Site-specific integration of Lmx1a, FoxA2, & Otx2 to optimize dopaminergic differentiation

Grant Award Details

Site-specific integration of Lmx1a, FoxA2, & Otx2 to optimize dopaminergic differentiation

Grant Type: Tools and Technologies II

Grant Number: RT2-01880

Project Objective: The objective of this project is to use the PIs novel genetic recombination strategy to express particular transcription factors that will lead to more efficient differentiation to midbrain dopaminergic (DA) neurons. The strategy is to use the PI's phiC31 integrase technology to perform site-specific integration of three TFs involved in DA neuronal differentiation into a transcriptionally active locus in human iPSCs derived from a Parkinson's Disease (PD) patient. The investigators hypothesize that forced expression of these TFs will achieve more efficient differentiation of DA neurons.

Investigator:

Name:	Michele Calos
Institution:	Stanford University
Type:	PI

Disease Focus: Neurological Disorders, Parkinson's Disease

Human Stem Cell Use: iPS Cell

Cell Line Generation: Embryonic Stem Cell, iPS Cell

Award Value: $1,592,897

Status: Closed

Progress Reports

Reporting Period: Year 1

View Report

Reporting Period: Year 2

View Report

Reporting Period: Year 3

View Report

Reporting Period: Year 4/NCE

View Report

Grant Application Details

Application Title: Site-specific integration of Lmx1a, FoxA2, & Otx2 to optimize dopaminergic differentiation

Public Abstract:

The objective of this study is to develop a new, optimized technology to obtain a homogenous population of midbrain dopaminergic (mDA) neurons in a culture dish through neuronal differentiation. Dopaminergic neurons of the midbrain are the main source of dopamine in the mammalian central nervous system. Their loss is associated with one of the most prominent human neurological disorders, Parkinson's disease (PD). There is no cure for PD, or good long-term therapeutics without deleterious side effects. Therefore, there is a great need for novel drugs and therapies to halt or reverse the disease.

Recent groundbreaking discoveries allow us to use adult human skin cells, transduce them with specific genes, and generate cells that exhibit virtually all characteristics of embryonic stem cells, termed induced pluripotent stem cells (iPSCs). These cell lines, when derived from PD patient skin cells, can be used as an experimental pre-clinical model to study disease mechanisms unique to PD. These cells will not only serve as an 'authentic' model for PD when further differentiated into the specific dopaminergic neurons, but that these cells are actually pathologically affected with PD.

All of the current protocols for directed neuronal differentiation from iPSCs are lengthy and suboptimal in terms of efficiency and reproducibility of defined cell populations. This hinders the ability to establish a robust model in-a-dish for the disease of interest, in our case PD-related neurodegeneration. We will use a new, efficient gene integration technology to induce expression of midbrain specific transcription factors in iPSC lines derived from a patient with PD and a sibling control. Forced expression of these midbrain transcription factors will direct iPSCs to differentiate into DA neurons in cell culture. We aim at achieving higher efficiency and reproducibility in generating a homogenous population of midbrain DA neurons, which will lay the foundation for successfully modeling PD and improving hit rates of future drug screening approaches. Our study could also set a milestone towards the establishment of efficient, stable, and reproducible neuronal differentiation using a technology that has proven to be safe and is therefore suitable for cell replacement therapies in human.

The absence of cellular models of Parkinson's disease represents a major bottleneck in the scientific field of Parkinson's disease, which, if solved, would be instantly translated into a wide range of clinical applications, including drug discovery. This is an essential avenue if we want to offer our patients a new therapeutic approach that can give them a near normal life after being diagnosed with this progressively disabling disease.

Statement of Benefit to California:	The proposed research could lead to a robust model in-a-dish for Parkinson's disease (PD)-related neurodegeneration. This outcome would deliver a variety of benefits to the state of California. First, there would be a profound personal impact on patients and their families if the current inevitable decline of PD patients could be halted or reversed. This would bring great happiness and satisfaction to the tens of thousands of Californians affected directly or indirectly by PD. Progress toward a cure for PD is also likely to accelerate the development of treatments for other degenerative disorders. The technology for PD modeling in-a-dish could be applied to other cell types such as cardiomyocytes (for heart diseases) and beta-cells (for diabetes). The impact would likely stimulate medical progress on a variety of conditions in which stem cell based drug screening and therapy could be beneficial. An effective drug and therapy for PD would also bring economic benefits to the state. Currently, there is a huge burden of costs associated with the care of patients with long-term degenerative disorders like PD, which afflict tens of thousands of patients statewide. If the clinical condition of these patients could be improved, the cost of maintenance would be reduced, saving billions in medical costs. Many of these patients would be more able to contribute to the workforce and pay taxes. Another benefit is the effect of novel, cutting-edge technologies developed in California on the business economy of the state. Such technologies can have a profound effect on the competitiveness of California through the formation of new manufacturing and health care delivery facilities that would employ California citizens and bring new sources of revenue to the state. Therefore, this project has the potential to bring health and economic benefits to California that is highly desirable for the state.

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