Diabetic Complications Consortium

Application Title: MicroRNA Regulation in the Type 2 Diabetic Human Heart

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1. Project Accomplishments:

Diabetic cardiomyopathy is an impairment of heart muscle, characterized by metabolic disturbance and contractile dysfunction. The mitochondrion is central in the development of diabetic cardiomyopathy however, examination of mitochondria is complicated by the fact that two subpopulations are present in the cardiac myocyte, interfibrillar mitochondria (IFM) which situate between the contractile apparatus, and subsarcolemmal mitochondria (SSM), which exist beneath the plasma membrane. MicroRNAs (miRNA) are non-coding RNAs that regulate protein expression of target mRNAs. Data from our laboratory indicates that the mitochondrion possesses a pool of miRNAs that translocate into and out of the organelle in a dynamic fashion in the type 2 diabetic mouse (*db/db*) heart. Nevertheless, it is unclear whether similar phenomena exist in type 2 diabetic human heart. The *objectives of this application* were: (1) to assess the impact of type 2 diabetes mellitus on cardiac transcriptome profiles in atrial appendage tissue from diagnosed type 2 diabetic patients and non-diabetic patients; and (2) to assess the impact of type 2 diabetes mellitus on cardiac mitochondrial subpopulation proteomes in atrial appendage tissue from diagnosed type 2 diabetic patients and non-diabetic patients. Our results revealed distinct patterns of mitochondrial miRNAs that we detected in a functional context with mitochondrial genome encoded mRNAs. Further, preliminary evaluation of mitochondrial subpopulation proteomic signatures was assessed. From a broad perspective, the findings provided confirmation of the mitochondrial miRNA translocation phenomenon which in some cases showed similar patterns to the *db/db* mouse. These data will provide translationally-driven specific aims that will complement mechanistic specific aims for future grant applications.

2. Specific Aims:

Specific Aim 1: Assess the impact of type 2 diabetes mellitus on cardiac transcriptome profiles in atrial appendage tissue from type 2 diabetic patients and non-diabetic patients.

Results: We tested our *working hypothesis* that changes in the mitochondrially-encoded and nuclear-encoded transcriptome and riscome contributing to mitochondrial proteomic make-up is negatively impacted by type 2 diabetic insult and that these effects would have greatest impact on the SSM. We utilized a unique *approach* via next generation sequencing, in which we assessed heart and mitochondrial subpopulation transcriptomic (mRNA) and riscomic (miRNAs present in the RNA-induced silencing complex) profiles, as well as protein partners that are part of the RISC complex in human atrial appendage tissue from type 2 diabetic and non-diabetic patients. Transcriptomic and riscomic changes were observed in both mitochondrial subpopulations of type 2 diabetic patients relative to controls (**Figures 1A and 1B**).

Figure 1

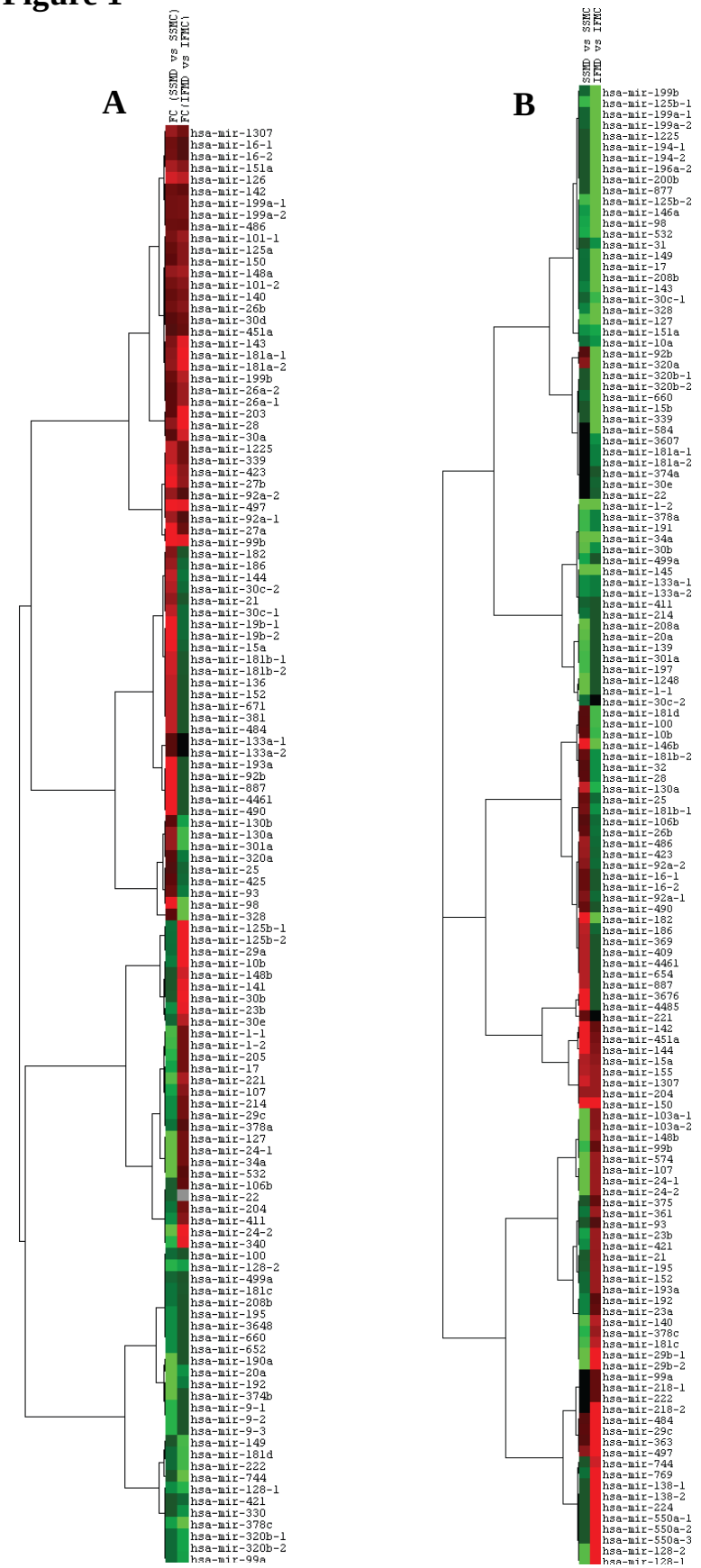

Figure 1. Mitochondrial MiRNA Response in Type 2 Diabetic Patient Atrial Appendage Tissue. (A) MiRNA heat map derived from next generation sequencing of small RNAs identifying enrichment and depletion patterns within the mitochondrial transcriptome of diabetic SSM and diabetic IFM relative to respective non-diabetic controls. **(B)** MiRNA heat map derived from next generation sequencing of small RNAs identifying enrichment and depletion patterns within the mitochondrial RISCome of diabetic SSM and diabetic IFM relative to respective non-diabetic controls.

Currently, we are focused on repeating the experiment to increase the sample size and due to the greater variability in the manifestation of diabetes mellitus in the patient population. We are also applying prediction analysis algorithms developed in our laboratory to determine potential predicted mitochondrial mRNA binding partners for the mitochondrial miRNAs identified in the mitoRISCome analyses (**Figure 1B**). Finally, we are assessing potential candidate mitochondrial miRNAs that showed changes in both type 2 diabetic patient and *db/db* mouse models to determine predictive targets in which molecular manipulation approaches can be applied. These preliminary data are the basis of upcoming grant submissions focusing on the regulation of the mitochondrial genome through miRNA interaction.

Specific Aim 2: Assess the impact of type 2 diabetes mellitus on cardiac mitochondrial subpopulation proteomes in atrial appendage tissue from type 2 diabetic patients and non-diabetic patients.

Results: We tested our *working hypothesis* that changes in mitochondrial proteomic make-up is greatest in type 2 diabetic patients as compared to non-diabetic patients, and that these effects are most pronounced in the SSM. We utilized an iTRAQ proteomic *approach* to assess protein abundances in cardiac mitochondrial subpopulation proteomes isolated from human atrial appendage tissue of type 2 diabetic and non-diabetic patients. As with Specific Aim 1, the results revealed distinct patterns of mitochondrial proteomic changes that were specific for a given mitochondrial subpopulation. Unfortunately, the data revealed a significant portion of cytosolic protein contamination that we did not expect based upon our previous studies. Nevertheless, similar patterns of protein loss were observed as compared to our *db/db* studies, with a large portion of proteomic loss occurring the SSM of the type 2 diabetic patient atrial appendage tissue. Because the data were extensive, we have only included a table that highlights a subset of proteomic changes observed in the type 2 diabetic patient atrial appendage tissue (**Table 1**). As **Table 1** indicates, both subpopulations displayed alterations in the mitochondrial proteomes following type 2 diabetes with SSM showing the greatest levels of protein loss.

Table 1

Protein Name	SSM Dia/Con	IFM Dia/Con
Succinyl-CoA ligase [GDP-forming] subunit beta	0.69	NS
Isocitrate dehydrogenase [NAD] subunit alpha	0.81	NS
Pyruvate dehydrogenase E1 component subunit beta	0.73	1.12
ATP synthase subunit e	0.77	1.40
Acetyl-CoA acetyltransferase	0.83	NS
Coiled-coil-helix-coiled-coil-helix domain-containing protein 3	0.78	NS
NipSnap homolog 2	NS	1.38
Lipoamide acyltransferase alpha-keto acid dehydrogenase	0.73	NS

Table 1. Proteomic Analyses of Mitochondrial Subpopulations in Human Type 2 Diabetic Heart. A limited set of iTRAQ data in SSM and IFM of type 2 diabetic (Dia) and non-diabetic (Con) patients. Not significant (NS). Red boxes = significant decrease, green boxes = significant increase.

As with Specific Aim 1, we are repeating these experiments to increase the sample size and due to the greater variability in the manifestation of diabetes mellitus in the patient population. Also, we are adjusting tissue homogenization approaches and our mitochondrial isolation techniques to limit cytosolic protein contamination. Nevertheless, these preliminary data will be included in upcoming grant submissions focusing on the regulation of the mitochondrial proteome through manipulation of the mitochondrial protein import system.

3. Publications:

No current manuscripts have resulted from the funding though we anticipate that future manuscripts will reference the grant.