Directed Evolution of Novel AAV Variants for Enhanced Gene Targeting in Pluripotent Human Stem Cells and Investigation of Dopaminergic Neuron Differentiation

Grant Award Details

Directed Evolution of Novel AAV Variants for Enhanced Gene Targeting in Pluripotent Human Stem Cells and Investigation of Dopaminergic Neuron Differentiation

Grant Type: Tools and Technologies I

Grant Number: RT1-01021

Investigator:

Name:	David Schaffer
Institution:	University of California, Berkeley
Type:	PI

Disease Focus: Neurological Disorders, Parkinson's Disease

Human Stem Cell Use: Embryonic Stem Cell

Cell Line Generation: iPS Cell

Award Value: $918,000

Status: Closed

Progress Reports

Reporting Period: Year 1

View Report

Reporting Period: Year 2

View Report

Reporting Period: NCE

View Report

Grant Application Details

Application Title: Directed Evolution of Novel AAV Variants for Enhanced Gene Targeting in Pluripotent Human Stem Cells and Investigation of Dopaminergic Neuron Differentiation

Public Abstract: Human embryonic stem cells (hESCs) and induced pluripotent stem (iPS) cells have considerable potential as sources of differentiated cells for numerous biomedical applications. The ability to introduce targeted changes into the DNA of these cells – a process known as gene targeting – would have very broad implications. For example, mutations could readily be introduced into genes to study their roles in stem cell propagation and differentiation, to analyze mechanisms of human disease, and to develop disease models to aid in creating new therapies. Unfortunately, gene targeting efficiency in hESCs is very low. To meet this urgent need, we propose to develop new molecular tools and novel technologies for high efficiency gene targeting in hES and iPS cells. Importantly, this approach will be coupled with genome-wide identification and functional analysis of genes involved in the process in dopaminergic neuron development, work with fundamental implications for Parkinson's disease.

Barriers to targeted genetic modification include the effective delivery of gene targeting constructs into cells and the introduction of defined changes into the genome. We have developed a high throughput approach to engineer novel properties into a highly promising, safe, and clinically relevant gene delivery vehicle. For example, we have engineered variants of this vehicle with highly efficient gene delivery to neural stem cells (NSCs), and the resulting vehicles can mediate efficient gene targeting. We now propose to engineer novel gene delivery and targeting vehicles optimized for use in hESCs and iPS cells. One application of such an improved vector system will be to study the mechanism of ESC differentiation into dopaminergic neurons aided by the key transcription factor Lmx1a. We propose to identify target genes that are regulated by Lmx1a during dopaminergic neuron differentiation using the newly developed technique of ChIP-seq, in combination with RNA expression and bioinformatics analysis. This work will identify essential control genes that drive dopaminergic neuron differentiation. Furthermore, our improved gene delivery and targeting system will be used for overexpressing candidate genes, knocking them down via RNA interference, and knocking in reporter genes to analyze gene expression networks during neuronal differentiation.

The generation of efficient targeting technologies, in combination with genome wide analysis of gene regulation networks, will provide a general method for identifying and testing key regulatory genes for stem cell self-renewal and differentiation, as well as generating stem cell-based models of human disease. This blend of bioengineering and cell biology therefore has strong potential to create an important new capability for basic and applied stem cell research.

Statement of Benefit to California:

This proposal will develop novel molecular tools and methodologies that will strongly enhance the scientific, technological, and economic development of stem cell therapeutics in California. The most important net benefit will be for the treatment of human diseases.

Efficiently introducing specific genetic modifications into a stem cell genome is a greatly enabling technology that would impact many downstream medical applications. This capability will further enable investigations of self-renewal and differentiation, two defining properties of human stem cells. New tools to introduce targeted alterations of ES and iPS cells will also yield key model systems to elucidate mechanisms of human disease, and most importantly enable the generation of mutant cell lines to serve as models of human disease and systems for high throughput screening to develop novel therapies. Finally, the reverse process, the repair of genetic lesions responsible for disease, can in the long run enable the generation of patent-specific stem cell lines for therapeutic application.

Each of these applications will directly benefit biomedical knowledge and human health. Furthermore, this proposal directly addresses several research targets of this RFA – the development and utilization of efficient homologous recombination techniques for gene targeting in human stem cells, the development of safer and more effective viral vectors for gene transduction in human stem cells, and the development and analysis of human embryonic stem cell lines with reporter genes inserted into key loci – indicating that CIRM believes that the proposed capabilities are a priority for California's stem cell effort. While the potential applications of the proposed technology are broad, we will apply it to a specific and urgent biomedical problem: elucidating mechanisms of ES cell differentiation into dopaminergic neurons, part of a critical path towards developing therapies for Parkinson's disease. While hESCs clearly have this capacity, the underlying mechanisms are incompletely understood, and the efficiency of this process must be improved. We will elucidate transcriptional networks that underlie this process, and utilize our novel gene targeting system to identify and analyze key components of these networks. This work will lead to a better fundamental understanding of mechanisms regulating stem cell differentiation, as well as enhance our ability to control this complex process for biomedical application.

The co-investigators have a strong record of translating basic science and engineering into practice through interactions with industry, including the founding of biotech companies in California. Finally, this collaborative project will focus diverse research groups with many students on an important interdisciplinary project at the interface of science and engineering, thereby training future employees and contributing to the technological and economic development of California.
