Homologous recombination in human pluripotent stem cells using adeno-associated virus.

Grant Award Details

Homologous recombination in human pluripotent stem cells using adeno-associated virus.

Grant Type: Tools and Technologies II

Grant Number: RT2-02064

Project Objective: Major goal of the project is to develop AAV mediated homologous recombination for gene targeting and to generate panel of marker hESC cell lines.

Investigator:

Name:	Karl Willert
Institution:	University of California, San Diego
Type:	PI

Human Stem Cell Use: Embryonic Stem Cell

Cell Line Generation: Embryonic Stem Cell

Award Value: $1,659,043

Status: Closed

Progress Reports

Reporting Period: Year 1

View Report

Reporting Period: Year 2

View Report

Reporting Period: Year 3

View Report

Reporting Period: NCE Year 4

View Report

Grant Application Details

Application Title: Homologous recombination in human pluripotent stem cells using adeno-associated virus.

Public Abstract: Since their discovery in 1998, human embryonic stem cells (hESCs) have been considered to hold great potential for the treatment of many currently incurable diseases. Possibly the most exciting application of hESC in the clinic is in the arena of regenerative medicine where hESC-derived cell populations are used to replace diseased, damaged or dead tissues. A major safety concern in developing hESC-based cell replacement therapies has been the potential risk of tumor growth, which is due to residual primitive, or undifferentiated, hESCs within the cell graft. Eliminating these undifferentiated and tumor promoting cells has proven to be difficult.

In this grant application, we propose to develop a technology to identify and enrich the cells of interest while eliminating undesired and contaminating cell populations. Using an elegant method to introduce genes into hESCs, we will engineer cell lines that will express a marker only when a particular cell type has been produced. Such “marker lines” will be used to develop and optimize protocols to efficiently derive specific mature and specialized cell types suitable for transplantation. Cell purification methods will be applied to enrich the cells of interest and eliminate primitive tumor-promoting cells. Therefore, the proposed research will yield critical tools to overcome safety concerns of tumor growth associated with hESC-based cell replacement therapies.

In addition to this crucial contribution to regenerative medicine, this technology is of immense value to basic biologist who wish to dissect developmental processes from undifferentiated to mature and specialized cell types. Such studies lie at the heart of developmental biology and will shed light on the intricate processes that guide a single cell, the fertilized egg, to divide, grow and acquire the thousands of cell types and characteristics of a complex multi-cellular organism. Another application of this technology is to append tags onto genes of interest to facilitate the studies of gene function during cellular growth and differentiation. Scientists studying particular genes and their roles in cellular and developmental biology and biochemistry will benefit tremendously from our engineered hESC lines carrying tagged genes. Finally, this technology will allow us to engineer specific mutations into genes associated with human diseases. HESCs carrying specific gene alterations can then be used to model human diseases in a petri dish, to screen efficacy and safety of drugs, and to devise methods to correct the defects.

Together, the proposed technology will yield valuable tools to the stem cell field to overcome multiple roadblocks in basic, translational or clinical stem cell research.

Statement of Benefit to California:	The rise in life expectancy to over 80 years will likely be associated with a corresponding increase in the number of people suffering from age-related diseases, such as cancer, heart disease and neurodegenerative disorders. Current medical treatments can alleviate symptoms and control, but not cure, such diseases. Human embryonic stem cells (hESC) provide a unique opportunity to develop novel cell replacement therapies for the treatment of many such diseases. Development of cell-based therapies will also overcome the inadequacy of conventional drug-based treatments. A major scientific challenge in the development of hESC-based therapies is the directed differentiation of hESC into functionally mature and pure cell types suitable for transplantation. The technology we propose to develop and disseminate to the greater scientific community utilizes an elegant gene replacement approach to create so-called “marker lines,” which are critical to the derivation and purification of mature cell populations. Such marker lines will be instrumental at gaining insight into the mechanisms that drive directed differentiation. Additionally, this technology will be applied to specifically modify individual genes, thereby enabling analysis of gene and protein functions. The broad stem cell research community will benefit tremendously from the development and streamlining of this technology and from the various engineered hESC marker lines, which will serve as critical building blocks to study and understand human development and disease. Translational and clinical stem cell research will likewise benefit from the tools developed under the proposed research as novel methods for cell isolation and purification will be identified. This research will not only benefit the health of Californians, but also the California economy by creating new reagents, protocols and technologies that will be adopted by existing companies as well as seed and complement novel business ideas. The outcome of this project will contribute to the development of a biotechnology platform that can provide great benefits to the advancement of California biotechnology. The patents, royalties and licensing fees that result from the advances in the proposed research will provide California tax revenues. Thus, the current proposed research provides not only the essential foundation for the scientific advances in regenerative medicine to improve health and quality of life, but also potential technology advancement and financial profit for the people in California.