2017 Pilot & Feasibility Award Recipients

The Vanderbilt Diabetes Research and Training Center (DRTC) announces the awardees of Pilot and Feasibility Grants for 2017 in the areas below:

Diabetes Research & Training Center Grant
Covers Basic and/or Clinic Research related to Diabetes, Metabolism and/or Obesity
Please click HERE for more details »

Vanderbilt Diabetes Center Discovery Program Grant
Covers Diabetes and/or Obesity-related Pilot studies that utilize high-throughput
Facility Bio VU and/or the Mass Spectrometry Research Centers
Please click HERE for more details »

Expand

2017 DRTC Pilot & Feasibility Award Recipients

Rolanda Lister, MD
Department of Obstetrics & Gynecology

I hypothesize that maternal hyperglycemia induces changes in DNA methylation in the developing heart thus increasing the risk of congenital heart defects due to abnormal mRNA expression of cardiac important genes. I have three specific aims to test this hypothesis. Aim 1: To analyze the cardiac phenotypes of embryos born to dams with hyperglycemia compared with pups of euglycemic pregnancies. Aim 2: To identify methylationsensitive DNA loci within developing fetal hearts that are more vulnerable to maternal hyperglycemia using a
high-resolution genome-wide cytosine methylation profiling assay. Aim 3: To juxtapose differential mRNA expression in developing fetal hearts with changes in genome wide DNA methylation.

Research strategy:
Diabetes will be induced in standard 8 week old CD-1 WT female mice with a one time intraperitoneal injection of 150 mg/kg of streptozotocin (STZ). Histological analysis of fetal cardiac morphology will be performed on the hearts of the hyperglycemic and the euglycemic pupps at day E16.5. We will extract the hearts at different timepoints and use genome wide cytosine methylation profiling to delineate the difference between pupps of hyperglycemic mothers versus controls. We will then juxtapose differential mRNA expression. With this information, we can then in the future obtain gene candidates that may be the targets for intervention.

Expected Results:
We anticipate that maternal diabetes will alter DNA methylation of specific genes related to cardiac development. We believe that by sequencing the entire genome of the extracted DNA after creating our library following the modified HELP-tagging assay and sequence, we will identify candidate genes that are implicated in dysregulation of the methylation patterns that ultimately predispose

these embryos to congenitally acquired cardiac lesions.

2017 Vanderbilt Diabetes Center Discovery Program Recipients

David Jacobson, PhD
Department of Molecular Physiology & Biophysics

The overall objective of this study is to identify selective activators and inhibitors of the two-pore-domain potassium channel, TALK-1. TALK-1 channels are key regulators of pancreatic ↑-cell electrical excitability, Ca2+ homeostasis and insulin secretion. KCNK16 the gene that codes for TALK-1 is the most abundant K+ channel transcript of the islet and TALK-1 is the most islet-restricted ion channel. Moreover, a mutation in KCNK16 results in neonatal diabetes and a nonsynonymous polymorphism in KCNK16 causes an increased predisposition for type-2 diabetes. However, our understanding of the role(s) of TALK-1 channel in human islets remains obscure due to a lack of specific and potent pharmacology. Therefore, this project will utilize a robust thallium (Tl+) based fluorescent assay in a high throughput screen (HTS) to identify pharmacological probes of the human TALK-1 channel. The assay will be performed on a tetracycline inducible TALK-1 cell line, which was selected for based on its performance in the Tl+ assay. The TALK-1 Tl+ assay was validated with primary screens of two small molecule libraries including the Spectrum Collection (~2320 molecules) and a bioactive lipid library (~928 molecules), which identified a small cohort of activators and inhibitors of TALK-1. The primary screens were utilized to optimize the Tl+ assay for use with the TALK-1 cell line in a large HTS. Building on these preliminary studies, this proposal plans to perform a HTS on the human TALK-1 channel with 35,000 structurally diverse small molecules from the Vanderbilt HTS small molecule library. This will be accomplished using 1. A Tl+ flux based HTS, which will be followed by 2. Secondary assays utilizing Tl+ flux, electrophysiology and Ca2+ imaging to support rapid hit-to-lead progression. This will be followed by assays to determine the mechanism of action of TALK-1 regulation including TALK-1 in single
channel inside out membrane patches as well as inhibition of G-protein-coupled receptor signaling, and finally 3.Molecular regulators of TALK-1 activity identified in this HTS will be utilized to test the influence of TALK-1 channels on human and mouse islet cell electrical activity, Ca2+ homeostasis and insulin secretion.

Anne Kenworty, PhD

Department of Molecular Physiology & Biophysics

Macroautophagy (hereafter referred to as autophagy) is an intracellular catabolic pathway involved in recycling of cellular components. The normal functioning of a variety of cell types important in both diabetes and obesity, including pancreatic beta cells and adipocytes, depends heavily on autophagy. Thus, it is critically important to understand the mechanisms that underlie this process. MAP1LC3B (LC3) is one of the central proteins involved in autophagy, playing essential roles in both the formation of autophagosomes as well as the capturing cargo for degradation by autophagy. Emerging evidence suggests nuclear forms of LC3 are also important in autophagy. However, the functions of nuclear LC3 are still largely unexplored and little information is currently available regarding LC3's interacting partners in the nucleus. Work from our group has shown that in the nucleus, LC3 is contained within ~1000 kDa complexes. We also identified an initial list of candidate LC3-interacting proteins using mass spectrometry. These studies have revealed a number of intriguing candidate interacting partners of nuclear LC3, including ribosomal subunits and tubulin. These findings lead us to hypothesize that additional functions of nuclear LC3 remain to be discovered, and that the identification of bona fide nuclear LC3 interacting proteins could provide new insights into how autophagy maintains cellular and organismal homeostasis. Here, we propose to leverage and expand on our initial findings by i) determining how the nuclear LC3 interactome is physiologically regulated and ii) validating key interacting partners of nuclear LC3 and exploring the functional significance of their interactions. These studies will thus set the stage for obtaining external funding for future work focused on identifying new functions of nuclear LC3. Ultimately, these studies should provide a framework that will inform our understanding of how autophagy functions as a general regulator of metabolism and how this pathway can be exploited to help prevent and treat diabetes and obesity.