

Coeli Lopes, Ph.D. - 2016 Awardee

University of Rochester School of Medicine and Dentistry

“Effect of Statin therapy in combination with KCNH2 targeting drugs: identifying cardiac arrhythmia risk in coronary artery disease patients.”

Statins are among the most commonly prescribed drug classes for prevention and treatment of coronary artery disease and their use is expected to increase due to recent changes in therapy guidelines. Statins are well known to reduce cardiovascular events and mortality in patients with coronary artery disease. Nonetheless, the effect of this drug class on cardiac electrophysiology, in particular for non heart-failure patients, has been incompletely studied. Indeed, some reports show statins decreasing repolarizing potassium channel currents in the heart, an effect expected to prolong QTc and increase the risk for arrhythmias. Our preliminary clinical data in Long QT syndrome patients suggest that statins may be detrimental to patients with mutations in the KCNH2 gene, increasing their cardiac risk, but may be protective to Long QT patients with mutations in other genes. The KCNH2 gene is the most common target for QT prolonging drugs. Our data indicates that statins inhibit KCNH2 channel trafficking to the plasma membrane and exacerbates the effect of drugs that block the channel. Thus, we propose that statins may serve as a pro-arrhythmic modulator of KCNH2-targeting drugs. The main goal of this project is to understand the molecular mechanism of action of statins in combination with commonly prescribed KCNH2-targeting drugs by studying the effect of statins at the cellular level. The ultimate goal of this project is to tailor statin therapy to the patient genetic profile and avoid detrimental drug-interactions. For that, we will first test the hypothesis that statins can increase the pro-arrhythmic effects of KCNH2 common genetic variants present in the general population and associated with increased risk of drug-induced arrhythmias. Second, we will investigate the molecular mechanism of channel regulation by statins by testing the hypothesis that statins increase channel ER-retention via the hERG chaperone, Hsp90. Finally, we will test the hypothesis that statins exacerbate the pro-arrhythmic effects of commonly used cardiovascular drugs, including anti-hypertensive, anti-arrhythmic and anti-anginal drugs, known to inhibit hERG function. Understanding the molecular effect of statins will allow tailoring of therapy to patients who would benefit the most and to avoid drug combinations or particular patient population for which statins may be harmful.

Javier Lopez, M.D. - 2016 Awardee
University of California, Davis School of Medicine

“Matching Stem Cell-derived Myocytes to Individual Patients' Heart Tissues: Personalizing Future Regenerative Treatments for Ischemic Heart Disease”

In the last decade, access to early revascularization, cardioverter-defibrillators and medications have lowered mortality rates from heart attacks. However, available interventions do not adequately address the myocardial insufficiency that stems from a lack of cardiac regeneration after infarction. In the future, patients suffering from progressive heart failure after a heart attack may instead have their myocardial insufficiency treated with regenerative strategies to recover the function of their native hearts.

The goal of this proposal is to develop, as a proof-of-concept, a novel in vitro 3D culture system of human tissue scaffolds that will allow future testing of new human derived myocytes for cardiac regenerative therapies. We propose that our new approach will help personalize the selection of human cell graft(s) from pluripotent stem cells to individual patients suffering of heart failure after a myocardial infarction. At the core of individualizing these therapies lies the testing of candidate cell grafts onto a patient's own cardiac tissue in the laboratory and before clinical use.

We hypothesize that the make-up of cardiac native tissues from patients and the size of implanted myocytes are critical determinants for the successful translation into the clinic of regenerative therapies. This project will apply a unique tissue-engineering approach developed at UC Davis to obtain extracellular tissue matrices from patient's own hearts. These matrices will serve as 3-dimensional scaffolds for testing the engraftment propensity of potential candidate cell grafts onto a patient's own heart. The key innovations of this proposal are: (1) the implementation of a tissue antigen-retrieval paradigm to generate cardiac tissue scaffolds that preserves the biophysical growth signals of an individual patient in vitro; 2) the testing of new small and mature human myocytes derived in Dr. Lopez's lab from pluripotent stem cells; and (3) a detail per-cell analysis of myocyte maturation in 3D cultures. This proposal aims to establish the early benchmarks of a future 3D culture system in a rat model first, and then, for the first time, using human tissues for future clinical translation.

Aim 1. To optimize 3D culture conditions for human myocytes derived from hiPSCs (hiPSC-CMs) grown over our unique adult rat cardiac 3D scaffolds (AR-ECM).

Aim 2. To produce human cardiac 3D scaffolds (AR-ECM) from surgical specimens and test their ability to engraft optimized human iPSC-CMs.

“Immune Checkpoint Inhibition and Atherosclerosis”

Deficiency of the scavenger receptor class B type I (SR-BI) protein in mice is associated with significantly increased plasma HDL-C levels and paradoxically accelerated atherosclerosis. We were the first to report associations with these same phenotypes in humans with SR-BI gene (SCARB1) single nucleotide polymorphisms (SNPs). Among these SNPs, rs10846744, a noncoding SNP (G>C) within intron 1 located at a regulatory site, was significantly associated with increased subclinical atherosclerosis (SCA) and increased odds for cardiovascular disease (CVD) in Multi-Ethnic Study of Atherosclerosis (MESA) participants. Inclusion of traditional CVD risk factors in a multivariate regression model did not attenuate the association of rs10846744 with CVD, strongly suggesting that other pathway(s), including an inflammatory one, likely drive this important clinical association.

We used RNA-Seq to examine transcriptional differences in gene expression between lymphocytes isolated from hyperalphalipoproteinemic (HALP) carriers homozygous for the SCARB1 rs10846744 reference (GG) allele with carriers homozygous for the risk (CC) allele. We identified lymphocyte activation gene-3 (LAG-3), a gene that encodes the immune checkpoint inhibitor LAG-3 protein, and binds major histocompatibility complex II to suppress expansion of T effector cells, as a strong candidate in the SCARB1 rs10846744 CVD risk pathway. Additionally, carriers of the LAG-3 missense rs870849 SNP had significantly lower plasma LAG-3 protein levels and increased SCA. The hypothesis to be tested in this application is that LAG-3 protein deficiency exerts a major influence in the risk for SCA and CVD events. The two aims will be: (1) To determine LAG-3 protein as an independent predictor of SCA and CVD risk in MESA, and (2) To determine the mechanism of LAG-3 protein on HDL-cholesterol efflux in macrophages. The long term objective of this project is to unravel the novel intersection of the SCARB1 rs10846744 variant and the immune checkpoint inhibitor LAG-3 protein in CVD risk.

William Wagner, Ph.D. - 2015 Awardee

Wake Forest University Health Sciences

“Biofabrication of a Novel Elastomeric Substitute for Autologous Veins in Coronary Artery Bypass Surgery”

Coronary artery disease is the main cause of death in the western world and is due primarily to atherosclerosis and thrombosis. Coronary artery bypass surgery utilizes autologous vein grafts; however due to compliance mismatch and intimal hyperplasia, the grafts typically fail due to atherosclerosis and thrombosis. We have synthesized and developed PFC, a novel viscoelastic composite polymeric material composed of poly (glycerol-sebacate), highly tensile silk fibroin, and Type I collagen. PFC was shown to have promising properties for use as a blood contacting material due to its non-thrombogenicity, tensile properties (2.3-5 Mpa elastic modulus), degradation (0.01% per week over 27 weeks), and cellular adhesion. This proposal details studies to develop PFC into a small diameter coronary artery vascular graft. The aims of the proposal include producing a PFC conduit by electrospinning and conducting in vitro mechanical property tests; fine tuning the nanofibrous conduit with extracellular matrix from cell sources; evaluating cell growth and performance on the conduit; and evaluating the performance of the conduit with and without arterial wall type cells through studies in a bioreactor. Completing these results should provide us with data to demonstrate the conduit facilitates cell growth, the cells function on the conduit, and the conduit is not subject to thrombosis and can service as a substitute for autologous veins in coronary artery bypass surgeries. These studies should demonstrate feasibility for future studies in an animal coronary artery bypass graft model.

Annarita Di Lorenzo, Ph.D. - 2014 Awardee
Weill Medical College of Cornell University

“Endothelial-derived Sphingolipids in Coronary Atherosclerosis”

The overall goal of this proposal is to explore novel ways of targeting endothelial cells to impact vascular inflammation in the pathogenesis of coronary artery disease (CAD) and heart failure. Despite multiple evidences linking the alteration of sphingolipid signaling to vascular inflammation, critical event in the development of cardiovascular diseases, specific molecular mechanisms are poorly understood.

The project focuses on our recent discovery that endothelial sphingolipid synthesis, and in particular sphingosine-1 phosphate (S1P) is negatively regulated by Nogo-B, a membrane protein of the endoplasmic reticulum. Our findings evidence a critical role of local endothelial sphingolipid production to regulate endothelial barrier functions in myocardial inflammation and reveal a novel regulation of endothelial sphingolipid synthesis by Nogo-B.

Thus, we hypothesize that Nogo-B governs the production of local sphingolipids to impact vascular inflammation and the development of CAD during pressure overload and hypercholesterolemia. As corollary to this hypothesis, we predict that modulating local sphingolipid synthesis to enhance homeostatic S1P signaling will protect the heart from CAD and failure.

To test this hypothesis we are proposing the following three specific aims:

- 1) To examine the role of endothelial specific Nogo-B in the pathogenesis of CAD by conditional deletion of Nogo-B in endothelial cells on Apo-E^{-/-} background.
- 2) To correlate the expression and localization of Nogo-B with vascular inflammation in coronary plaque formation at different time point post-transverse aortic constriction (TAC).

Collectively, these studies will define the role of endothelial-derived sphingolipids, particularly S1P, in the pathogenesis of CAD and heart failure. Finally, the results of these proposed investigations may provide the foundation for novel approaches towards the treatment of CAD, in which vascular dysfunction and inflammation leads to or exacerbates this pathological state.

Timothy Fitzgibbons, M.D., Ph.D. - 2014 Awardee

University of Massachusetts Medical School

“Rationale and Discovery of A New Class of Anti-Atherosclerotic Compounds; Ligands for the Orphan Nuclear Receptor Nurr77/NR4A1”

Coronary artery disease (CAD) has surpassed infectious disease as the leading cause of death worldwide and it is imperative that we discover new cures. For at least 30 years CAD has been recognized as an inflammatory disorder. Arterial cholesterol deposition stimulates foam cell formation, growth of atherosclerotic plaque, and ultimately plaque rupture. Although traditionally considered detrimental, specific components of immunity are beneficial. For example, a recently described "intermediate class" of monocytes, are anti-inflammatory and protect the endothelium. We have found increased expression of genes required for the development of intermediate monocytes in the peri-coronary fat of patients without CAD. In mouse models, deletion of one of these genes (orphan nuclear hormone receptor, Nr4a1), results in progression of CAD. Our central hypothesis is that expression of Nr4a1 in macrophages is protective against CAD. We propose three specific aims to study this hypothesis.

First, we will create a transgenic mouse that overexpresses Nr4a1 in macrophages, and then cross transgenic mice with Apolipoprotein E (EKO) mice to determine if Nr4a1 overexpression protects against CAD. Second, using Nr4a1^{+/+} or Nr4a1^{-/-} bone marrow transplants in EKO mice, we will test the hypothesis that the non-specific Nr4a1 ligand azathioprine prevents CAD, and whether or not this protection is dependent upon macrophage Nr4a1. Finally, in collaboration with the Broad Institute, we will perform a high-throughput screen for ligands of Nr4a1 in a human monocyte cell line (THP-1).

In summary, this is an innovative and ambitious proposal that will confirm the importance of Nr4a1 as a therapeutic target, determine the cellular hierarchy of Nr4a1 activity, and discover a heretofore un-described ligand for this receptor. Completion of this project may result in an entirely new class of medications to prevent disability and death from CAD by promoting activity of vasculo-protective monocytes.

“Nanoparticle Formulations of Cannabinoids to Treat Coronary Artery Disease”

Cardiovascular disease is the leading cause of morbidity and mortality in developed nations with an enormous societal and economic burden. Since the development of novel treatment strategies for atherosclerosis has stagnated in recent years there exists an urgent need to explore new and potent therapeutic approaches.

A major driving force in the progression to vulnerable plaques is a macrophage-driven maladaptive inflammatory response characterized by a defect in the resolution of inflammation (RI) phase. Cannabinoids are a class of hydrophobic compounds that can activate either the cannabinoid 1 (CB1) or CB2 receptor, or both. The main and most thoroughly investigated cannabinoid is tetrahydrocannabinol (THC). THC has been shown to exhibit interesting anti-inflammatory actions, while similar anti-inflammatory properties have been found for cannabidiol (CBD). Despite their enormous potential as an anti-inflammatory treatment their exploitation is hampered by poor bioavailability, water insolubility and, most notably, their psychotropic properties. Importantly, the latter and the widespread use of cannabinoids for (illegal) recreational use causes their medical application to be approached with great skepticism. Therefore, developing formulations with known and titratable amounts of cannabinoids that can improve bioavailability and reduce psychoactive effects may have a significant impact on their therapeutic and clinical potential as well as their acceptance as therapeutics.

The exploitation of nanotherapies in cardiovascular disease has been largely unexplored, but may have unprecedented benefits as they have the potential to help increase the efficacy of drugs with significantly reduced adverse side effects.

The research proposed in the application is aimed at improving the anti-inflammatory properties and specificity of cannabinoids for atherosclerotic plaques. We propose 1) a new therapeutic (and potential clinical) paradigm by developing nanoparticle formulations of cannabinoids and 2) applying and evaluating these in preclinical atherosclerosis. This application is highly innovative and is designed such that it facilitates clinical translation.

Margaret Doyle, Ph.D. - 2010 Awardee
University of Vermont

“The Role of Adaptive Immunity in Atherosclerosis and Coronary Disease”

Atherosclerosis directly contributes to coronary heart disease, heart failure, peripheral arterial disease, stroke and dementia. The adaptive immune response is a critical part, with Th1 and Th17 cells accelerating this process, and Th2 and Treg cells playing moderating roles. Many details are currently unknown about the adaptive response to atherogenic lipids, especially the degree of genetic control and the roles of the environment and health-related behaviors, indicating that epidemiological approaches are critically needed. Current methods for studying these cells are expensive and difficult to use, severely limiting progress. We propose to develop and apply improved tools for estimating Th bias, and Th17 and Treg cell responses in more readily available BioSample Repository samples. In Aim 1 we will use existing biomarkers to develop a panel that accurately estimate these adaptive response parameters. To optimize the number of candidate biomarkers we will determine the full set of cytokines expressed in cell culture supernatants from cells stimulated to yield optimal T Helper cell expression. And we will biochemically characterize several important panel candidates which are soluble forms of normally non-soluble transmembranous proteins. In Aim 2 we will determine if cryo-preserved cells are useful for flow cytometric analysis potentially increasing the number of subjects with cell-based measurements that might prove useful for correlation studies of our biomarker panels. In Aim 3 we will begin to utilize this biomarker panel of Th bias, and Th17 and Treg responses in epidemiological populations; specific applications will include determining age-, sex-, and ethnicity-related differences, determining the degree and nature of genetic control, and determining whether this panel improves CVD risk prediction beyond that observed with traditional CVD risk factors. This work will have a major impact by improving CVD risk prediction beyond that available from traditional CVD risk factors, and helping move towards immune modulation therapy for atherosclerosis.

Bernhard Kuhn, M.D. - 2010 Awardee
Boston Children's Hospital

“A Novel Strategy to Regenerate Heart Muscle after Myocardial Infarction and Ischemic Cardiomyopathy”

Myocardial infarction and ischemic cardiomyopathy are severe forms of coronary artery disease (CAD), leading via myocardial injury to heart failure and premature death. Injured human heart muscle regenerates very little: in fact, the current dogma is that human heart muscle cells (cardiomyocytes) cannot regenerate. Our finding of extracellular factors that stimulate cardiomyocyte regeneration presents a fundamental challenge to this belief. We have identified and characterized peptides of periostin (PN) and neuregulin1 (NRG1), and documented that they stimulate successful cardiomyocyte proliferation. Preliminary results indicate that PN is effective in rats and in pigs with myocardial infarction. Others have shown that NRG1 is safe and effective for treating patients with cardiomyopathy. These results raise the exciting possibility that these factors might also be effective regenerative factors in humans.

I propose translational research aimed at advancing a potentially curative strategy for treating heart failure. By stimulating controlled heart muscle regeneration, this strategy has the potential to prevent the development of heart failure, and greatly improve the quality of life and survival of patients with CAD.

My central hypothesis is that patients with CAD have altered cardiomyocyte proliferation and that specific molecular interventions stimulate cardiomyocyte proliferation. We take advantage of a unique international collaboration between my team and that of Cris dos Remedios, University of Sydney (Australia). We will contribute our established methodology for inducing cardiomyocyte regeneration, and our expertise in quantifying the proliferation of these cells. Dr. dos Remedios' team will contribute a large repository of human myocardial samples. Aim 1 is to quantify the activity of cardiomyocyte proliferation in CAD patients, relative to age-matched controls. Aim 2 is to determine the extent to which cardiomyocytes from CAD patients can be stimulated to proliferate with PN and NRG1. Collectively, our results should provide crucial pre-clinical data to support future clinical trials in humans.