Mitochondrial miRNAs in Diabetes: Just the Tip of the Iceberg

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<td>Baradan, Rohini; Johns Hopkins University; B.S. Abdur Rahman University, School of Life Sciences Hollander, John M.; West Virginia University School of Medicine, Human Performance - Exercise Physiology (SOM) Das, Samarjit; Johns Hopkins University, Pathology</td>
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Mitochondrial miRNAs in Diabetes: Just the Tip of the Iceberg

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ABSTRACT

Over the last two decades mi(cro)RNAs have emerged as one of the key regulators of metabolic homeostasis. Most of the studies have highlighted that in the cytoplasm, miRNAs directly bind to the 3’-UTR (untranslated region) of a mRNA. Conventional RISC (RNA Inducing Silencing Complex) formation results in the post-transcriptional inhibition. This process is known to contribute to the development of metabolic diseases, including diabetes mellitus. Recent advancements with small RNA detection technologies have enabled us to identify miRNAs in the mitochondrial compartment of the cells. We have termed these miRNAs, which translocate into the mitochondria as mitochondrial miRNA, MitomiR. It has been demonstrated that MitomiRs can regulate gene expression, with some evidence even suggesting that after translocation, MitomiRs can bind to the 3’-end of a mitochondrial gene, altering its regulation. Our main focus in this review is to highlight the potential role of MitomiR in the pathogenesis of metabolic disorders such as diabetes mellitus.

Keywords: Mitochondria, Mitochondrial microRNA, MitomiR, Diabetes, Metabolism
INTRODUCTION

Diabetes mellitus, a metabolic disorder, is a condition in which either the insulin secretion of pancreatic β islet cells is impaired (Type-1), there is a defect in insulin action (Type 2), or a combination of the two (Kharroubi and Darwish 2015). Type 2 diabetes (T2D) is one of the most common causes of morbidity and disability (Villard et al. 2015), and it is influenced by environmental and genetic factors (Villard et al. 2015). T2D leads to vascular complications such as neuropathy, nephropathy, retinopathy [microvascular]; ischaemic heart disease and peripheral vasculopathy [macrovascular] (Villard et al. 2015). The number of affected patients with T2D complications is increasing at an alarming rate. Based on a recent American Diabetic Association’s report, it was estimated that the economic burden diabetes poses is about $132 million in the United States alone (Cade 2008). It is estimated that the number of affected individuals will reach 592 million by the year 2035, with a global prevalence of 10.1% (Kharroubi and Darwish 2015).

A continuous relation exists between chronic hyperglycemia and the incidence and progression of vascular complications. These vascular complications lead to tissue and organ damage in approximately one-third to one-half of diabetic patients, and are a major cause of morbidity and mortality (Cade 2008). Some of the known molecular mechanisms that are involved in the etiology of micro- and macro-vascular complications include, advanced glycation end products (AGE), aberrant activation of protein kinase C (PKC), increased reactive oxygen species (ROS) production, and abnormal stimulation of hemodynamic regulation systems (Cade 2008; Fong et al. 2004). According to a recent report, adults with diabetes have an annual mortality of about 5.4% (double the rate for non-diabetic adults), and their life expectancy is decreased on average by 5-10 years (Donnelly et al. 2000). Even though the primary cause of death due to diabetes is cardiovascular defects, deaths from non-cardiovascular causes are also increased.
One of the most common microvascular complications of T2D is neuropathy. It has been estimated that about half of people with diabetes are affected by neuropathy (Cade 2008). The pathogenesis of diabetic neuropathy includes the symptoms of peripheral nerve dysfunction where all other causes of peripheral nerve dysfunction have been ruled out (Bansal et al. 2006). The molecular mechanism involves a decrease in angiogenic and neurotropic factors such as VEGF, IGFs, NGFs and angiopoietins with the progression of T2D. This condition alters the blood supply in the nerves and impairs neural function. This leads to changes in the nerve structure, ultimately resulting in neuropathy (Tahergorabi and Khazaei 2012).

Derangement of angiogenesis has also been identified as one of the factors contributing to vascular complications. Angiogenesis is a process in which new blood vessels arise from pre-existing ones. Neovascularization is achieved by maintaining a balance between the angiogenic (VEGF, FGF2, TGF-β) and anti-angiogenic factors (angiostatin, endostatin and thrombospondins) (Tahergorabi and Khazaei 2012). Angiogenesis in the eye due to diabetic retinopathy elevates reactive oxygen species (ROS) production, which directly or indirectly activates HIF-1α (hypoxia inducible factor-1α) (Tahergorabi and Khazaei 2012). ROS production coupled with activated HIF-1α signalling stimulates VEGF expression (Tahergorabi and Khazaei 2012). Diabetic retinopathy affects the peripheral retina, macula or both, eventually leading to blindness (Cade 2008). Diabetic retinopathy is one of the prime consequences of angiogenesis. Harris et al., (Harris 1993) has demonstrated that onset of diabetic retinopathy can begin seven years before a patient is even diagnosed with T2D. Each year, greater than 10,000 diabetic patients in the US develop blindness due to diabetic retinopathy, despite many medical advancements (Fong et al. 2004; Moore et al. 2009).

Diabetic nephropathy is characterized by the presence of albumin in urine (albuminuria), which is indicative of renal dysfunction (Cade 2008). Nephropathy leads to
end stage renal disease that requires dialysis and ultimately, a kidney transplant (Moore et al. 2009). “Proteinuria”, also known as albuminuria or urine albumin, is a pathological condition where urine contains an abnormal amount of protein, mainly albumin. Proteinuria is a sign of chronic kidney disease (CKD), which can arise from chronic hyperglycemia. Approximately 15-40% patients with T1D, and 5-20% patients with T2D are affected by proteinuria (Chawla et al. 2016). It has been estimated that a quarter of patients with T2D develop microalbuminuria within 10 years of the diagnosis of diabetes (Adler et al. 2003). Unlike diabetic neuropathy, elevated angiogenesis is observed in diabetic nephropathy. It has been shown that the factors leading to enhanced angiogenesis are overexpression of VEGF, tumor growth factors (TGFs) and glomerular hypertension (Tahergorabi and Khazaei 2012).

The macrovascular complications like coronary artery disease, stroke and peripheral vascular disease contribute greatly to morbidity and mortality associated with diabetes (Moore et al. 2009). In 2013, cardiovascular disease due to diabetes accounted for 30.8% of deaths in the United States (Writing Group et al. 2016). The risk of developing stroke increases 2 to 5 fold in diabetic patients (Cade 2008). About 14% of peripheral artery disease prevalence is due to diabetes (Criqui and Aboyans 2015). In addition, several myocardial abnormalities such as cardiomyopathy, myocardial hypertrophy, fibrosis, and myofibril defects, result from hyperglycemia (Dhalla et al. 2014; Joshi et al. 2014). One of the major T2D-induced complications is diabetic cardiomyopathy. Diabetic cardiomyopathy, a major cause of mortality in T2D patients, may be defined as alterations in the structure and function of the left ventricular myocardium that is not directly attributable to coronary artery disease or hypertension (Boudina and Abel 2010). It has been described that there are several molecular factors contributing to the development of diabetic cardiomyopathy, which include hyperglycemia, lipotoxicity, ROS, mitochondrial dysfunction, impaired calcium metabolism,
renin angiotensin system (RAS) activation, altered substrate metabolism, and endothelial dysfunction (Joshi et al. 2014).

ROLE OF MITOCHONDRIA IN DIABETIC CARDIOMYOPATHY

Mitochondria, a cellular organelle, are the major site for energy production. Multiple energy pathways, such as the electron transport chain (ETC) which occurs in the intermembrane space of the mitochondria, and the Tricarboxylic acid cycle (TCA cycle) which occurs in the mitochondrial matrix, generate ATP as a source of energy (Steenbergen et al. 2009). In addition, parts of amino acid and nucleic acid metabolism also occur inside the mitochondria (Duncan 2011). It has been reported that T2D can affect mitochondrial function in various ways. Fatty acid and β-oxidation are the major sources of energy in the heart. The substrate choice (fatty acid or glucose) of a normal heart is usually dynamic and balanced, with approximately 70% of the energy obtained from fatty acid oxidation (FAO). Mitochondrial function in T2D-hearts has been altered due to changes in the rate of both FAO and glucose oxidation (GO) (Duncan 2011). Under diabetic conditions, FAO is the only source of energy for the heart. T2D impairs the ability of the heart to switch its substrate from glucose to fatty acid, which results in mitochondrial dysfunction (Duncan 2011). With T2D, targeting heart-mitochondria may lead to alterations in the rate of GO over FAO, which may ultimately lead to a therapeutic intervention in diabetic cardiomyopathy.

It has been demonstrated that hyperglycemic conditions induce cardiac mitochondrial swelling, reducing the number of cardiac-mitochondria (Joshi et al. 2014). Impaired cardiac insulin signalling leads to mitochondrial dysfunction (Boudina et al. 2009; Dhallal et al. 2014). Additionally, oxidative stress plays an important role in cardiac insulin signalling dysfunction during T2D stress (Boudina et al. 2009). Apart from hyperglycemia, an increase in free radical production can stimulate glucose oxidation, protein glycation, and subsequent
degradation of glycated proteins, which are involved in the pathogenesis of diabetic complications (Gillery et al. 1988; Hunt et al. 1990; Wolff and Dean 1987). Oxidative stress is one of the key regulators of sub-cellular abnormalities, including sarcoplasmic reticular and sarcolemmal functions (Dhalla et al. 1998). The excessive utilization of long-chain fatty acids for prolonged periods during the hyperglycemic condition results in the excessive production of free radicals. Thus, altered mitochondrial function can influence other sub-cellular organelles such as the sarcolemma, sarcoplasmic reticulum, and myofibrils in the progression of cardiac dysfunction.

In addition to oxidative stress, alterations in mitochondrial function with diabetic complications can lead to Ca$^{2+}$-handling abnormalities. Chronic diabetes has been associated with abnormalities in the sarcoplasmic reticular and sarcolemmal Ca$^{2+}$-transport processes (Dhalla et al. 1998; Dhalla et al. 2014; Machackova et al. 2005). Cardiac dysfunction in diabetes is associated with poor myofibrillar function due to inferior sarcoplasmic reticular and sarcolemmal Ca$^{2+}$-handling (Dhalla et al. 1998; Dhalla et al. 2014; Machackova et al. 2005). Long-term diabetes can lead to myofibrillar remodelling by altering the contractile and regulatory proteins in myofibrillar assembly. This alteration can be adaptive or maladaptive, based on the functional and metabolic demands of the heart (Machackova et al. 2005). On the basis of these observations, it is clear that mitochondria play an important role in the pathogenesis of cardiac dysfunction during the development of diabetic cardiomyopathy.

Recently, it has been described that miRNAs can translocate into the mitochondrial compartment of cardiomyocytes, and play an important role in mitochondrial energy production by targeting mitochondrial genes (mito-mRNAs) or nuclear genes (mRNAs) in the mitochondrial matrix (Das et al. 2014; Das et al. 2012; Jagannathan et al. 2015; Srinivasan and Das 2015).
MITOCHONDRIAL miRNAs IN DIABETIC CARDIOMYOPATHY

miRNAs are a group of small, 19-25 nucleotides long, non-coding RNAs (Bartel 2004). These evolutionally conserved miRNAs have the ability to negatively regulate the expression of various genes by the degradation of target mRNA or by inhibiting the translational process (Bartel 2004). The biogenesis of a miRNA includes formation of a primary-miRNA transcript (pri-miRNA) from the nuclear genome. The pri-miRNA then gets digested by a RNA polymerase II, DROSHA/DGCR8, into a premature miRNA transcript (pre-miRNA), which is then translocated to the cytoplasm by exportin 5. Pre-miRNA in the cytoplasm then gets cleaved by another RNase III enzyme, DICER, into double-stranded mature miRNA (Srinivasan and Das 2015). In the cytoplasm, Ago2, an RNA binding protein, first binds to miRNA. This Ago2 bound miRNA transcript then finds the miRNA target mRNA (Kawamata and Tomari 2010). The Ago2 bound miRNA forms a ribonucleoprotein complex, the RNA induced silencing complex (RISC), by binding to the 3'-untranslated region (3'-UTR) of the mRNA. The RISC also includes other RNA binding proteins such as GW182, TRBP1, and TRBP2 (Srinivasan and Das 2015). The involvement of Dicer as one of the RISC components is still controversial (Gregory et al. 2005; Kawamata and Tomari 2010; Kim and Kim 2012; MacRae et al. 2008; Rivas et al. 2005). Usually, the formation of the RISC complex occurs in the cytoplasmic compartment of a cell; however, recent studies have shown that miRNAs are also found in mitochondrial (Barrey et al. 2011; Das et al. 2014; Das et al. 2012; Dasgupta et al. 2015; Jagannathan et al. 2015; Srinivasan and Das 2015; Zhang et al. 2014), nuclear (Hwang et al. 2007), and endoplasmic reticulum (ER) (Li et al. 2013; Montgomery and Ruvkun 2013) components of the cell.

Ago2 has been found to act as a carrier protein, transporting a miRNA such as miR-1 (Zhang et al. 2014) or miR-181c (Das et al. 2012), into the mitochondria after the miRNAs mature in the cytoplasm. In addition, Barrey et al. have suggested that both miRNAs and pre-
miRNAs are encoded within the mitochondrial genome (Barrey et al. 2011). However, the biogenesis of these mitochondrial genomic miRNAs is not known. Nevertheless, the functional aspects of these mitochondrial miRNAs are not well studied. Thus, in this article, we focus on the miRNAs which have been found in the mitochondrial compartment, and target either mitochondrial or nuclear genes, leading to an alteration of mitochondrial function during T2D. We term this subset of miRNAs as MitomiRs. MitomiRs can influence various metabolic pathways such as the TCA, electron transport chain, lipid metabolism, and amino acid metabolism. These mitochondrial metabolic pathways are actively involved in energy metabolism during T2D.

**miRNA INFLUENCES THE TRICARBOXYLIC ACID CYCLE**

The TCA cycle is a key metabolic pathway involved in glucose oxidation. It is a series of biochemical reactions that oxidizes acetyl coA to release energy in the form of ATP. Glucose oxidation occurs in multiple steps, starting from glycolysis in the cytoplasm followed by pyruvate decarboxylation to acetyl coA (An and Rodrigues 2006). Acetyl coA, then enters into the TCA cycle. Since the TCA cycle is an indispensable process for GO, any alterations in the TCA cycle may lead to altered energy metabolism in the heart. Recently, miRNAs have been found to regulate the TCA cycle by targeting key enzymes in the pathway.

Pyruvate dehydrogenase (PDH) is an enzyme which links glycolysis to the TCA cycle by converting pyruvate into acetyl Co-A. It has been shown that miR-26a targets PDH subunit X (PDHX), a non-catalytic component of PDH (Chen et al. 2014). By targeting PDHX, miR-26a reduces PDH enzyme activity, leading to accumulation of pyruvate and decreased levels of acetyl-coA in the mitochondria (Chen et al. 2014).
Several miRNAs, such as miR-152, miR-148a, miR-148b, miR-299-5p, miR-19a-3p, miR-19b-3p, miR-122a, miR-421, and miR-494, target citrate synthase. By modulating the expression of citrate synthase, these miRNAs affect roughly 78 pathways that are involved in lipid, nucleotide, carbon, and amino acid metabolism (Tibiche et al. 2008).

Succinate co-A ligase GDP forming beta Subunit (SUCLG2) catalyses the conversion of succinate to succinyl co-A. In a study, miR-124 was found to downregulate the expression of SUCLG2 (Wang and Wang 2006).

**miRNAs INFLUENCE THE ELECTRON TRANSPORT CHAIN**

The electron transport chain (ETC) is a redox pathway carried out by ETC complexes; I, II, III, IV and V (ATP synthase), which is located in the inner membrane of the mitochondria. The ETC complexes I, III and IV generate a proton gradient by oxidizing NADH/NADPH. This proton gradient is then utilized by ATP synthase to produce ATP. Oxidative stress has been implicated in the etiology of diabetic cardiomyopathy. Mitochondrial ETC complexes I and III generate ROS as a by-product of their respiratory function. Generation of ROS has been found to have deleterious effects on mitochondria and eventually leads to mitochondrial dysfunction (Das et al. 2014; Das et al. 2012).

Iron-sulfur clusters (Fe-S) are essential cofactors for the transfer of electrons in oxidative phosphorylation (OXPHOS) (Tong and Rouault 2000). Thus, the function of Complex I and Complex IV is highly dependent on (Fe-S). The Fe-S assembly enzyme (ISCU) plays an important role in the synthesis of these Fe-S clusters (Tong and Rouault 2000). Under hypoxic conditions, it has been demonstrated that miR-210-5p can directly target the ISCU (Chan et al. 2009; Chen et al. 2010). Succinate dehydrogenase subunit D (SDHD), a subunit of Complex II, has been identified as a target of miR-210 (Puisségur et al. 2011). The authors have concluded that miR-210 can ultimately alter complex II activity
(Puissegur et al. 2011). COX10, another nuclear encoded subunit of complex IV has been reported as a target of miR-210-5p (Chen et al. 2010). Nuclear encoded, cytochrome c oxidase subunit IV (COXIV), one of the complex IV subunits, plays a vital role during the assembling process of complex IV and in its respiratory function (Li et al. 2006). In vitro studies on neuronal cells showed miR-338-5p alters complex IV activity by targeting the 3′-UTR of COXIV mRNA (Aschrafi et al. 2008).

Complex IV of the ETC has three subunits which are encoded in the mitochondrial genome - mt-COX1, mt-COX2, and mt-COX3. The remaining subunits; IV, Va, Vb, VIa, VIb, VIc, VIIa, VIIb, VIIc and VIII are encoded by the nuclear genome, and are translocated into the mitochondria after their maturation. In two independent studies, it has been shown that the mitochondrial genomic subunit of complex IV, mt-COX1, can be targeted by two different miRNAs, miR-181c (Das et al. 2014; Das et al. 2012), and miR-1 (Zhang et al. 2014). Mitochondrial transcripts are polycistronic in nature, and thus, the effects of miRNA binding to the 3'-UTR of mt-mRNA are still not fully understood. On the one hand, it has been shown that by binding to a miRNA, mt-mRNA expression is downregulated (Das et al. 2014; Das et al. 2012; Jagannathan et al. 2015). Conversely, it has been shown that miRNA acts as an activator of post-translational processes when it binds to a mitochondrial encoded-mRNA (Li et al. 2016; Zhang et al. 2014). Chronic overexpression of a miRNA that targets mitochondrial mRNA confirmed this observation (Das et al. 2014; Li et al. 2016).

Zheng et al. reported that miR-101-3p negatively regulates the expression of ATP synthase subunit β (ATP5B) (Zheng et al. 2011). In another study, miR-127-5p was also shown to target the 3′-UTR of the ATP5B transcript, and alters protein content (Willers et al. 2012). Additionally, miR-338-5p has been found to target ATP5G1, which ultimately augments ATP synthase activity (Aschrafi et al. 2008). Finally, miR-378 has been shown to target and bind to the mitochondrial transcriptome at the ATP6 locus, causing down-
regulation of the protein in the type 1 diabetic heart (Jagannathan et al. 2015). ATP6 is a subunit of the F0 complex of the ATP synthase, and its repression impacts ATP generating capacity.

**EFFECT OF miRNA ON FATTY ACID METABOLISM**

In the heart, energy in the form of ATP is obtained from various sources including fatty acids, glucose, lactate and ketone bodies (Rodrigues et al. 1998). The major source of energy is obtained by oxidation of fatty acids. During diabetes, FAO in the heart is augmented, and GO is reduced. This altered state leads to contractile dysfunction that initially begins with diastolic dysfunction, eventually developing into systolic dysfunction. This ultimately leads to diabetic cardiomyopathy (An and Rodrigues 2006). In several studies, miRNAs have been shown to target key components of the FAO pathway. A peroxisomal enzyme, Carnitine Octanoyl Transferase (CROT), allows short fatty acid chains to enter into the mitochondria by coupling them with carnitine. CPT1A converts acyl-CoA to acylcarnitine, thereby allowing fatty acids to enter the mitochondria where they are oxidized (Bonnefont et al. 2004). miR-33 has been found to target both CROT and CPT1A, which eventually affects fatty acid β oxidation (Gerin et al. 2010). miR-33a-5p and miR-33b-5p is encoded from the intronic region of SREBP2 (Bommer and MacDougald 2011), and SREBP1 (Xu et al. 2013) genes, respectively. By targeting important enzymes like CROT, CPT1A, and 3-Ketoacyl-CoA thiolase, the two miRNAs, miR-33a and miR-33b, affect fatty acid metabolism (Rottiers and Naar 2012). Interestingly, the complementary strand of miR-33a-5p is miR-33a-3p, which has been found to play an important role in FAO by targeting the same targets as miR-33a: CROT and CPT1A (Goedeke et al. 2013). In another study, miR-370 has been shown to reduce FAO by targeting the 3’-UTR of CPT1A transcript (Iliopoulos et al. 2010). Peroxisome proliferator activated receptor δ (PPARδ) plays an
important role in energy metabolism by switching the metabolism from FAO to glycolysis (Burkart et al. 2007). miR-199a-5p has been found to decrease the FAO by targeting PPARδ in both the heart and liver mitochondria (el Azzouzi et al. 2013). Azzouzi et al. (el Azzouzi et al. 2013) also concluded that by targeting PPARδ, miR-199a alters mitochondrial content and increases lipid deposition in the heart and liver cells. Another miRNA, miR-29a-3p, also has been found to target PPARδ and affects FAO (Kurtz et al. 2014). It has been demonstrated that in liver miR-122 affects lipid metabolism (Esau et al. 2006). The mRNA level of aldolase-A has been found to be reduced upon transfecting miR-122 in the hepatocellular carcinoma cell line, AML2 (Esau et al. 2006). Pantothenate kinase 1 (PANK) is an enzyme which is involved in the synthesis of coenzyme A (Leonardi and Jackowski 2007). Coenzyme A is a key cofactor involved in lipid metabolism. miR-107 and miR-103, located in the intronic sequence of the PANK1α gene, can influence lipid metabolism (Wilfred et al. 2007). miR-224-5p inhibits translation of acyl-CoA synthetase long chain family (ACSL4) (Wilfred et al. 2007). Acyl Co-A ester is an intermediate complex of lipid synthesis. ACSL4 regulates the synthesis of Acyl Co-A from free long chain fatty acids (Peng et al. 2013). Peng et al. (Peng et al. 2013) demonstrated that miR-224-5p alters FAO by regulating the mRNA of ACSL4.

ROLE OF miRNA IN AMINO ACID METABOLISM

Amino acid metabolism refers to the synthesis and breakdown of amino acids, and it mainly occurs within the mitochondria. Diabetic cardiomyopathy has been shown to alter amino acid metabolic pathways. The amino acid levels within the heart not only alters the energy stores of the heart, but also influences numerous contractile proteins that are essential for proper contractile function of the heart (Avogaro et al. 2004). Recently, multiple miRNAs have been implicated in the regulation of amino acid metabolism. For example, in a recent
study, miR-193b has been found to bind to the 3’-UTR of Serine Hydroxyl transferase (SHMT2) (Leivonen et al. 2011), which is responsible for converting serine to glycine. Glutaminase (GLS) is an enzyme that converts glutamine to glutamate by deamination. In human neuronal progenitor cells (NPCs), GLS was found to play an important role in cell proliferation and cell death (Wang et al. 2014). The lower stand component of the miR-23 family, miR-23a-3p and miR-23b-3p, have been found to inhibit GLS by binding to the 3’UTR of GLS (Gao et al. 2009). In another study, miR-29b was found to target the dihydrolipoyl branched chain acyltransferase (DBT). DBT is a component of branched chain α-ketoacid dehydrogenase (BCKD). BCKD plays an important role in the catabolism of branched chain amino acids leucine, isoleucine and valine (Mersey et al. 2005).

ROLE OF miRNA IN NUCLEOTIDE METABOLISM

The synthesis and the breakdown of nucleotides is referred to as nucleotide metabolism. Some parts of nucleotide metabolism occur inside the mitochondria, and it has been shown that various miRNAs can influence this process by regulating mRNAs in multiple nucleotide metabolism pathways (Desler et al. 2010). Three miRNAs, miR-149 (Wu et al. 2013), miR-125 (Stone et al. 2011), and miR-22 (Stone et al. 2011), have been found to target the Methylentetrahydrofolate (MTHFR) transcript. By negatively regulating MTHFR, all three miRNAs have been shown to slow the process of the conversion of homocysteine to methionine. Mitochondrial dihydroorotate dehydrogenase enzyme (DHODH) is an enzyme that plays an important role in the de-novo pathway of pyrimidine biosynthesis (Rawls et al. 2000). Zhai et al. showed that miR-502 decreases both protein levels and mRNA expression of DHODH (Zhai et al. 2013). Thus, miR-502 negatively regulates nucleotide metabolism by attenuating pyrimidine biosynthesis (Zhai et al. 2013).
MITOCHONDRIAL TRANSPORT

The mitochondrial genome only has 37 genes, 13 of which are the major subunits of mitochondrial ETC complexes (Pearce et al. 2013). A major portion of the proteins responsible for proper mitochondrial functions are encoded within the nuclear genome. These proteins, after being translated, are imported into the mitochondria through translocases such as translocase of outer membrane (TOM) and translocase of inner membrane (TIM) (Neupert and Herrmann 2007). Mitochondrial transport of proteins is therefore essential for the proper functioning of the organelle. Impaired mitochondrial transport leads to cardiac mitochondrial dysfunction and eventually cardiomyopathy. By targeting the proteins responsible for transporting mitochondrial proteins, miRNAs are able to influence mitochondrial function.

Slc25a3 is a mitochondrial phosphate carrier which carries inorganic phosphate from cytosol into the mitochondrial matrix. Slc25a3 supplies phosphate for the process of ATP synthesis by the ETC. In the myoblast cell line, HL-1, Slc25a3 has been found to be the target of miR-141 (Baseler et al. 2012). Consequently, overexpression of miR-141 can reduce ATP synthase. Baseler et al., demonstrated that in T1D, the expression of miR-141 goes up, influencing the ATP level in the heart (Baseler et al. 2012). It has been shown that in pancreatic β cells, miR-184 reduces insulin secretion by targeting Slc25a22 (Morita et al. 2013). Slc25a22 is a mitochondrial glutamate carrier. Consistent with the fact that glutamate can induce insulin secretion, repression of Slc25a22 by miR-184 resulted in a reduction in glucose-induced insulin secretion (Morita et al. 2013). Carnitine-acylcarnitine translocase (CACT) transports long-chain acyl carnitines into the mitochondria for GO. Two miRNAs, miR-212 and miR-132, can directly bind to the 3'-UTR of the CACT gene (Soni et al. 2014). By post-translational regulation of CACT, overexpression of both miR-212 and miR-132 can result in the accumulation of fatty acids in the cytoplasm and reduction in FAO (Soni et al. 2014). Arl2 is an interacting protein of adenine nucleotide transporter 1 (ANT1). Arl2 alters
adenine nucleotide transport by binding to the region on ANT1 called binder of Arl2 (BART) (Sharer et al. 2002). In another study by Nishi et al., it has been shown that the miR-15/16 cluster, miR-15b, miR-16, miR-195, and miR-424, target the 3′-UTR of Arl2 in the heart (Nishi et al. 2010). Furthermore, the authors described that overexpression of miR-15b results in decreased Arl2 protein and mRNA levels; resulting in reduced cellular ATP levels (Nishi et al. 2010). The role of the miR-15 family has been further evaluated in the context of multiple cardiac disorders, and the indispensable role of Arl2 has been highlighted in these studies (Hullinger et al. 2012; Porrello et al. 2011; Porrello et al. 2013).

CONCLUSIONS AND FUTURE DIRECTIONS

Multiple studies have clearly demonstrated the influence of miRNAs on numerous metabolic pathways, see table 1. A growing amount of evidence suggests a direct influence of miRNAs on the function of mitochondria. By targeting mitochondrial transcripts, miRNAs are able to influence various aspects of mitochondrial metabolism, resulting in the alteration of mitochondrial function. This alteration ultimately leads to mitochondrial dysfunction, which plays an important role in the development of diabetic cardiomyopathy. Targeting these miRNAs may lead to therapeutic options for patients with diabetic cardiomyopathy. The discovery of miRNAs in the mitochondrial compartment is possibly due to recent advancements in technologies. RNA-sequencing platforms are more sensitive. As methodologies improve, more and more researchers are attempting to isolate other subcellular fractions, such as sarcolemma and sarcoplasmic reticulum. Encouragingly, it has been shown that miRNAs can be found in the sarcoplasmic reticulum fraction of plant cells. More studies are necessary to explore the existence of miRNAs in various subcellular organelles. More importantly, the functional consequences of miRNAs isolated in these subcellular compartments needs to be well characterized.
As this field continues to expand, a number of pertinent questions are critical for understanding how miRNAs contribute to the regulation of mitochondrial proteins. In general, the continued identification of miRNAs that regulate mitochondrial proteins is of great interest. Further, identification of mechanisms and participants responsible for the import of miRNAs into the mitochondria as well as the cellular cues influencing their actions in extra-mitochondrial locales are of great interest. Finally, because the diabetic phenotype influences the cellular milieu, understanding whether these changes precipitate miRNA regulation of mitochondrial proteins as well as the impact to mitochondria located at different subcellular fractions is essential. The answers to these questions will facilitate the development of miRNA-based therapeutic options for the diabetic patient suffering from cardiac-related morbidities.

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REFERENCES


### Table 1: Influence of MitomiRs on Metabolic Pathways

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<td><strong>TCA</strong></td>
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<td></td>
<td>miR-124</td>
<td>SUCLG2</td>
<td>(Wang and Wang 2006)</td>
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<td><strong>ETC</strong></td>
<td>miR-210-5p</td>
<td>ISCU, SDHD, COX10</td>
<td>(Chan et al. 2009; Chen et al. 2010; Puissegur et al. 2011)</td>
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<td>miR-338-5p</td>
<td>COX IV, ATP5G1</td>
<td>(Aschraft et al. 2008)</td>
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<td>miR-181c</td>
<td>COX 1</td>
<td>(Das et al. 2014; Das et al. 2012)</td>
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<td>COX 1</td>
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<td>ATP5β</td>
<td>(Zheng et al. 2011)</td>
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<td>ATP5β</td>
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<td>miR-378</td>
<td>ATP6</td>
<td>(Jagannathan et al. 2015)</td>
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<td><strong>Fatty Acid Metabolism</strong></td>
<td>miR-33</td>
<td>CROT, CPT1A</td>
<td>(Gerin et al. 2010)</td>
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<td>miR-370</td>
<td>CPT1A</td>
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<td>PPARδ</td>
<td>(el Azzouzi et al. 2013)</td>
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<td>miR-193b</td>
<td>SHMT2</td>
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<td>miR-23-3p</td>
<td>GLS</td>
<td>(Gao et al. 2009)</td>
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<td>miR-23b-3p</td>
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<td>miR-149</td>
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