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Project Summary:

A major unmet need in cardiac transplantation is a non-invasive biomarker that can replace the endomyocardial biopsy and coronary angiogram to diagnose allograft rejection and cardiac allograft vasculopathy (CAV), respectively. We propose to build upon previous work suggesting that blood-based microRNAs (miRs) may serve this purpose. Within the scope of this proposal, we employ a global unbiased approach to annotate the miR transcriptome using next-generation sequencing (NGS). We will sequence over 2,200 miRs annotated in the human miR transcriptome to identify miRs that underlie the pathologic processes of allograft rejection and CAV.

We will leverage the clinical trial infrastructure and biorepository of the existing Genomic Research Alliance for Transplantation (GRAfT). This study, established in 2014, recruits cardiac transplant recipients from five mid-Atlantic transplant centers for the purposes of investigating a different genomic biomarker, donor-derived, cell-free DNA (dd-cfDNA). Using a case-control study format, we will select patients with and without rejection or CAV. The plasma miR transcriptome will be sequenced in approximately 250 subjects and the sequence data will be analyzed with machine learning techniques. The miR panel that distinguishes allograft rejection and CAV will be validated in a separate cohort using reverse transcriptase polymerase chain reaction (RT-PCR). As dd-cfDNA measurements are made as part of the GRAfT study, we will compare the biomarker characteristics of dd-cfDNA and our newly developed miR panel. Importantly, dd-cfDNA has no ability to distinguish the two major subtypes of rejection, T-cell and antibody-mediated; early data suggests miRs can accomplish this goal.

Our work will identify novel miRs that are implicated allograft rejection and CAV. The biological mechanisms by which these miRs regulate RNA expression, and which gene targets are modulated will be explored using bioinformatic tools. These miRs can then be studied further through functional analyses and may be targeted as part of a drug development program to prevent or treat allograft rejection and CAV. We expect to identify a panel of miRs that will be the basis for a phase III, clinical validation study. Our long-term goal is to develop a miR biomarker panel that entirely replaces the endomyocardial biopsy and coronary angiogram as a diagnostic tool to accurately detect allograft rejection and CAV.

Specific aims:

The endomyocardial biopsy remains the clinical gold standard to screen for allograft rejection in cardiac transplant recipients. This procedure is invasive, costly, associated with morbidity and has variability in histopathologic interpretation. **There are currently no reliable blood-based biomarkers that accurately detect allograft rejection** and distinguish between the two subtypes: T cell-mediated rejection (TCMR) and antibody-mediated rejection (AMR). These rejection subtypes have distinct clinical management implications and long-term outcomes. Although the risk of rejection is highest in the first-year post-transplant, there is a subsequent growing risk of other post-transplant co-morbidities that limit post-transplant survival, including: malignancy, cardiac allograft vasculopathy (CAV) and chronic kidneydisease.

An emerging class of circulating genomic biomarkers for detecting allograft rejection is plasma microRNAs (miRs). These small, highly-conserved, 22 nucleotide-long, non-coding RNAs target messenger RNA (mRNA) to inhibit protein translation. MiRs are highly stable in plasma even at extremes of temperature making them ideal circulating biomarkers. **Given their ability to regulate gene expression, miRs are implicated in disease pathogenesis and could serve as therapeutic targets to prevent or treat rejection and CAV.** The research proposed in this grant application involves an ongoing collaboration with the Genomic Research Alliance for Transplantation (GRAfT). The GRAfT research consortium, formed in 2014, investigates donor-derived, cell-free DNA (dd-cfDNA) as a marker of graft rejection. GRAfT will be the resource for subjects to be studied as part of this proposed research. Although dd-cfDNA is an excellent biomarker of total graft necrosis, it has no ability to distinguish the rejection subtypes of TCMR or AMR. Further, dd-cfDNA does not appear to mediate the rejection phenotype. Previous work has shown that specific miR subsets are markers of the distinct processes of TCMR and AMR. By identifying specific miRs involved in rejection and in CAV, we will identify potential targets for the future development of antagomir or short interfering RNAs (siRNA) therapeutics.

We propose a global unbiased analysis of the human plasma miR transcriptome in patients after cardiac transplant using next-generation sequencing (NGS) to discern miR expression profiles that typify allograft rejection and distinguish the major subtypes of rejection. We hypothesize that miRs are involved in the key molecular pathways that underlie TCMR, AMR and CAV and can serve as a genomic biomarker of these conditions.

Our specific aims are:

Aim 1: Using NGS, determine the miR transcriptome of cardiac transplant recipients and distinguish miR biomarkers of cardiac allograft rejection.

Hypothesis: Specific plasma miRs will be differentially expressed in patients with allograft rejection.

Aim 2: Contrast the ability of two genomic biomarkers to accurately diagnose TCMR and AMR.

Hypothesis: A set of miRs has superior ability to diagnose allograft rejection and can distinguish the subtypes of rejection when compared to dd-cfDNA.

Aim 3: Identify miRs implicated in the pathophysiological underpinnings of CAV.

Hypothesis: Specific miRs are involved in the development of CAV and can serve as non-invasive biomarkers of CAV.

Following completion of these aims, we expect to have a panel of miRs that will be the basis for functional analyses and clinical validation studies. Our long-term goal is to develop a miR-based in vitro diagnostic multivariate index assay (IVDMIA) that replaces the endomyocardial biopsy and coronary angiogram as a diagnostic tool to accurately detect allograft rejection and CAV.

Lay Summary:

1. What is the major problem being addressed by this study?

In cardiac transplantation, there are no reliable, blood-based markers to accurately detect heart transplant rejection or coronary disease. After transplant, patients are subjected to repetitive invasive procedures (~10-17) to screen for rejection. These procedures are associated with patient discomfort, significant healthcare cost, and carry a risk of complications. Also, the tissue obtained at the time of the biopsy is often interpreted incorrectly. Coronary disease of the transplanted heart is often silent clinically until it is too advanced to be treated effectively; this accounts for 30% of the mortality after transplant. The angiogram is not sensitive enough to detect disease until it is too advanced. A non-invasive marker of earlier coronary disease is urgently needed.

2. What specific questions are you asking and how will you attempt to answer them?

We propose developing a blood-based microRNA panel to replace two common invasive procedures employed after heart transplant to detect rejection and coronary disease: the endomyocardial biopsy and coronary angiogram. We will use next-generation sequencing technologies to identify specific microRNAs that can be detected in the blood and accurately diagnose rejection and coronary disease. We will accomplish this by evaluating blood-based microRNAs in 500 heart transplant patients from an existing study. Using statistical tools, we will identify key microRNAs that can be used in a blood-based panel to screen patients after heart transplant for rejection and coronary disease. This panel of microRNAs could replace the biopsy and angiogram to manage patients after heart transplant.

3. Overall, what is the potential impact of this work to the mission of the AHA?

Our work will lead to the development of a diagnostic test that can screen the blood of patients after heart transplant for rejection and coronary disease. This will significantly reduce patient morbidity and healthcare costs, as it will eliminate the need for an invasive endomyocardial biopsy or heart catheterization. Additionally, because microRNAs are not only markers of disease but can mediate disease as well, this proposal will identify microRNAs that might cause heart transplant rejection and coronary disease. These microRNAs can then be targeted in the future to develop new drugs to prevent or treat rejection and coronary disease. Additionally, these microRNAs may be implicated in rejection of other transplanted organs (e.g. kidneys, lung).