TAT Cell-Permeable Protein Delivery of siRNAs for Epigenetic Programming of Human Pluripotent and Adult Stem Cells

Grant Award Details

TAT Cell-Permeable Protein Delivery of siRNAs for Epigenetic Programming of Human Pluripotent and Adult Stem Cells

Grant Type: Tools and Technologies I

Grant Number: RT1-01063

Project Objective: To develop a nontoxic siRNA delivery system that can be used in proliferating or non-proliferating cells

Investigator:

Name:	Steve Dowdy
Institution:	University of California, San Diego
Type:	PI

Human Stem Cell Use: Embryonic Stem Cell

Award Value: $720,000

Status: Closed

Progress Reports

Reporting Period: Year 1

View Report

Reporting Period: Year 2

View Report

Grant Application Details

Application Title: TAT Cell-Permeable Protein Delivery of siRNAs for Epigenetic Programming of Human Pluripotent and Adult Stem Cells

Public Abstract:

The rapid progress of embryonic stem cell, induced-pluripotent cell, and adult stem cell research opens the door to thousands of promising, new medical applications and discoveries. However, one of the major obstacles in translating these basic science discoveries into safe therapies for patients is the risk of acquiring mutations from viral and DNA vectors. Exposure of stem cells to DNA vector can result in integration of the DNA element into the chromosome of the stem cell and thereby potentially induce a malignant mutation. Consequently, to clinically develop stem cell therapies, there is a great need to develop reagents and protocols that do not expose the stem cells to DNA vectors.

Over the last 10+ years our lab has developed small domains from proteins called cell-permeable peptides or peptide transduction domains (PTDs) that enter cells, including embryonic stem cells and non-dividing adult stem cells, in a non-cytotoxic manner that is independent of exposing the stem cells to DNA vectors. We have generated over 50 transducible proteins that enter the entire population of all cell types tested, including human embryonic stem cells and adult stem cells. We have also used this approach to introduce tumor suppressor proteins into pre-clinical mouse models of cancer. Moreover, PTDs are currently being tested in multiple clinical trials in the United States for heart disease and cancer.

One non-genetic (no DNA vector) approach with great potential to manipulate stem cells in specific cell types, such as heart muscle or neurons for spinal cord injury, is by RNA Interference (RNAi). RNAi, which was won the Nobel prize in 2006, allows for the selective degradation of mRNA and hence their protein product by introduction of short interfering dsRNAs (siRNA). However, siRNAs are difficult to deliver into cells and current delivery approaches result in cytotoxicity, poor percentage of cells, changes in the overall transcription and biology of the cells, and even DNA damage. Recently, our labs have developed an approach to combine the advances in cell-permeable PTD peptides with siRNA delivery. Pilot experiments show that we can efficiently deliver siRNAs into human embryonic stem cells and adult stem cells and induce specific RNAi responses. In this proposal, we will determine the overall efficiency of siRNA delivery using cell-permeable proteins in pluripotent and adult stem cells and will test for possible negative effects of this approach on their pluripotency and regenerative capacity. We will further test the feasibility of this approach to stimulate pluripotent stem cells to produce clinically relevant cells such as nerve, heart muscle and blood. If successful, the use of cell-permeable peptides to deliver siRNA into cells has great potential for scientists and biotech companies to remove a gap between stem cell discoveries and safe, medical treatments.

Statement of Benefit to California:	Our California universities and biomedical industries are poised for the development of an entirely new spectrum of potential patient therapies based on discoveries in the stem cell field. However, a major obstacle in bringing these discoveries to the clinics is the absence of safe, non-DNA based approaches. Consequently, an alternative approach to manipulate stem cells that avoids the use of DNA vectors is critical to advance stem cell therapies into the clinics. Over the past 10 years, our labs have pioneered alternative epigenetic, non-DNA based approach that allows for the introduction of proteins into embryonic and adult stem cells. We have used this approach to introduce more than 50 active proteins into a broad spectrum of human and animal cell types. In this proposal, we will develop this non-genetic approach to manipulate embryonic and adult stem cells by introducing RNA Interference (RNAi) inducing short interfering dsRNAs (siRNAs) in differentiate these pluripotent cells and adult stem cells into specific cell lineages such as heart muscle and neurons. Proof-of-Concept experiments have demonstrated the potential of approach to efficiently deliver siRNAs into stem cells in a non-cytotoxic fashion to induce RNAi response in the absence of DNA vectors (No DNA). Here we propose to expand this approach and ascertain its potential to manipulate stem cells to differentiate into therapeutically important cell types and understand the consequences of RNAi on stem cells therapeutics. Any breakthrough from this proposal could have an immediate impact on biomedical field. Furthermore, these technologies will have an immense economic benefit to California by removing a major obstacle for biotechnology and pharmaceutical companies in California and reducing the barriers between new discoveries and new treatments.