NEBRASKA

Good Life. Great Mission.

DEPT. OF HEALTH AND HUMAN SERVICES

Annual Report on the Nebraska Stem Cell Research Act (LB 606) (Neb.Rev.Stat. §71-8801 et seq)

Presented to the State of Nebraska Legislature

Nebraska Stem Cell Research Advisory Committee and the Nebraska Department of Health and Human Services

March 26, 2021

Introduction

The Nebraska Stem Cell Research Act (LB 606) was passed in the 2008 Legislative Session (Neb.Rev.Stat. §71-8801 et seq).

Stem Cell Research Advisory Committee

This Act created the Stem Cell Research Advisory Committee. Members include the dean of each medical school in Nebraska accredited by the Liaison Committee on Medical Education (Creighton University School of Medicine and the University of Nebraska Medical Center), or his/her designee. Four scientists from outside Nebraska also serve as members of the Advisory Committee. The process is underway to fill a scientist vacancy on the Committee. The current membership includes:

- Bradley Britigan, M.D., Dean, University of Nebraska Medical Center, College of Medicine
- Robert Dunlay, M.D., Dean, Creighton University School of Medicine
- Alysson Muotri, Ph.D., University of California – San Diego
- Dennis Roop, Ph.D., University of Colorado – Denver
- Rui Yi, Ph.D., Northwestern University

The Committee is responsible for developing the grant process and making recommendations on grants to the Division of Public Health Chief Medical Officer. Institutions or researchers may not receive stem cell funding if using human embryonic stem cells. The Committee is also responsible for submitting an annual report to the Legislature on the progress of awarded projects.

Eligibility

Awards are granted as defined below:

- Sponsoring Institution. Preference will be given to funding proposals submitted by an institution in Nebraska that has an ongoing, large-scale research program that is conducive to the completion of a complex project in stem cell research that does not use human embryonic stem cells.

- Principal Investigator. The leader of a project is the “principal investigator” (PI). Researchers with a doctoral degree in science (PhD or equivalent), or a professional degree in a medical field (MD, DMD, DVM, or similar), are eligible to submit a proposal to the Stem Cell Research Advisory Committee as a PI. The PI must be employed at an institution in Nebraska that meets the criteria for “Sponsoring Institution” (see above). Researchers that are classified as Post-doctorates or Fellows are not eligible.

Availability of Funds and Matching Requirements

The amount of money available each year is determined by the Legislature. As provided in Neb.Rev.Stat. §71-8805, no single institution or researcher is eligible to receive more than 70 percent of the funds available for distribution.

Each Sponsoring Institution or researcher must provide a dollar-for-dollar match. See Neb.Rev.Stat. §71-8805. The matching funds must be obtained from sources other than funds provided by the Stem Cell Research Act (e.g., principal investigator's salary provided by the sponsoring institution, other research grants from federal sources, stipends for students, and post-doctorates).

Submission Requirements

Each proposal must be vetted and approved by a local committee appointed by the Sponsoring Institution, or its equivalent, before it is accepted by the Stem Cell Research Advisory Committee for full review. Approval of the application by the Sponsoring Institution should be based upon the degree to which the proposal appears to meet the selection criteria.

Proposals that are vetted and approved by local committee or its equivalent, must be submitted to the Division of Public Health of the Nebraska Department of Health and Human Services. Each Sponsoring Institution may submit a maximum of five proposals in a given funding cycle and no Principal Investigator may hold more than a single award.

Progress Report of Funded Grants

Some major highlights of the Nebraska Stem Cell Research Project during 2017-2021:

- Nebraska researchers have received additional funds exceeding $10 million from NIH, the American Cancer Society, DoD, and the University of Nebraska Collaboration Initiative that were directly related to their project
- Nebraska researchers also have NIH proposals pending/submitted/under review
- 40 publications (i.e., abstracts, articles, manuscripts, papers) have been prepared, are under consideration for publication, or published
- 15 research positions resulted from these grants (full and/or part-time)
- Over 50 national and international presentations relating to funding from the Nebraska Stem Cell Research Project
- 5 patents (includes provisional and pending)
- 4 inventions
- Technology led to a start-up company

COVID-19 has impeded the progress of the 2019 and 2020 stem cell grants.

2020 Stem Cell Grants

After reviewing seven applications, five grants were funded totaling $436,500. These grants will end June 30, 2021. The Principal Investigators provided the summaries.

Shannon Buckley, PhD (University of Nebraska Medical Center): Regulation of Stem Cell Fate Decisions by FBXO21: received $110,000 for one year

Project Summary: Ubiquitin E3 ligase protein, FBXO21, which serves as the substrate recognizing component for protein degradation by ubiquitin proteosome system, is highly expressed in hematopoietic stem and progenitor populations (HSPC). However, the molecular mechanism of FBXO21 in hematopoiesis is unknown. Our preliminary data shows silencing of *FBXO21* in HSPC leads to increased myeloid differentiation and loss of immature progenitors. In order to further study FBXO21 in hematopoiesis, we generated the first conditional knockout mouse. Our findings suggest that the ubiquitin E3 ligase FBXO21 and its substrates regulate hematopoietic stem cell quiescence and maintenance of the stem cell pool.

Laura Hansen, PhD (Creighton University): Targeting Cancer Stem Cells for Cutaneous SCC: received $110,000 for one year

Project Summary: Among non-melanoma skin cancers, squamous cell carcinoma (SCC) is common and can progress to metastasis. SCCs are populated by variable numbers of cancer stem cells (CSC) that have retained or acquired aberrant self-renewal properties. These CSC play an essential role in tumor initiation, and SCCs with higher numbers of CSC metastasize more frequently and are associated with higher mortality. Our preliminary data suggest that Flower-Win expression may help maintain stemness in skin cancer cells through a Notch-ΔNp63 signaling axis. The project is designed to test this hypothesis in order to identify new strategies for the treatment and prevention of malignant skin cancer.

Sidharth Mahapatra, MD, PhD (University of Nebraska Medical Center): NFIB's Role in Medulloblastoma Stem Cell Renewal: received $57,954 for one year

Project Summary: The proposed objective of this study was to investigate the stem cell renewal and maintenance roles of Nuclear Factor I/B (NFIB) in group 3 medulloblastoma. The central hypothesis of this proposal was that NFIB facilitates an oncogenic role in group 3 MB by driving cancer stem cell renewal and maintenance. We are exploring this hypothesis by first using an *in silico* approach to isolate top differentially dysregulated cancer stem cell pathways triggered by NFIB expression. Then, we hope to define the role of NFIB in cancer stem cell maintenance and tumor progression via *in vitro* and *in vivo* NFIB knock-out studies.

Holly Stessman, PhD (Creighton University): KMT5B Role in Maintaining Neural Stem Cell Pools; received $110,000 for one year

Project Summary: In 2017, we and another research group highlighted enrichment for germline heterozygous truncating mutations in the gene, KMT5B, among humans, suggesting that this gene may play a significant role in neurodevelopment. In the adult mouse brain, KMT5B shows high expression in regions known to contain neural stem cell (NSC) pools, and heterozygous KMT5B knockout results in significantly reduced overall weight, suggesting that KMT5B may affect cell growth and/or proliferation. Our overall objective in this proposal is to identify whether KMT5B is required for the proper function of NSCs using a mouse model. Our central hypothesis is that KMT5B is required for maintaining the balance between quiescent (non-dividing) and activated (dividing) NSC pools. We are testing this by quantifying adult NSC populations and by testing neurosphere-forming capacity of adult NSCs associated with KMT5B expression.

Siwei Zhao, PhD (University of Nebraska Medical Center): Electrical Stimulation of Endogenous Adipose-Derived Stem Cells for Chronic Wound Healing: received $48,546 for one year

Project Summary: The goal of our project is to develop a novel electrical stimulation device to more effectively recruit endogenous adipose-derived stem cells (ASCs) to facilitate chronic wound healing. ASCs are capable of regenerating skin tissues and have been shown to reduce wound size and associated pain in both animal studies and human clinical trials. Our first task is to develop an electrical stimulation device that can safely apply high electrical current intensities without causing cell death. Our second task is to determine the efficacy of high-intensity electrical stimulation in enhancing the directional migration of *in vitro* cultured ASCs. Our third task is to determine the efficacy of high-intensity electrical stimulation in mobilizing ASCs in freshly isolated living tissues.

2019 Stem Cell Grants

After reviewing eleven applications, five grants were funded totaling $436,500. These grants ended June 30, 2020. The Principal Investigators provided the summaries.

Kyle Hewitt, PhD (University of Nebraska Medical Center): Signaling Mechanisms in Hematopoietic Stem Cells; received $110,000 for one year

Project Summary: In this proposal we used primary hematopoietic stem and progenitor cells (HSPC) ex vivo to test Samd14 mechanisms in mediating cell signaling through the pro-survival, pro-self-renewal SCF/c-Kit signaling pathway, with the long-term goal of developing strategies to improve ex vivo HSPC expansion from primary non-embryonic sources. We made substantial progress delineating Samd14 mechanisms. The objectives of this aim are completed and accepted for publication in the *Journal of Biological Chemistry* (Apr 2, 2020). These findings provide several innovative biological/technical advances to the field of hematopoietic stem/progenitor cells.

R Katherine Hyde, PhD (University of Nebraska Medical Center): Evaluation of Novel Agents for HSC Mobilization; received $72,166 for one year

Project Summary: In this project, we tested the potential for a polymeric CXCR4 inhibitor (PCX) to be used for hematopoietic stem cell (HSC) mobilization. We found that PCX induced comparable HSC mobilization to the parent compound, AMD3100 (plerixafor) in mice. However, we found that PCX induced more toxicity in the HSC population than AMD3100. This indicates that PCX will likely not be appropriate for the mobilization of healthy HSCs from donors, but has potential for use in disease states where the goal is to eliminate affected HSCs prior to bone marrow transplantation.

Xiaowei Li, PhD (University of Nebraska Medical Center): Injectible Antioxidative Resilient Composite for Peripheral Artery Disease Treatment; received $72,167 for one year

Project Summary: We are working on optimization of our antioxidative hydrogel to support vascularization *in vitro*. We are verifying a new PAD model based on different ligation patterns of femoral or iliac arteries. We are testing biomaterials in the new PAD model.

Shashank Dravid, PhD (Creighton University): Targeting Stem Cells to Facilitate Remyelination in Disease States; received $110,000 for one year

Project Summary: The goal of this project is to address the role of GluD1-Cbln1 signaling in oligodendrocyte precursor cell (OPC) differentiation into oligodendrocytes and in myelination in normal and disease state. Our data so far demonstrates that GluD1 is enriched in the OPCs and ablation of GluD1 leads to an age dependent change in the number of oligodendrocytes and myelination characteristics. In addition, we find that loss of GluD1 makes mice more vulnerable to myelin loss and behavioral impairment suggesting that GluD1 is important for recovery from demyelination.

William Velander, PhD (University of Nebraska – Lincoln): Combinational Stem Cell Therapy for Chronic Wounds; received $72,167 for one year

Project Summary: To elucidate the combined effect of human plasma derived fibrinogen, fibronectin, recombinant factor XIII, recombinant thrombin and recombinant A1AT in producing an optimized matrix for MSC delivery and wound healing. This includes generation from human blood plasma and characterization of pro-healing matrix precursors, structure and mechanical properties. The viability and growth rate of MSCs, endothelial cells and fibroblasts within the matrix made from precursors is also characterized.

2018 Stem Cell Grants

After reviewing eight applications, five grants were funded totaling $436,500. These grants ended June 30, 2019. The Principal Investigators provided the summaries.

Iqbal Ahmad, PhD (University of Nebraska Medical Center): Human Disease Modeling of Glaucoma; received $101,850 for one year

Project Summary: The progressive degeneration of the output retinal neurons-called the retinal ganglion cells (RGC)-in glaucoma leads to irreversible vision loss. Our goal is to understand mechanism underlying RGC degeneration, which will help early diagnosis and treatment of glaucoma, by establishing a disease in a dish model of primary open angle glaucoma associated with SIX6 risk allele, using the induced pluripotent stem cell technology. Our results demonstrated that glaucoma-patient specific RGCs are developmentally and functionally immature, compared to their healthy control counterparts, which may make them susceptible to degenerative changes in adult.

Matthew Dilisio, MD (Creighton University): Stem Cell Derived Exosome Delivery for Tendon Repair; received $20,950 for one year

Project Summary: Stimulation of Mesenchymal Stem Cells (MSCs) with appropriate growth factors to manipulate exosomes for tendon regeneration has promising therapeutic application for the management of shoulder tendon injuries (STI). The hydrogel based approaches for drug and cell delivery has been found to be promising for the regeneration of various tissues owing to their hydrophilicity, biomimetic nature and close resemblance with native ECM. We developed a hydrogel patch loaded with pre-determined exosomes which can facilitate sustained delivery of exosome at the site of tendon injury.

Sung-Ho Huh, PhD (University of Nebraska Medical Center): Mechanism Regulating Sensory Stem Cell Generation; received $101,850 for one year

Project Summary: This project is to understand the role of Wnt/beta-catenin signal in auditory sensory stem cells. Auditory epithelia, which are responsible to hearing, are not able to regenerate after damage resulting in unrecoverable hearing loss. By under-standing mechanisms of auditory stem cell generation and maintenance, we may develop a new strategy to regenerate damaged auditory epithelium promoting ultimate hearing restoration.

Jung Yul Lim, PhD (University of Nebraska – Lincoln): Role of YAP Mechanotransduction in MSC Fate Decision; received $110,000 for one year

Project Summary: This project aimed to determine the role that Yes-Associated Protein (YAP) plays in Mesenchymal Stem Cell (MSC) fate decision to osteoblastic cell phenotype under physiologically relevant mechanical cell stretch loading conditions. This study further tested the mediation of such stretch-YAP pathway by cytoskeleton signaling through stressed actin filaments.

Jingwei Xie, PhD (University of Nebraska Medical Center): ADSCs-seeded 3D Aerogels for Bone Regeneration; received $101,850 for one year

Project Summary: We have established a method for effective coupling of modified BMP2 peptides with 3D hybrid nanofiber aerogels. Such BMP-2 peptide immobilized nanofiber aerogels can enhance cranial bone regeneration in a rat critical-sized calvarial bone defect model. The results have been published. One NIH R21 grant was awarded based on this work.

2017 Stem Cell Grants

After reviewing 11 applications, four grants were funded totaling $414,500. These grants ended June 30, 2018. The Principal Investigators provided the summaries.

Bin Duan, PhD (University of Nebraska Medical Center): MSC Derived Myelinating Schwann Cell for PN Regeneration; received $101,500 for nine months

Project Summary: We have successfully and repeatedly induced human adipose derived mesenchymal stromal cells (hADMSC) into myelinating Schwann cell (SC) like cells on our novel nanofiber yarns. We have confirmed the culture condition to improve the myelination properties of hADMSC-SC on both 2D culture and nanofiber yarns. We have also found that electrical stimulation process can synergize the chemical induction to promote the hADMSC differentiation into myelinating SC like cells. We have implanted the conduits with nanofiber yarns into a rat model with peripheral nerve defect and demonstrated that the implementation of nanofiber yarns could improve peripheral nerve regeneration.

Anna Dunaevsky, PhD (University of Nebraska Medical Center): Modeling Fragile X Syndrome with Stem Cells; received $101,500 for nine months

Project Summary: We have identified a novel deficit in astrocytes derived from Fragile X Syndrome (FXS) human induced pluripotent stem cells (hiPSC-astrocytes). We found that FXS hiPSC-astrocytes have shorter cell cycle and result in more proliferative cell population. We have also discovered that when engrafted into mouse cortex hiPSCS-astrocytes can develop into intralaminar astrocytes. These astrocyte are unique to humans and nonhuman primates and don’t exist in mice. Thus, our new model provides an opportunity to study the function of these cells in health and disease.

Yuguo Lei, PhD (University of Nebraska – Lincoln): A Method for Culturing Induced Pluripotent Stem Cells; received $110,000 for nine months

Project Summary: Specific Aim 1 is to systematically study how the parameters of AlgTubes including the alginate hydrogel concentration, tube diameter, hydrogel shell thickness and initial cell seeding density influence iPSC viability, growth, yield and pluripotency. Specific Aim 2 is to systematically compare culturing iPSCs in AlgTubes and the current 2D and 3D suspension culturing.

Jingwei Xie, PhD (University of Nebraska Medical Center): 3D Scaffolds Homing Stem Cells for Cartilage Repair; received $101,500 for nine months

Project Summary: We have invented new methods to convert 2D nanofiber membranes into 3D objects. We also generated 3D nanofiber objects with compositional and structural gradients. 3D nanofiber objects with compositional and structural gradients can effective regulate cell behavior (e.g., cell migration, cell differentiation).

Conclusions

The Nebraska Stem Cell Research Project continues to show substantial progress, establishing a solid stem cell research foundation. Research includes: glaucoma, facilitation of chronic wound healing, cranial bone regeneration, peripheral nerve regeneration, tendon regeneration to manage shoulder tendon injuries, strategies to regenerate damaged auditory epithelium promoting ultimate hearing restoration, Fragile X Syndrome, bone marrow transplantation, cancer stem cell renewal, treatment/prevention of malignant skin cancer, and innovative biological/technical advances to the field of hematopoietic stem/progenitor cells.

Researchers have also used their Nebraska stem cell funds as leverage in applying for new grant applications from the National Institutes of Health (including the National Eye Institute, National Institute of General Medical Sciences, R01, R21, R33, STTR), the American Cancer Society, the DoD, and the University of Nebraska Collaboration Initiative.