Maximizing the Safety of Induced Pluripotent Stem Cells as an Infusion Therapy: Limiting the Mutagenic Threat of Retroelement Retrotransposition during iPSC Generation, Expansion and Differentiation

Grant Award Details

Maximizing the Safety of Induced Pluripotent Stem Cells as an Infusion Therapy: Limiting the Mutagenic Threat of Retroelement Retrotransposition during iPSC Generation, Expansion and Differentiation

Grant Type: Early Translational I

Grant Number: TR1-01227

Project Objective: The objective of this project was to better understand whether retroelement retrotransposition represents a genome-destablizing event during reprogramming and in pluripotent stem cells, and to use that knowledge to enhance cellular protective functions in these cells.

Investigator:

Name:	Warner Greene
Institution:	Gladstone Institutes, J. David
Type:	PI

Human Stem Cell Use: Embryonic Stem Cell, iPS Cell

Cell Line Generation: iPS Cell

Award Value: $1,280,001

Status: Closed

Progress Reports

Reporting Period: Year 1

View Report

Reporting Period: Year 2

View Report

Reporting Period: Year 3 + NCE

View Report

Grant Application Details

Application Title: Maximizing the Safety of Induced Pluripotent Stem Cells as an Infusion Therapy: Limiting the Mutagenic Threat of Retroelement Retrotransposition during iPSC Generation, Expansion and Differentiation

Public Abstract: The ability to convert human skin cells to induced pluripotent stem cells (IPSCs) represents a seminal break-through in stem cell biology. This advance effectively circumvents the problem of immune rejection because the patient's own skin cells can be used to produce iPSCs. This exciting technology could accelerate treatments for a number of presently incurable diseases. However, a paramount unanswered question is whether these cells or their derivatives are truly safe for administration. Specifically, it is unknown whether the integrity of the iPSC genome is maintained during the tissue culture steps required to generate, maintain, expand and differentiate iPSCs. Every cell contains roughly 3 million "jumping genes" or mobile genetic retroelements that comprise up to 45% of the human genome. This contrasts with the fact that the roughly 21,000 human genes occupy only 1.5% of genome. While many of these retroelements have been permanently silenced during evolution, many others remain active and capable of replicating and moving to new chromosomal locations potentially producing disease-causing mutations or cancer. Somatic cells limit the jumping of these mobile genetic elements (retrotransposition) chiefly by methylating the DNA in and around these elements. Strikingly, the process of converting a skin cell to an iPSC results in a profound loss of DNA methylation potentially opening the door for high level retroelement activity that could corrupt genomic integrity. These insertions can disrupt key genes, create double strand DNA breaks or lead later to loss of large sections of DNA. Whether retroelement activity contributes to the fact that only 0.01% of skin cells are successfully reprogrammed to iPSCs is unknown. Thus, key questions regarding the safety of these cells remains. We now propose to determine the level of retroelement retrotransposition occurring in iPSCs and hESCs and to develop potentially safer ways to generate and maintain iPSCs in culture by blocking a key retroelement enzyme. Further, we will assess whether differentiation of these cells triggers retroelement activity. Finally, we will explore potential additional cellular defenses brought into action to oppose these retroelements with the goal of further enhancing these defenses.

Statement of Benefit to California:	The use of pluripotent stem cells derived either from the inner cell mass of developing blastocysts or by reprogramming of skin cells holds great therapeutic promise. These cells could provide exciting new approaches for a number of incurable human diseases like Parkinson's and Alzheimer's disease, type 1 diabetes, and cardiac failure. However, a paramount unanswered question in the field is whether these cells can be used in a completely safe manner. One major threat that could undermine these exciting stem cell therapies is the appearance of genetic mutations during their generation, expansion or differentiation. Such mutations could be induced by mobile genetic elements. Every cell contains roughly 3 million mobile genetic retroelements that comprise up to 45% of the genome. Active retroelements are capable of reproducing themselves and then jumping to a new chromosomal location potentially causing devastating disease-causing mutations or cancer-promoting changes. In normal differentiated cells, the jumping of these retroelements is highly constrained by DNA methylation. However, when skin cells are reprogrammed to become induced pluriopotent stem cells (iPSCs), DNA methylation is essentially erased. Human embryonic stem cells (hESCs) also exhibit dynamic changes in DNA methylation characterized by rapid losses and gains. These events open the door for repeated waves of retroelement retrotransposition that could greatly undermine the genome integrity of these cells. A real gap in our understanding of iPSC biology is that the potential activity and damaging effects of these retroelements has not been explored. To determine if hESCs and iPSCs and their cellular progeny can be safely used in patients, we propose to study the expression of retroelement RNA, the frequency of physical jumping events, and the impact of potential cellular defensive mechanisms opposing these retroelements. Since stem cells will be differentiated in vitro prior to their use in patients, we will also study levels of retroelement jumping in cells induced to differentiate into the three germ cell layers, endoderm, mesoderm and ectoderm. Additionally, we propose to explore potentially safer ways to generate and maintain iPSCs in culture where the retrotransposition process is interrupted using an FDA-approved HIV antiviral drug. Such an approach could protect the genome of these cells during their culture and manipulation in the laboratory prior to infusion into patients. The results of these studies will have both important scientific and practical value for the future therapeutic use of stems cells. As such, we believe these studies will benefit the citizens of California certainly at a societal level and potentially at a personal level.
