Subcellular microRNAs in diabetic cardiomyopathy

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Abstract: Cardiovascular complications are the leading causes of diabetes-related morbidity and mortality. The high incidence and poor prognosis of heart failure in diabetic patients have been associated, in part, to the presence of an underlying cardiomyopathy characterized by cardiac hypertrophy, cardiomyocytes apoptosis, and fibrosis. It has been unclear about the mechanism that connects diabetes mellitus to the development of cardiovascular dysfunction. Micro(mi)RNAs represent a class of small, 18- to 28-nucleotide-long, non-coding RNA molecules. MiRNAs typically suppress gene expression at the post-transcriptional levels by binding directly to the 3’-UTR of the target miRNAs in the cytoplasm. Interestingly, recent studies suggest that miRNAs may also regulate gene expression in a positive manner. Our recent studies have shown that subcellular miRNAs, such as cytosol-, mitochondria- and nucleus-localized miRNAs, were dramatically dysregulated in diabetic cardiomyopathy. Specifically, cytoplasm localized miRNAs regulate genes expression in a post-transcriptional manner. Nuclear localized miRNAs regulate gene transcription or chromosomal reconstruction through the non-canonical mechanism. Mitochondrial miRNAs stimulate, rather than repress, the translation of specific mitochondrial genome-encoded transcripts. By reviewing these latest discovered functions of subcellular miRNAs in diabetic animal models, we identified new mechanistic insights for diabetic cardiomyopathy. Understanding the nature of subcellular miRNAs will provide new therapeutic targets against diabetes-associated cardiac complications in the near future.

Keywords: Subcellular miRNAs; diabetic cardiomyopathy; transcription; translation

By 2025, the number of individuals with diabetes worldwide is predicted to reach ~300 million. Cardiovascular disease, which often leads to heart failure, accounts for more than 80% of mortality resulting from diabetes. Therefore, this study reviewed the mechanistic insights into diabetes-induced cardiac dysfunction in order to promote the development of treatment.

Diabetes-induced cardiac dysfunction: vascular or cardiomyocyte disorder?

Diabetes is a major risk factor for the development of heart failure (1). The Framingham Study reported approximate two- and five-fold increases in the risk of heart failure in diabetic men and women, respectively (2). The pathogenesis of heart failure in diabetes can be largely attributed to the cardiotoxic tetrad of coronary artery disease (CAD): hypertension, diabetic cardiomyopathy, and extracellular fluid volume expansion (3,4). Among these cardiovascular complications, CAD is regarded as the leading cause of morbidity and mortality in patients with diabetes (5). Indeed, together with the primary prevention United Kingdom Prospective Diabetes Study (UKPDS), the Action
to Control Cardiovascular Risk in Diabetes (ACCORD) trial, the Action in Diabetes and Vascular Disease (ADVANCE) trial, and the Veterans Affairs Diabetes Trial (VADT), all showed that individuals who were subjected to intensive, rather than standard glycemic, control had a statistically significant reduction in myocardial infarction-associated morbidity (6). However, intensive glycemic control did not reduce the risk of hospital admission for heart failure, which is somewhat surprising given the importance of CAD in the pathogenesis of heart failure (3,6).

Several studies have provided clues for the observation that an increase in the frequency of congestive heart failure in diabetic individuals is more likely be caused by cardiomyopathy than ischemic heart disease (7). Traditionally, diabetic patients have an increased incidence of heart failure, which has been attributed to coexisting ischemic or hypertensive heart disease (8). However, recent scientific evidence suggests that diabetic cardiomyopathy is being considered more as a distinct nosologic entity, independent of the co-existence of CAD, hypertension, or other risk factors (8,9). Although coronary heart disease, hypertension and diabetic cardiomyopathy seem to mutually enhance the other's progression, possibly due to shared pathophysiological processes, such as reactive oxygen species (ROS) and inflammation (10-14), several differences have been noted between the two. Specifically, diabetic cardiomyopathy has been reported to show similar features of diastolic dysfunction (14), which is especially apparent in asymptomatic individuals as the earliest sign of heart failure. Most imaging evidence in patients with diabetes has not been found to show a significant decrease in ejection fraction/systolic dysfunction (15-17).

The common histological characteristic of diabetic cardiomyopathy is the presence of interstitial and/or perivascular fibrosis. Diabetic cardiomyopathy can lead to global hypertrophy and apoptosis of cardiomyocytes (18), while ischemic heart disease usually induces cardiomyocyte apoptosis and fibrosis localized to the ischemic site (19). Diastolic dysfunction is one of the early manifestations in both diabetic cardiomyopathy and hypertensive heart disease (20). However, previous studies reported that hypertensive animals showed greater vascular changes but less myocardial damage than the more severely affected hypertensive-diabetic animals (21).

Currently, diabetic cardiomyopathy is typically defined as structural and functional abnormalities of the myocardium in diabetic patients without CAD or hypertension (22). However, myocardial abnormalities might also present in diabetic patients with CAD and/or hypertension; in such cases, assessing the specific contribution of diabetic cardiomyopathy to overall ventricular dysfunction is challenging (23). Clinically, it is unrealistic to diagnose diabetic cardiomyopathy only in the absence of CAD, hypertension, or any other form of cardiac disease. On this basis, researchers have recently proposed that diabetic cardiomyopathy might be defined as “cardiac abnormalities not wholly explained by other cardiovascular or non-cardiovascular causes and likely to be due to diabetes” (24).

Diabetic cardiomyopathy most often occurs alongside other cardiovascular conditions, although it may also be the sole cause of cardiac disease (24). Therefore, in this review, we focus on the frequent, but often forgotten, occurrence of diabetic cardiomyopathy as a cardiovascular complication of diabetes characterized by cardiac hypertrophy and myocardial fibrosis (25).

Mechanisms that contribute to the development of diabetic cardiomyopathy

Several hypotheses have been proposed to explain the mechanisms underlying the development of diabetic cardiomyopathy.

Lipotoxicity

Increased myocardial free fatty acid (FFA) usage and reduced glucose oxidation have been observed in patients with type 1 and type 2 diabetes (26,27). Griffin et al. reported that glucose could directly activate the translation of fatty acid translocase (CD36) by regulating the upstream open reading frame in the 5’-untranslated region (UTR) of CD36 mRNA (28); consequently, an increased amount of FFA was imported into cardiomyocytes, even when the levels of circulating FFA were normal. Metabolism of high levels of FFA in cardiomyocytes requires increased oxygen consumption and leads to intracellular accumulation of toxic intermediates that may negatively influence myocardial performance through reduced availability of adenosine triphosphate (ATP) (29,30).

Mitochondrial dysfunction

A study by Bugger and Abel revealed impaired respiratory capacity, mitochondrial oxidative stress, and abnormal mitochondrial ultrastructure in rodent models of insulin-dependent and non-insulin-dependent diabetes (31).
Metabolic stress-induced mitochondrial dysfunction increases mitochondrial ROS generation and impairs oxidative phosphorylation resulting in cardiomyocyte hypertrophy and cell death.

**Impaired calcium (Ca\(^{2+}\)) handling**

In their study, Jia et al. found that the hearts of type 1 and 2 diabetic mice had elevated intracellular resting Ca\(^{2+}\), prolonged intracellular Ca\(^{2+}\) decay, slowed Ca\(^{2+}\) transients, reduced sarcoplasmic reticulum Ca\(^{2+}\) pumping, and impaired sarcoplasmic reticulum Ca\(^{2+}\) reuptake (32). These observations suggest that impaired cardiomyocyte Ca\(^{2+}\) handling may play a key role in the development of cardiac diastolic dysfunction.

**Myocardial fibrosis and myocardial remodeling**

Fibrosis, which can be either perivascular or interstitial, is the most typical cardiac-related finding in diabetic patients (33). Hyperglycemia induces abnormal gene expression and altered signal transduction, leading to cardiac hypertrophy and cardiomyocyte apoptosis. Hyperglycemia may also directly induce cardiomyocyte necrosis, resulting in increased collagen deposition, fibrosis, and cardiac remodeling in the heart (30). Dysregulation of extracellular matrix degradation due to the remodeling of matrix metalloproteinases (MMPs), in particular reduced expression of MMP-2, contributes to increased connective tissue content in diabetic hearts (34,35).

**Signaling pathways**

A number of signaling pathways have been identified as important contributors to the development of diabetic cardiomyopathy, including the adenosine monophosphate protein kinase (AMPK), peroxisome proliferators-activated receptors (PPARs), O-linked beta-N-acetylglucosamine (O-GlcNAc), and protein kinase C (PKC) pathways (32). The AMPK pathway, for instance, is impaired in diabetic cardiomyopathy. AMPK enhances the expression of glucose transporter type 4 (GLUT4) and, subsequently, insulin-induced glucose uptake. Therefore, AMPK activation plays a protective role against the progression of diabetic cardiomyopathy (36). Peroxisome proliferator-activated receptor γ (PPAR-γ) has also shown cardiac anti-hypertrophic and anti-inflammatory effects, with PPAR-γ agonists enhancing insulin sensitivity and improving glucose uptake in cardiomyocytes (37). Dassanayaka and Jones observed sustained O-GlcNAc signaling in the diabetic heart, which could exert detrimental effects on mitochondrial function and energy generation in cardiac function (38). PKC signaling pathways are activated in diabetic cardiomyopathy in response to hyperglycemia and insulin resistance. PKCa, β, ε, θ, and δ isoforms have been suggested to be involved in the development of diabetic cardiac hypertrophy (39). PKC β2 has been shown to induce diastolic cardiac dysfunction in diabetic rats through caveolin-3 and insulin metabolic Akt/eNOS signaling (40). Furthermore, other signaling pathways, such as SGLT2, MAPK, NFκB, nuclear factor erythroid 2-related factor 2 (Nrf2), and cyclic adenosine 5’-monophosphate-responsive element modulator (CREM), have also been put forward as participants in diabetic cardiomyopathy (32).

**Other mechanisms**

Inflammation, autophagy, apoptosis/necrosis, the telomere-telomerase system, advanced glycation end-products, and endothelial dysfunction may also play roles in the development of diabetic cardiomyopathy (41,42). Although these pathways are considered as separate mechanisms, they may interact with each other in complex ways. For instance, elevated FFA oxidation and lipotoxicity may promote mitochondrial dysfunction and oxidative stress; mitochondrial dysfunction and endoplasmic reticulum stress may increase apoptosis in cardiomyocytes; and oxidative stress, increased advanced glycation end-product signaling, and inflammation may promote apoptosis or the increased expression of pro-fibrotic genes (43). Overall, multiple potential mechanisms have been proposed and investigated for the development of diabetic cardiomyopathy. Novel mechanisms, such as miRNA and epigenetic mechanisms, remain relatively under-investigated and need to be further investigated (43). In the subsequent sections, we review the roles of various miRNAs, especially newly discovered subcellular localized miRNAs, in diabetic cardiomyopathy.

**miRNAs and diabetic cardiomyopathy**

miRNAs represent a class of small, 18- to 28-nucleotide-long, non-coding RNA molecules. MiRNAs typically suppress gene expression at the post-transcriptional level by binding directly to the 3’-UTR of target mRNAs (44). Interestingly, recent studies have suggested that miRNAs may also be positive regulators of gene expression (45,46).
Early studies reported the associations between altered miRNA expression and the pathologies of multiple diseases, including heart failure, atherosclerosis, diabetes, chronic kidney disease, and cancer (47-50). A recent study in mice revealed that dysregulation of 316 miRNAs was observed in diabetic hearts compared with controls. Among them, the expressions of 268 miRNAs remained significantly altered in diabetic mice, even after subsequent normoglycemia (51), indicating that miRNAs potentially contribute to the development of diabetic cardiomyopathy.

**Canonical mechanism**

For decades, miRNAs were reported as post-transcriptional regulators in the cytoplasm of multicellular organisms. MiRNAs typically exert their inhibitory effects by base pairing with the 3’-UTR of target mRNAs through their seed sequences (52,53), which constitutes the canonical mechanism of miRNA function.

**Cytoplasmic miRNAs in diabetic cardiomyopathy**

Various animal studies have suggested that many cardiac-enriched miRNAs are involved in the development of diabetic cardiomyopathy. MiR-133a, one of the predominantly expressed miRNAs in cardiac tissue, was found to be dramatically decreased in the hearts of streptozotocin (STZ)-induced type 1 diabetic animals. Chen et al. observed that miR-133a re-expression prevented a diabetes-induced increase in the expression of extracellular matrix protein and focal cardiac fibrosis, most likely by targeting transforming growth factor β1 mRNA (54). In the hearts of mice with high-fat diet–induced type 2 diabetes, the levels of miR-451 were found to be significantly increased, while cardiomyocyte-specific miR-451 knockout mice showed ameliorated cardiac hypertrophy and contractile dysfunction. Calcium Binding Protein 39 (Cab39) was identified by Kuwabara et al. as a direct target of miR-451 in the heart (55). MiR-195 expression was increased in STZ-induced type 1 and db/db type 2 diabetic mouse hearts. Knocking down of miR-195 expression in the heart was shown to significantly attenuate myocardial hypertrophy and improve myocardial function in STZ-treated mice via direct targeting of Sirt1 and Bcl-2 mRNA (56). Meanwhile, upregulation of miR-195 sufficiently induced apoptosis in cardiomyocytes and promoted cardiac dysfunction. MiR-155 was enhanced in the macrophages and the hearts of ovariectomy-induced diabetic mice. The delivery of gold nanoparticle-based miR-155 antagonist macrophages restored cardiac function in these mice (57). Upregulation of miR-195 sufficiently induced apoptosis in cardiomyocytes and promoted cardiac dysfunction in diabetic hearts (56). MiR-34a overexpression impaired autophagy in high-glucose-induced cardiomyocytes in the hearts of diabetic mice; however, the downregulation of miR-34a restored autophagy, and thus ameliorated diabetic cardiomyopathy (58). In cardiomyocytes, miR-34a induced telomere shortening by reducing the level of protein phosphatase 1 nuclear targeting subunit, whereas the inhibition of miR-34a significantly enhanced telomere length and telomerase activity (59,60). Interestingly, miR-34a inhibition also reduced high glucose-induced apoptosis in cardiomyocytes.

Our group systematically investigated the roles of miRNAs in diabetic cardiomyopathy. For example, miR-30c was significantly decreased in db/db type 2 diabetic model mice. MiR-30c overexpression by a recombinant adeno-associated virus type 9 (rAAV9) vector reversed cardiac functional and structural changes in diabetic mice (61,62). Mechanistically, miR-30c repressed beclin-1 expression by directly binding to its 3’-UTR. MiR-30c overexpression inhibited the induction of beclin-1 and subsequent autophagy in diabetic hearts (61). Moreover, miR-30c also directly targeted and repressed the expression of PGC-1β, which reduced the transcriptional activity of PPAR-α. Glucose-fatty acid use, oxidative stress, lipid accumulation, ATP production abnormalities, and apoptosis mediated by PPAR-α were eliminated by treatment with miR-30c (61). Another miRNA enriched in the heart, miR-21, was specifically down-regulated in cardiomyocytes but remained unchanged in non-cardiomyocytes of db/db and high-fat diet–induced type 2 diabetic mice. Remarkably, treatment with miR-21 effectively protected against the early impairment in cardiac diastolic dysfunction in db/db mice, which was shown by decreased ROS production, increased bioavailable nitric oxide, and improved diabetes-induced cardiomyocyte hypertrophy. Through bioinformatics analysis and Ago2 co-immunoprecipitation, we identified gelsolin, a member of the actin-binding protein family, which acted as a transcriptional cofactor in signal transduction, as a direct target of miR-21 (63). These findings suggest that changes to miRNAs localized in the cytoplasm contribute to the development of diabetic...
Non-canonical mechanism

Some miRNAs might localize in the nucleus or the mitochondria, as well as in the cytoplasm (46, 64). Stavast and Erkeland obtained strong evidence that nuclear miRNAs regulate the transcription of target genes by binding to reverse complementary sequences in promoter regions in their functional in vitro experiments using mammalian cells (65). Instead of negatively regulating gene transcription by binding to the 3'-UTR of target mRNAs, miRNAs localized in the mitochondria stimulate, rather than repress, the translation of specific mitochondrial genome-encoded transcripts. These subcellular localized miRNAs usually function through non-canonical mechanisms.

Nuclear miRNAs in diabetic cardiomyopathy

Most miRNAs can be found in both the nucleus and the cytoplasm, with some showing selective nuclear enrichment (66). For instance, in HeLa cells, miR-29b is mainly nuclear, whereas miR-29a is predominantly cytoplasmic (67). However, in comparison to many studies examining cytoplasm-localized miRNAs, the pathophysiological roles of nuclear miRNAs remain largely under-investigated. Our recent study demonstrated that a specific miRNA, miR-320, was significantly up-regulated in the diabetic myocardium of mice and human patients, and translocated into the nucleus to directly enhance CD36 expression (68).

MiR-320 acts as a small activating RNA in the nucleus at the transcriptional level. CD36 is a key target gene of miR-320, and the induced expression of CD36 is responsible for increased FFA uptake and cardiac lipid accumulation, thereby causing cardiac lipotoxicity and increased cardiomyocyte apoptosis. The delivery of rAAV9-mediated miR-320 TUD (Tough Decoy) was shown to restore cardiac dysfunction in diabetic mice. These findings indicate that the miR-320/CD36 pathway links glucose toxicity to lipotoxicity in the heart (Figure 1). This study offered the first in vivo example of transcriptional activation by a natural small activating RNA and uncovered a novel mechanism for cardiac dysfunction induced directly by diabetes. Furthermore, this finding suggests a potential strategy for developing miRNA-based therapy for diabetes-associated cardiovascular complications (68).

Mitochondrial miRNAs in diabetic cardiomyopathy

Recently, miRNAs were reported to be present in the mitochondria. As early as 2009, several groups reported the detection of nuclear-coded miRNAs in rat liver mitochondria and HeLa cells (69-71). Later, Das et al. reported that miR-181c in rat mitochondria was involved in electron chain complex IV remodeling in...
cardiomyocytes (72). Another study of interest showed the redistribution of mitochondrial miRNAs in diabetic hearts. In STZ-induced type 1 diabetic hearts, Jagannathan et al.’s miRNA array analysis indicated that 78 miRNAs were differentially expressed in the mitochondrial subpopulation relative to the controls (73). However, the significance and functional consequences of these changes were not characterized. Our recent study demonstrated that 14 miRNAs were downregulated in the mitochondria of db/db type 2 diabetic mouse hearts (74). Of these miRNAs, miR-92a-2-5p and let-7b-5p targeted mitochondrially encoded cytochrome B (mt-Cytb) and positively modulated mt-Cytb expression. Overexpression of miR-92a-2-5p or let-7b-5p was also observed to decrease apoptosis and the level of mitochondrial ROS through the upregulation of mt-Cytb. Interestingly, rAAV9-mediated delivery of miR-92a-2-5p, but not let-7b-5p, could rescue cardiac diastolic dysfunction in the hearts of db/db mice. Let-7b-5p not only upregulated mt-Cytb, but also downregulated insulin receptor substrate 1 in cytosol, resulting in a failure to improve diastolic dysfunction in db/db mice (74). Our study gave the first in vivo example of mitochondria-localized miRNAs in diabetic cardiomyopathy and revealed a complicated pattern of miRNA regulation in the subcellular organelles during the disease.

Conclusions and perspectives

MiRNAs are commonly believed to serve as fine-tuning tools that play regulatory roles in virtually all diabetic cardiomyopathy mechanisms. Especially in type 1 and 2 diabetic hearts, an accumulation of lipids is frequently observed, which negatively influences myocardial performance through the excessive production of toxic intermediates. However, the mechanism by which glucose toxicity leads to lipotoxicity has not been well defined. Our recent study reveals that nuclear miR-320 might be one of the “missing links” between glucose toxicity and lipotoxicity in diabetic hearts. MiRNAs might also be associated with the signaling pathways involved in diabetic cardiomyopathy such as inflammation, autophagy, and apoptosis/necrosis. MiRNAs can act as the nodes of signaling networks that regulate the progression of diabetic cardiomyopathy. Signaling pathways are prime candidates for miRNA-mediated regulation, and signaling complexes are the ideal targets for the degree of quantitative fluctuations imposed by miRNAs. Instead of focusing on protein-coded signaling pathways, which are difficult to target therapeutically, one could focus on their target miRNAs. However, facing the potential complexity of the miRNA-signaling network, future studies are needed to reveal the complicated cross-talk between miRNAs and diabetic cardiomyopathy-related signaling pathways using unambiguous and pathway-specific readouts in cultured cell or other model systems.

Until recently, little was known about the subcellular localization, functions, turnover, and dynamics of miRNAs. In fact, most miRNAs are present in both the nucleus and the cytoplasm. However, their pathophysiological functions and mechanisms are largely undetermined. Furthermore, miRNAs are also localized in other subcellular compartments. For instance, mitochondrial miRNAs that control the translation of mitochondrial genes could provide new mechanisms for mitochondrial dysfunction in diabetic cardiomyopathy.

Conclusively, miRNAs localized in the cytoplasm usually regulate gene expression through a post-transcriptional manner, which fell into the canonical mechanism of miRNAs. Nuclear miRNAs regulate gene transcription or chromosomal reconstruction through a non-canonical mechanism (68,75). Mitochondrial miRNAs stimulate, rather than repress, the translation of specific mitochondrial genome-encoded transcripts by targeting their coding sequences, instead of the 3’-UTRs of target miRNAs, which represents another non-canonical mechanism of miRNAs (46).

A major drawback current studies on miRNAs is the absence of a strategy to investigate their cytosolic, mitochondrial, or nuclear effects independently. Thus, more sophisticated experimental systems must be developed to resolve the roles and functions of miRNAs in subcellular compartments (76). It is still unclear how subcellularly localized miRNAs work together to function in diabetic cardiomyopathy. Moreover, although many potential mechanisms have been proposed for diabetic cardiomyopathy, including lipotoxicity, ROS induction, and mitochondrial dysfunction, their effects are rather unclear, and questions remain regarding the factors involved in the initiation and progression of diabetic cardiomyopathy. A deeper understanding of the nature of subcellular miRNAs is needed to reveal new mechanisms and to identify potential novel therapeutic targets for diabetes-associated cardiac complications.

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