulation of mTOR signaling, which is crucial for neural stem cell proliferation, differentiation, and neurogenesis and has implications for autism. The specific aims and a more detailed explanation of the research studies are attached.

We appreciate the opportunity to administer this grant program and look forward to working with these grantees. If you have any questions, please feel free to contact me.

Sincerely,

Joseph M. Acierno, M.D., J.D.
Chief Medical Officer – State of Nebraska
Director, Division of Public Health
Department of Health and Human Services

Principal Investigator/Program Director (Last, first, middle): Rizzino, A. Angie
Applicant Organization: University of Nebraska Medical Center
Abstract Title: Reprogramming Human Somatic Cells Without Transgenes

**Abstract: List the application's specific aims, and make a clear statement of the project's relevancy to stem cell research.** Describe concisely the research design and methods for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. This abstract is meant to serve as a succinct and accurate description of the proposed work when separated from the application. **There is a maximum of 30 lines of text.**

Soon after the paradigm-shifting studies that reprogrammed mouse somatic cells into induced pluripotent stem cells (iPSC), human iPSC (hiPSC) were generated from various cell types using similar protocols. Despite significant progress, many challenges associated with hiPSC must be overcome before this technology can be used to produce patient-specific pluripotent stem cells from which desired differentiated cell types could be generated and used clinically. Currently, reprogramming protocols that rely on the use of viral vectors or plasmid-based approaches run a significant and unacceptable risk of insertional mutagenesis. *Thus, a major goal in regenerative medicine is the development of protocols that are able to produce patient-customized hiPS cells without the possibility of insertational mutagensis*. Toward this goal, impressive progress has been made in developing protocols for the generation of hiPSC using small molecules. Several years ago, human somatic cells that express OCT4 from a transgene were reprogrammed into hiPSC using a cocktail of six small molecules: sodium butyrate, A83-01, CHIR99021, PS48, parnate and PD0325901 - collectively referred to as BACPPP. Although the BACPPP formulation represents a significant advance in the reprogramming of human somatic cells, the requirement for expression of OCT4 from a transgene remains. Thus, the final step in the reprogramming of human somatic cells using only small molecules should be achievable by eliminating the requirement for exogenous OCT4 expression. This can be accomplished by reactivating expression of the endogenous OCT4 gene, which is silenced in somatic cells due to DNA methylation. In this application, we propose several strategies for eliminating the requirement for exogenous OCT4 expression and testing **our overall hypothesis: supplementation of the BACPPP formulation with small molecules that *precociously* activate the endogenous OCT4 gene will enable the reprogramming of human somatic cells into hiPSC without the use of transgenes or nucleic acid-based protocols.** To test this hypothesis, we propose **a multipart Specific Aim**, which seeks to identify small molecules that substitute for exogenous OCT4 expression and successfully reprogram primary human somatic cells when used in combination with the six small molecule BACPPP formulation. Successful completion of the translational studies proposed here will not only surmount a major hurdle in regenerative medicine, it could also have a significant impact on the timeline for testing the therapeutic potential of cells derived from human pluripotent stem cells.

**Mini-abstract**: please provide one sentence that best describes this grant.

The research proposed addresses a major goal in regenerative medicine: reprogramming primary human somatic cells to induced pluripotent stem cells without the use of viral vectors or plasmid-based protocols.

Principal Investigator/Program Director (Last, first, middle): Rennard, Stephen I.
Applicant Organization: University of Nebraska Medical Center
Abstract Title: Reprogramming airway fibroblasts in asthma

Fibrotic airway remodeling leads to permanent loss of lung function in about 20% of asthmatics that may cause up to 20-30,000 deaths in the United States each year[1,2], far more than the 3,000 deaths occurring from acute asthma attacks. Current treatment does not meaningfully affect either tissue remodeling or progressive loss of lung function.

The current proposal will explore the hypothesis that **reprogramming of airway fibroblasts is a therapeutic option to alter the natural history of asthma**. We will pursue two independent specific aims:

**Aim #1: Determine if the altered function of asthmatic airway fibroblasts can be "corrected" by epigenetic reprogramming by de-differentiation into iPSCs followed by re-differentiation into fibroblasts *in vitro*.**

Fibroblasts isolated from the airways of asthmatic subjects and controls will be reprogrammed by formation of induced pluripotent stem cells (iPSCs) followed by re-differentiation into fibroblasts. The functional phenotype of the parental cells will be compared with that of the re-differentiated fibroblasts allowing for a direct test of whether reprogramming can "correct" functional alterations. We will also confirm that the iPSCs are derived from airway fibroblasts by lineage tracking and video microscopy.

**Aim #2: Identify a defined and limited set of factors that directly trans-differentiate "asthmatic" fibroblasts into "normal" fibroblasts.**

Using established methodology, differentially expressed transcription factors that can directly trans-differentiate asthmatic fibroblasts to fibroblasts with a normal functional phenotype will be identified. This will set the stage for animal studies of direct trans-differentiation, a potentially tractable therapeutic intervention.

Thus, the current proposal will use advances in understanding transitions to and from stem cells to advance the development of a novel treatment for a major healthcare problem.

**Mini-abstract:**

The concept that the natural history of asthma can be improved by restoring normal function to airway fibroblasts by epigenetic reprogramming will be tested using induced pluripotent stem cell biology.

Principal Investigator/Program Director (Last, first, middle): Dudley, Andrew T.
Applicant Organization: University of Nebraska Medical Center
Abstract Title: Calcium-optimized cartilage formation from MSCs

**Abstract: List the application's specific aims, and make a clear statement of the project's relevancy to stem cell research.** Describe concisely the research design and methods for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. This abstract is meant to serve as a succinct and accurate description of the proposed work when separated from the application. **There is a maximum of 30 lines of text.**

**Applicant's Specific Aims**

Cartilage degeneration is a significant clinical issue that affects the majority of older Americans and shows higher prevalence and disability among veterans, who are a key constituency in the Nebraska community. Surgical transplantation of whole cartilage or cartilage cells (chondrocytes) from "less important" regions of the joint can restore function, but the lack of donor tissue from the patient often limits use when cartilage destruction is significant. Autologous mesenchymal stem cells (isolated from the patient) offer a good source of cells that can be differentiated into chondrocytes. However, mesenchymal stem cell-derived cartilage exhibits decreased extracellular matrix formation and reduced biomechanical properties relative to chondrocyte-derived cartilage. Numerous approaches have been tried – including addition of growth factors and generating artificial matrix (scaffolds) into which stem cells are seeded. More recently, mechanical stimulation has shown promise, but this approach requires complex equipment and is not easily scalable. An alternative approach is to chemically manipulate the mechanotransduction pathways to induce changes in cellular physiology. This proposal tests modulation of intracellular calcium, a mediator of mechnotransduction, by treatment of mesenchymal stem cell cultures with the calcium ionophore A23187 during scaffold-mediated differentiation into chondrocytes and utilizes gene expression and protein analysis, and histological and ultrastructural outcomes in conjunction with magnetic resonance imaging and magnetic resonance elastography to quantitatively assess tissue development under stimulated conditions in two aims: 1) Define the optimal treatment regimen to stimulate chondrogenesis and 2)Test if calcium signaling imporves biomechanical properties of MSC-derived constructs. Results with mesenchymal stem cells will be compared to isolated human chondrocyte cultures. These studies will test if modulation of intracellular calcium promotes chondrogenesis in three-dimensional mesenchymal stem cell cultures and generates tissue constructs that exhibit biomechanical properties similar to chondrocyte-derived cartilage cultures. Successful execution of these studies will advance cartilage tissue engineering using autologous mesenchymal stem cells and with therefore accelerate development of new clinical options for patients suffering from cartilage degeneration.

**Mini-abstract**: This proposal tests if regulation of calcium signaling can increase the potential of mesenchymal stem cells to differentiate into mature cartilage to advance treatment of cartilage degeneration such as in osteoarthritis.

Principal Investigator/Program Director (Last, first, middle): Dravid, Shashank, Manohar
Applicant Organization: Creighton University
Abstract Title: mTOR pathway and glutamate delta-1 in neural stem cells

**Abstract: List the application's specific aims, and make a clear statement of the project's relevancy to stem cell research.** Describe concisely the research design and methods for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. This abstract is meant to serve as a succinct and accurate description of the proposed work when separated from the application. **There is a maximum of 30 lines of text.**

There is currently no effective treatment for autism spectrum disorders (ASDs) which affect 1 in 68 children in the United States (CDC). Overactivation of Akt-mTOR pathway by selective ablation of negative regulators PTEN or Tsc1 in neural stem cells (NSCs) leads to cellular abnormalities in subgranular zone (SGZ) in the hippocampus and ASD-like behavioral phenotypes [1-3]. Thus understanding whether and how ASD susceptibility genes modify Akt-mTOR pathway in NSCs may lead to discovery of novel therapeutic targets. GRID1 is an ASD associated gene and codes for glutamate delta-1 (GluD1) subunit, a member of ionotropic glutamate receptor family. We have found that loss of GluD1 leads to ASD-like social and cognitive deficits in mice [4,5]. Our *central hypothesis* is that loss of GluD1 dysregulates metabotropic glutamate receptor 1/5 (mGluR1/5) signaling leading to overactivation of Akt-mTOR pathway in the SGZ NSCs which is responsible for enhanced proliferation and differentiation of NSCs and social deficits. This hypothesis is supported by previous reports suggesting an interaction between glutamate delta receptors and mGluR1/5 and our preliminary data which indicate that GluD1 is a critical regulator of mGluR5 mediated Akt-mTOR signaling in the hippocampus. Using a combination of immunohistochemistry, *in vitro* NSC cultures, shRNA knockdown and behavioral methods together with GluD1 knockout model and pharmacological agents we will test the role of GluD1-mGluR1/5-Akt/mTOR pathway in the DG NSC proliferation, differentiation and social behavior. We have the following specific aims:

1. ***Determine the effect of loss of GluD1 on NSC proliferation and differentiation and granule neuron morphology.*** We hypothesize that loss/knockdown of GluD1 will increase proliferation/differentiation of NSCs and lead to moprhological defects in granule neurons.
2. ***Determine the effect of loss of GluD1 on Akt-mTOR pathway in the dentate gyrus cells and its dependence on mGluR1/5.*** We hypothesize that loss/knockdown of GluD1will lead to overactivation of Akt-mTOR in NSCs which will be dependent on mGluR1/5 function.
3. ***Determine the effect of inhibition of mTOR on social deficits and abnormalities in NSC proliferation and differentiation in GluD1 knockout mice.*** We hypothesize that inhibition of mTOR in GluD1 knockout mice will attenuate social deficits and abnormalities in NSC proliferation and differentiation.

The expected outcomes from the proposed studies will identify a novel mechanism of regulation of mTOR pathway in NSCs which has implications for etiology and therapy in ASDs.

**Mini-abstract**: please provide one sentence that best describes this grant.
The proposed studies will determine the role of glutamate delta-1 receptor in the regulation of mTOR signaling which is crucial for neural stem cell proliferation, differentiation and neurogenesis and has implications for autism.