



AMERICAN KENNEL CLUB
**CANINE HEALTH
FOUNDATION**
PREVENT TREAT & CURE

GRANT PROGRESS REPORT REVIEW

Grant: 01272: *Isolation and Characterization of Canine Induced Pluripotential Stem Cells (iPS)*

Principal Investigator: Dr. Jorge Piedrahita, PhD

Research Institution: North Carolina State University

Grant Amount: \$82,610.00

Start Date: 1/1/2010 **End Date:** 12/31/2011

Progress Report: 18 month

Report Due: 6/30/2011 **Report Received:** 6/28/2011

Recommended for Approval: Approved

(Content of this report is not confidential. A grant sponsor's CHF Health Liaison may request the confidential scientific report submitted by the investigator by contacting the CHF office. The below Report to Grant Sponsors from Investigator can be used in communications with your club members.)

Original Project Description:

Background: Stem cells have tremendous promise to alleviate clinical conditions in dogs such as spinal cord damage, hematopoietic malignancies, and cardiac and hepatic disease. While a range of adult stem cells have been isolated and studied, most of these have a limited capacity to differentiate outside a living organism and inside a living organism. Recently, approaches have been developed to convert differentiated cells into cells resembling embryonic stem (ES) cells by the use of "reprogramming" factors. These cells referred to as induced pluripotential stem cells (iPS) have the ability, like ES cell, to differentiate into multiple tissue types. As virtually any cell can be converted to an iPS cell this means that it is now possible to isolate patient-derived stem cells.

Objective: The researchers will utilize this technology for the development of canine iPS. Briefly, adipocyte-derived mesenchymal cells and keratinocytes will be transformed with the required reprogramming factors and plated under a condition that allows development of iPS cells. Colonies will be selected, expanded, and studied for their ability to differentiate outside a living organism into multiple tissue types. The development of patient-specific pluripotential stem cells is a critical step toward the successful scientific application of this promising technology.

Grant Objectives:

Objective 1: Development of canine iPS. Transfection of adipocyte-derived mesenchymal cell (AMC) and keratinocytes with canine OCT4, SOX2 and KLF4 for generation of iPS cells.

Objective 2: Testing the in vitro differentiation potential of canine iPS cells

Publications:**Report to Grant Sponsor from Investigator:**

Stem cells can make cell types as varied as neurons, heart cells, or bone cells. This ability to make multiple cells makes them valuable for clinical applications as they can replace damaged, or diseased cells. Broadly, there are two types of stem cells, those that can be isolated from embryos, or embryonic stem cells (ES), and those that can be obtained from adults. ES cells can make many cell types but cannot easily match the patient being treated as they can only be obtained from embryos. Adult stem cells can be matched to the patient, but they are not as good as ES cells as they can only make some cell types. Recently, in mice and humans, stem cells were isolated called induced pluripotent stem cells (iPS). These stem cells can be made from easily obtained skin or fat cells, yet they are as useful as ES cells. Their development is a critical step toward the successful clinical application of stem cells in dogs, so we proposed to develop canine iPS using fat or skin cells, and to study them for their ability to make multiple cell types in vitro.

In the eighteen months that we have been funded we have been able to isolated four new canine iPS cell lines and have maintained them for over 6 months in culture. These cell lines express the appropriate makers of stem cells and can differentiate into multiple tissues types in vitro and in vivo. We have also examined whether the cell lines have a normal karyotype (chromosomes) and found no evidence of abnormality. However, we have found by more detailed genetic analysis that the cells become unstable with prolonged culture. We are now completing the experiments to document how and when the cell lines change and to see if we can find way of preventing those changes.

In short, we have successfully developed canine iPS cell and have demonstrated that they can differentiate into several tissue types in vitro and in vivo. Presently, we are completing experiment related to the instability of the cell with prolonged culture. Moreover, we are examining what effect different culture conditions have on the ability of the cIPS to differentiate into multiple tissue types.