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Division of Public Health

Annual Report on the Nebraska Stem Cell Research Act

December 2023

Neb. Rev. Stat. § 71-8804

Introduction

The Nebraska Stem Cell Research Act (LB606) 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 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 School of Medicine
- David Owens, Ph.D., Columbia University Medical Center – New York
- Dennis Roop, Ph.D., University of Colorado Anschutz Medical Campus - Aurora
- Rui Yi, Ph.D., Northwestern University Feinberg School of Medicine - Chicago

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 (Ph.D. 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 who 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 (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 on the degree to which the proposal appears to meet the selection criteria.

Proposals that are vetted and approved by a 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.

Overview of the 2016 – 2022 Stem Cell Grants

Twenty-eight (28) stem cell grants were awarded between July 1, 2016 – June 30, 2022. These grants went to researchers at the University of Nebraska Medical Center, Creighton University, and the University of Nebraska–Lincoln. The amount of funding awarded to these 28 grants was $2,595,500.

As a direct result of the Nebraska Stem Cell Project:

- Nebraska researchers received new funds exceeding $15,594,246 from programs within the National Institute of Health (i.e., National Institute of Allergy & Infectious Diseases, National Institute of General Medical Sciences, National Eye Institute, National Institute of Arthritis & Musculoskeletal & Skin Diseases), the University of Nebraska Collaboration Initiative, the American Society of Gene & Cell Therapy, the Department of Defense, and the National Science Foundation (Engineering/Civil, Mechanical and Manufacturing Innovation/Biomechanics and Mechanobiology)
- Sixty-nine (69) publications (i.e., abstracts, articles, manuscripts, papers) have been prepared, are under consideration for publication, or have been published
- Forty-four (44) research positions were created
- Eighty-nine (89) national and international presentations were given
- Three (3) provisional patents filed with an appropriate agency
- Four (4) inventions filed with an appropriate agency

Research Topics

Nebraska stem cell research covers a wide range of topics. It includes, but is not limited to:

- Autism
- Bone marrow transplantation
- Cancer (skin cancer, cancer stem cell renewal and maintenance, colorectal cancer, glioblastoma [brain cancer])
- Cartilage regeneration
- Cranial bone regeneration
- Diabetic cardiomyopathy
- FBX08, FBX021
- Fragile X Syndrome
- Gastrointestinal diseases
- Glaucoma
- Hearing loss/hearing restoration
- Peripheral arterial disease
- Regeneration of peripheral nerve grafts
- Sepsis-induced intestinal tissue damage
- SOX2

The COVID-19 pandemic began to affect the progress of Nebraska stem cell research in late 2019. Although research has improved, COVID-19 still has some lingering effects.

2021 Stem Cell Grants

After reviewing nine applications, five grants were funded totaling $436,500. These grants ended June 30, 2022. The Principal Investigators provided their project goals and results.

Andrew Hamann, Ph.D. (University of Nebraska – Lincoln): *MSCs for miRNA-Loaded and Cell-Targeting Exosomes*: received $25,000 for one year.

Project Goals: This project aims to develop a system to genetically engineer adult stem cells to produce nanosized vesicles loaded with a class of biomolecules called microRNAs, which are promising new drug candidates. These vesicles, called exosomes, will specifically be loaded with microRNA-133a, which our collaborator has shown could be potentially used as a new therapeutic to treat a specific type of heart failure frequently caused by diabetes (i.e., diabetic cardiomyopathy). microRNA loading into exosomes will be optimized and the system will also be engineered to present molecules that can specifically target cells of the heart to deliver this potential new drug system. These microRNA-loaded and heart-targeted exosomes will then be investigated for their therapeutic properties using cell culture models of diabetic cardiomyopathy.

Project Results: This project has resulted in the design and characterization of new molecular systems that can be used to engineer cells to produce nanoscale vesicles containing microRNAs. Depending on the microRNA chosen, these engineered vesicles could be used as

more efficient delivery vehicles to diseased cells for treatments of many diseases that are associated with dysregulation of specific microRNAs. The project has generated knowledge that can be used to enhance loading of vesicles with microRNA and to further engineer vesicles that more specifically and efficiently deliver their microRNA cargoes to cells in disease models.

Robert Lewis, Ph.D. (University of Nebraska Medical Center): *Determinants of Stem Cell Formation in Colorectal Cancer*: received $110,000 for one year

Project Goals: The goal of this research is to identify mechanisms required for the generation of cancer stem cells, which are responsible for tumor initiation. Cancer stem cells are also major contributors to resistance to therapy, which limits treatment. Our preliminary data indicate that disruption of a protein called KSR1 prevents resistance to two clinically approved cancer therapeutics in both human colorectal cancer cells and human lung cancer cells. Ongoing experiments will determine how KSR1 contributes to cancer drug resistance with a long-term goal of testing approaches to inhibit KSR1 to improve cancer treatment.

Project Results: One of, if not the major problem with cancer treatment is resistance to therapy. Cancer stem cells are thought to be the source of therapy resistance. The data generated by this project reveal that resistance of human lung and colorectal cancer cells to a clinically approved drug (Trametinib) is prevented when the function of either of two proteins (SOS1 and KSR1) is inhibited. Coincident with the renewed drug sensitivity of lung and colorectal cancer cells, disruption of either of these proteins also depletes the stem cell population in these cancers. This observation is important because, in addition to their contribution to drug resistance, cancer stem cells are critical to the formation of new tumors (metastasis). Thus, these data suggest an approach to suppress tumor formation by both decreasing the potential to form new tumors and increasing the effectiveness of certain drugs that will kill them.

Brian North, Ph.D. (Creighton University): *Targeting SIRT2 in Glioblastoma Cancer Stem Cells*: received $95,750 for one year

Project Goals: Glioblastoma is a deadly brain cancer with poor survival. The severity of glioblastoma is characterized by a cancer stem cell population which promotes its growth and resistance to various therapeutic approaches. By studying the role of a SIRT2/β-TRCP/BubR1 molecular pathway that is involved in cell proliferation and survival, we hope to shed light on why glioblastoma cancer stem cells provide for an aggressive cancer and potentially identify mechanisms to target this disease.

Project Results: Glioblastoma is a deadly form of brain cancer with poor survival and limited treatment options. Cancer stem cells represent a portion of a tumor that promotes a cancer's growth and its inability to be effectively treated. Identifying molecular pathways that lead to cancer stem cells development is important to designing new therapies for glioblastoma. We study a group of genes, one of which is named SIRT2, that are involved in cell division, a process that is enhanced in cancer cells. We have found that the way this gene, and its protein product, is used by glioblastoma cells is different in the cancer stem cell portion of a tumor, and this difference may be important for enhancing the ability of cancer stem cells to promote cancer

growth. Future studies will determine how alterations in this gene in cancer stem cells is important for glioblastoma cancer growth and whether we can target this gene for better treatment options.

A. Angie Rizzino, Ph.D. (University of Nebraska Medical Center): *SOX2: MYC Axis and Drug-Tolerant Cancer Stem Cells*: received $95,750 for one year

Project Goals: Aim 1 was to determine whether increased SOX2 expression is required for the generation of cMYC-depleted drug-tolerant tumor cells (DTPs). Aim 2 was to screen a library of clinically used drugs to identify drugs that are able to kill SOX2-growth-arrested tumor cells.

Project Results: Our studies set out to better understand how drugs used to treat cancer reduce the expression of some cancer-promoting genes while increasing the expression of other cancer-promoting genes. We studied several drugs to address this question, including one used widely to treat melanoma. We determined that the increase in the cancer-associated stem cell transcription factor SOX2 occurring after drug treatment is not responsible for the concomitant decrease in the oncoprotein cMYC. In related studies, we set out to identify drugs that are capable of eradicating tumor cells that proliferate infrequently and are responsible for the recurrence of many types of human cancer. Our studies have identified five drugs used in the study of cancer that increase the death of infrequently proliferating tumor cells relative to their active proliferating counterparts.

Jian Zuo, Ph.D. (Creighton University): *Cochlear Stem Cells for Hearing Restoration*: received $110,000 for one year

Project Goals: Damage and loss of inner ear cochlear hair cells (HCs) leads to hearing loss. We have successfully induced the formation of new HCs (cHCs) in mouse models with therapeutic drugs. Here we propose to analyze transcriptional changes at the single-cell level via RNA sequencing to identify pathways and restore hearing pharmaceutically.

Project Results: We have analyzed the cocktail of two drugs in inducing new hair cell formation in adult mice and lineage-traced these new hair cells to supporting cells. We have performed single-cell RNA sequencing of drug-injected adult mouse cochleae and are in the process of analyzing the results.

Conclusions

Created in the 2008 Nebraska Legislative session, the Nebraska Stem Cell Research Project continues to show substantial progress, establishing a solid stem cell research foundation. The research includes the areas of autism, bone marrow transplantation, cancer (skin cancer, cancer stem cell renewal and maintenance, colorectal cancer, glioblastoma [brain cancer]), cartilage regeneration, cranial bone regeneration, diabetic cardiomyopathy, FBX08, FBX021, Fragile X Syndrome, gastrointestinal diseases, glaucoma, hearing restoration, peripheral arterial disease, regeneration of peripheral nerve grafts, sepsis-induced intestinal tissue damage, and SOX 2.

Nebraska researchers have also used their Nebraska stem cell funds as leverage in applying for new grant applications from the National Institute of Health, the University of Nebraska Collaboration Initiative, the American Society of Gene and Cell Therapy, the Department of Defense, and the National Science Foundation.