DCC Pilot and Feasibility Program
Progress Report
Carmella Evans-Molina, M.D., PhD

Project Title: Epigenetic Mechanisms of Metabolic Memory in Diabetes Mellitus

A. Specific Aims:

Aim 1: Generate and validate a mouse model of macrophage specific deletion of Set7/9 and expose this model to metabolic conditions analogous to sustained and intermittent diabetes mellitus.

Aim 2: Characterize the molecular and epigenetic effects of macrophage specific Set7/9 deletion in a mouse model of DM

No changes have been made to the Specific Aims as originally proposed and funded.

B. Studies and Results

Our experimental efforts to date have been focused on three main tasks. The first includes generation and validation of our mouse models of Set7/9 deletion. Therefore, a major emphasis of this award year has been to backcross our Set7/9$^{flox/flox}$ model onto a C57BL/6 background. To date, we have successfully backcrossed 8 generations and will complete backcrossing within the next two months. To begin to test the hypotheses proposed in this application, we have bred a robust colony of mice with a macrophage specific deletion of Set7/9 (macSet7/9KO). With support from this award, we have also been able to obtain a total body knockout mouse model of Set7/9. This mouse was obtained from Dr. Danny Reinberg, a world-renowned expert in epigenetics and will be used to augment our analysis of the conditional Set7/9 knockout model.

Our second major emphasis has included the initial phenotypic analysis of these novel transgenic models. Our *in vitro* data demonstrates that these mice possess less inflammatory macrophages with decreased expression of key NF-κB inflammatory targets like TNF-α and MCP-1. Future efforts will be focused on the characterization of chromatin architecture of the promoters of these key gene targets. We predict that Set7/9 is a chromatin modifying cofactor at these loci and loss of Set7/9 will lead to a "closed" chromatin architecture and inflammatory gene silening. These analyses will be complemented by functional macrophage assays, which are currently underway.

We hypothesize that in the context of diabetes, mice with a macrophage specific deletion of Set7/9 will be resistant to the development of atherosclerosis. To begin to address this question, we are finishing a 12-month dietary intervention. Mice were treated with or without streptozotocin to induce insulitis and glucose intolerance and then fed a normal or atherogenic diet. In August, we will end this experiment and perform *en face* assessment of atherosclerosis with Sudan IV staining and Oil-Red-O staining and analysis of the proximal aorta. We are mindful that wild-type mice are relatively resistant to the development of atherosclerosis, so plans are underway to perform bone marrow transplantation studies using macSet7/9KO donors and LDL-receptor deficient recipients followed by streptozotocin and atherogenic diet treatment.

Given our expertise in islet biology, we have broadened our experimental scope to examine the effect of Set7/9 deletion on the development of diabetes under inflammatory conditions. Interestingly, data obtained this year has shown that both the macrophage specific and total body Set7/9 knockout mice are protected against streptozotocin-induced diabetes and islet inflammation. Further, when challenged with streptozotocin, these mice have reduced T cell activation. This provides *in vivo* evidence of macrophages that are less inflammatory under diabetic conditions.

Our final goal has been to obtain key collaborations in order to increase our chance of obtaining continued extramural support. We have established three important collaborations that will be key to the success of this project

1. Lynne Hedrick, PhD (La Jolla Institute for Allergy and Immunology) - Dr. Hedrick's group has assisted us in investigating T cell activation in our transgenic models.
2. Rama Natarajan, PhD (City of Hope Hospital) - Dr. Natarajan's group will be examining the effect of Set7/9 loss on the development of diabetic nephropathy.
3. David Hui, PhD (University of Cincinnati) - Dr. Hui will be assisting us in our initial *en face* atherosclerosis assessment

C. Significance

Currently, nearly 24 million people in the U.S. are affected by DM. Patients with diabetes have a 2-10 fold increased risk of developing cardiovascular disease compared to non-diabetics. Understanding the factors that link elevated blood glucose with the development of heart disease is a key prerequisite for designing new treatment strategies for this problem. In order to fill this knowledge gap, we have generated a novel reagent that will allow us test the role of Set7/9 in the epigenetic activation of inflammatory macrophage gene expression. The studies presented here will allow us to define the role of Set7/9 in the development of metabolic memory and enhance our understanding of the role of epigenetics in diabetic heart disease

D. Plans

Future studies will be focused on three areas of emphasis:

1. Characterizing the effect of Set7/9 deletion on chromatin architecture at key inflammatory genes. Experiments are underway to characterize the chromatin architecture at key inflammatory genes in the context of Set7/9 loss. We predict the promoters of these targets will demonstrate a closed chromatin architecture consistent with data showing a loss of gene expression.

2. Assessment of the effects of a macrophage specific Set7/9 deletion on the development of atherosclerosis in mouse models of diabetes. We will perform bone marrow transplantation studies using macSet7/9KO donors and LDL-receptor deficient recipients followed by streptozotocin and atherogenic diet treatment.

3. Assessment of the effect of a macrophage specific Set7/9 deletion on islet inflammation and death. Results will be contrasted to mice with a β cell specific deletion of Set7/9

4. Characterization of alterations in macrophage and T-cell cross-talk in the context of Set7/9 deletion.

The preliminary data obtained under this award will be used to apply for an ADA Career Development Award in January 2012, an NIH R01 or R21 grant in February 2012.