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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, 2020

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 current membership of the Stem Cell Research Advisory Committee includes:

- Bradley Britigan, M.D., Dean, University of Nebraska Medical Center, College of Medicine
- Robert Dunlay, M.D., Dean, Creighton University School of Medicine
- Rebecca Morris, Ph.D., The Hormel Institute at the University of Minnesota
- Alysson Muotri, Ph.D., University of California – San Diego
- Dennis Roop, Ph.D., University of Colorado – Denver
- Rui Yi, Ph.D., University of Colorado – Boulder

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 2016-2020:

- Nebraska researchers have received additional funds exceeding $6 million from NIH, NSF, CoBRE, and the University of Nebraska Collaboration Initiative that were directly related to their project
- Nebraska researchers have multiple NIH proposals pending/under review
- 50 publications (i.e., abstracts, articles, manuscripts, papers) have been prepared, are under consideration for publication, or published
- 28 research positions resulted from these grants (full and/or part-time)
- 54 national and international presentations relating to funding from the Nebraska Stem Cell Research Project
- 5 patents have been filed (including a provisional); 2 patents under preparation
- Negotiating a licensing agreement with a company
- Discussions with the DOD and the NIH regarding therapeutic uses
- Technology led to a start-up company

2019 Stem Cell Grants

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

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

Project Summary: This project utilizes innovative genetic mouse models and human primary cells to reveal mechanisms of a poorly-studied gene, SAMD14, in hematopoietic stem cell (HSC) signaling and regenerative engraftment. Using robust non-embryonic stem cell sources, the discovery of a founding member of a unique class of regeneration-activated genes may unveil strategies for therapeutic gene editing to treat hematologic disease.

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

Project Summary: This project tests the novel CXCR4 inhibitors for potential use in hematopoietic stem cell (HSCs) mobilization. Currently, the CXCR4 inhibitor AMD3100 (plerixaflor) is used to mobilize HSCs from the bone marrow to the peripheral blood, where they can be collected and used for stem cell transplantation. Our collaborator, Dr. David Oupicky, UNMC, developed novel polymeric CXCR4 inhibitors that not only have the potential to be more effective CXCR4 inhibitors, but also can be used to deliver other molecules that promote HSC health. Therefore, these new agents have the potential to increase both the number and health of mobilized HSCs, and greatly impact the health of patients undergoing stem cell 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: In this project we aim to develop an injectible antioxidative composite to mimic mechanical properties of muscle tissue, and control release of trophic factors to facilitate vascularization of EPCs in vitro, and determine whether the optimized composite restores blood flow into ischemic hindlimb with functional recovery in a rat PAD model.

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

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 and recombinant thrombin 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 summaries were provided by the Principal Investigators.

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.

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 understanding 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 aims to determine the role that Yes-Associated Protein (YAP) plays in mesenchymal stem cell (MSC) fate decision to bone cell phenotype under

mechanical cell stretch loading conditions. This study further tested the mediation of such stretch-YAP pathway by cytoskeletal 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: The specific aims of our project are: 1) establish effective coupling of modified BMP2 peptides with 3D hybrid nanofiber aerogels and examine their adipose stem cell osteogenic differentiation *in vitro* and 2) determine the bone regenerative efficacy of adipose stem cells seeded, BMP2 peptides incorporated aerogels for healing critical-sized cranial bone defects in rats.

2017 Stem Cell Grants

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

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 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: Fragile X Syndrome (FXS) is a devastating neurodevelopmental disorder with no known cure. To identify potential therapeutic targets, we differentiated induced human Pluripotent Stem Cells (ihPSC) derived from FXS patients into astrocytes, non-neuronal brain cells and characterized their properties. We have confirmed phenotypes previously found in a mouse model of FXS as well as identified new phenotypes that could be human specific and potentially more relevant to the 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 fabricated the 3D nanofiber scaffolds. Kartogenin and SDF-1 have been incorporated to the nanofiber scaffolds. We have tested the release profiles of kartogenin and SDF-1 *in vitro*. We have implanted nanofiber scaffolds to the cartilage defect in a porcine cartilage defect model to test the idea we proposed. We have harvested the tissues after 4 and 8 weeks and do the MRI imaging and examine the histology.

2016 Stem Cell Grants

After reviewing 11 applications, four grants were funded, totaling $435,000. These grants ended June 30, 2017. The summaries were provided by the Principal Investigators.

Shannon Buckley, PhD (University of Nebraska Medical Center): Role of E3 Ubiquitin Ligase, FBX08, in Maintaining & Inducing Pluripotency; received $109,468 for one year

Project Summary: Pluripotent stem cells (embryonic stem cells; ESC and induced pluripotent stem cells; iPSC) have the unique characteristics that they can differentiate to all three germ layers and are capable of indefinite self-renewal. Our previous studies suggested that key elements of the pluripotency network (including the “master” regulators Nanog and Oct4) are controlled by ubiquitination and that a significant number of UPS members (mainly ubiquitin E3 ligases) regulate pluripotency and influence lineage differentiation. Within the E3 ligase family, FBXO8 is an intriguing ligase to propose for further study for a number of reasons: 1) very little is known about FBXO8; 2) loss of FBXO8 enhances efficiency of cellular reprogramming; and 3) it has recently been shown that E3 ligases, including F-box proteins represent a class of proteins that are “druggable” targets due to structure and number of downstream substrates. The goal of our studies is to understand the role of E3 ligase FBXO8 and its substrates in cellular reprogramming to achieve optimal cell reprogramming and accelerate clinical applications of induced pluripotent stem cells.

Xian-Ming Chen, MD (Creighton University): Intestinal Stem Cell Responses to C. Parvum Infection; received $110,000 for one year

Project Summary: With a long-term goal to better understand the molecular mechanisms of gastrointestinal diseases for more efficient therapeutic interventions, this application aims to investigate the responses of distinct intestinal stem cells (ISCs) to pathogen infection at the intestinal mucosal surface, using models of intestinal infection by *Cryptosporidium parvum* (*C. parvum*), a protozoan parasite that usually only infects epithelial cells (enterocytes) at the villus tip.

Andrea Cupp, PhD (University of Nebraska – Lincoln): Purification & Proliferation of Mouse Spermatogonial Stem Cells; received $110,000 for one year

Project Summary: Few markers distinguish Spermatogonial Stem Cells (SSCs) from other male germ cells. Additionally, SSCs require a feeder layer when cultured, and spermatogonial stem cell transplantation (SSCT: transplanting donor germ cells into endogenously depleted germ cell recipients and determining colonization efficiency) is the only assay to determine SSCs. Our long-term objective is to develop combinations of markers that identify the SSC population and a culture system to allow for increased proliferation of these rare cells from adult testes in vitro. Thus, our hypothesis is that a specific combination of proteins will define a more enriched population of SSCs that can be increased through in vitro proliferation with growth factor combinations added to a 3-D alginate culture system. The aims to test this hypothesis are: 1) Evaluate combinations of protein antibodies: ID4, PAX7, TSPAN8 and NRP1 for a purified SSCs through cell sorting and flow cytometry followed by SSCT to determine colonization efficiency. 2) Determine combinations of growth factors (GDNF, FGF) that could be utilized in a 3-D alginate culture system followed by SSCT to identify greater proliferation of SSCs.

Haitao Wen, PhD (University of Nebraska Medical Center): O-GlcNAc Signaling in Intestinal Stem Cell Mediated Epithelial Regeneration; received $105,532 for one year

Project Summary: The intestinal epithelium is the most rapidly self-renewing tissue in the mammalian body and its process is controlled by the intestinal stem cells (ISCs). This project is to investigate the role of the *O*-GlcNAc signaling in ISC-mediated epithelial regeneration following sepsis-induced intestinal tissue damage.

Conclusions

The Nebraska Stem Cell Research Project has shown substantial progress, and a solid stem cell research foundation has been established. Research has included glaucoma, therapy for chronic wounds, therapeutic intervention for gastrointestinal diseases, treatment of artery disease, bone regeneration, peripheral nerve regeneration, tendon repair, male fertility, strategies to regenerate damaged auditory epithelium promoting ultimate hearing restoration, Fragile X Syndrome, treatment of hematologic disease, and sepsis-induced intestinal tissue damage.

Researchers have also used their Nebraska stem cell funds as leverage in applying for new grant applications from various sections of the National Institutes of Health (National Eye Institute, National Institute of Allergy & Infectious Diseases [NIAID], National Institute of General Medical Sciences [NIGMS]), National Science Foundation (NSF), the Centers of Biomedical Research Excellence (CoBRE), and the University of Nebraska Collaboration Initiative.