Peer Review File

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Reviewer A

1. The language grammar and style should be re-edited；

Response: Thank you, as suggested we have re-edited the language grammar and style with the help of an English language native speaker.

2.Apoptosis, autophagy and telomere-telomerase system play an important role in diabetic cardiomyopathy. Do current researches support these mechanisms which are related to mi-RNA? Whether the further research are needed should be discussed；

Response: Thanks for bringing out this crucial issue to us. Emerging evidence has demonstrated that diabetes induced cardiomyocyte apoptosis and suppressed cardiac autophagy (J Mol Cell Cardiol. 2014;71:71-80). Restoration of autophagy reduced cardiac apoptosis and cardiac dysfunction in diabetes (Diabetes. 2013;62:1270-1281). In terms of telomere-telomerase system, recent studies have suggested that telomere shortening and abnormal telomerase activity occur in patients with diabetes mellitus, and targeting the telomere-telomerase system has become a prospective treatment for diabetes mellitus and diabetic cardiomyopathy (Expert Opin Ther Targets. 2015;19(6):849-864, Exp Clin Cardiol. 2010;15(4):e128-133).

In fact, miRNAs were reported to regulate pathways involved in apoptosis, autophagy and telomere-telomerase system. For example, upregulation of miR-195 sufficiently induced apoptosis in cardiomyocytes and promoted cardiac dysfunction in diabetic heart (Diabetologia. 2015;58(8):1949-1958). Upregulation of miR-34a impaired autophagy in high-glucose-induced cardiomyocytes and in the hearts of diabetic mice. Decreasing the expression of miR-34a restored impaired autophagy and thus ameliorated DCM (Cardiovasc Drugs Ther. 2020;34(3):291-301). In cardiomyocytes, miR-34a induced telomere shortening by reducing the level of protein phosphatase 1 nuclear targeting subunit (PNUTS), miR-34a inhibition significantly telomere length and telomerase activity (Nature. 2013;495:107-110, Physiol Genomics.

2016;48:42-49). Interestingly, miR-34a inhibition also reduced high glucose induced apoptotic cell death in cardiomyocytes (Cell Death Differ. 2018;25(7):1336-1349).

These data suggested that apoptosis, autophagy and telomere-telomerase system were both regulated by miRNAs. Moreover, a same pathway could be regulated by different miRNAs and a miRNA was able to regulate several different genes or pathways (Future Oncol. 2016;12(9):1135-1150). Therefore, future studies need to reveal the complicated crosstalk between miRNAs and genes involved in apoptosis, autophagy, telomere-telomerase system and other DCM related pathophysiologic processes. Unearth the initiative factors in the sophisticated network between miRNAs and pathophysiologic processes would be crucial to develop new therapeutic strategies for DCM.

We have added these new points (in red) in the revised manuscript (Page 7, line 24-33 and Page 10, line 18-21)

3.The differences and connections between diabetic coronary heart disease, hypertension and diabetic cardiomyopathy should be discussed more in detail；

Response: Thank you. Indeed, the differences and connections between diabetic coronary heart disease, hypertension and diabetic cardiomyopathy are very important. Therefore, we have added the following sentences in the revised manuscript:

Traditionally, diabetic traditionally patients have an increased incidence of heart failure which has been attributed to the concurrent presence of ischemic or hypertensive heart disease (J Diabetes Complications. 2009;23(4):273-282). Yet, nowadays, recent scientific evidence suggested that diabetic cardiomyopathy (DCM) were more being considered as a distinct nosologic entity, independent of the co-existence of coronary artery disease, hypertension or other risk factors (Heart Fail Rev. 2014;19(1):15-23, J Diabetes Complications. 2009;23(4):273-282). Though coronary heart disease, hypertension and diabetic cardiomyopathy seemed to mutually enhanced the progression of each other, which might be due to shared pathophysiological processes such as reactive oxygen species (ROS) and inflammation (Diabetes Care. 2003; 26(suppl 1): s80-s82, Am J Physiol Heart Circ Physiol. 2008; 295(4): H1634-H1641, Curr Atheroscler Rep. 2012;14(2): 160-166, J Hum Hypertens. 2002;16 Suppl 1:S61-

S63, Expert Rev Cardiovasc Ther. 2012;10(8):1051-1060), several differences were suggested for diabetic coronary heart disease, hypertension and diabetic cardiomyopathy. Specifically, diabetic cardiomyopathy has mainly been linked with features of diastolic dysfunction (World J Diabetes. 2019;10(10):490-510). This is especially apparent in asymptomatic individuals as the earliest sign of HF. Most of the evidence in imaging of patients with T2D have not shown a significant decrease in ejection fraction/systolic dysfunction (Circ Heart Fail. 2015; 8(3):448-454, Am J Cardiol. 2009;104:1398-1401, Vrach Delo, 1990;(2):61-3). The common histological characteristic of diabetic cardiomyopathy is the presence of interstitial and/or perivascular fibrosis. DCM led to global hypertrophy and apoptosis of cardiomyocytes (Int J Mol Sci, 2020;21(8):2896), while ischemic heart disease usually induced cardiomyocytes apoptosis and fibrosis Localized to the ischemia site (Eur J Heart Fail. 2017;19(2):177-191). In terms of the differences between hypertensive cardiomyopathy and diabetic cardiomyopathy, diastolic dysfunction is one of the early manifestations in both diabetic cardiomyopathy and hypertensive heart disease (Indian Heart J. 2018;70(1):75-81). However, previous studies have shown that hypertensive animals had greater vascular changes but less myocardial damage than the more severely affected hypertensive-diabetics (Am J Pathol. 1981;102(2):219-228).

The current typical definition of diabetic cardiomyopathy involves structural and functional abnormalities of the myocardium in diabetic patients, without coronary artery disease or hypertension. However, this type of cardiomyopathy should be present also in diabetics with coronary artery disease and/or hypertension, though it is difficult to separately assess the contribution of diabetic cardiomyopathy to overall ventricular dysfunction in such cases. Clinically, it seems unrealistic to require the absence of coronary artery disease, hypertension, or any other form of cardiac disease before a diagnosis of diabetic cardiomyopathy is made. Therefore, recently, researchers proposed that diabetic cardiomyopathy should be defined as “cardiac abnormalities not wholly explained by other cardiovascular or non-cardiovascular causes and likely to be due to diabetes”.

These sentences (in red) were presented in the revised manuscript (Page 3, line 10-30 and Page 4, 1-13).

4.The process of myocardial remodeling and the signal pathways which involved in diabetic cardiomyopathy are need to be listed；

Response: Thank you. We have added myocardial remodeling and the signal pathways which involved in diabetic cardiomyopathy in the revise manuscript as:

Myocardial remodeling and fibrosis: The most typical cardiac finding in diabetic patients is fibrosis, which may be both perivascular and interstitial. Hyperglycemia may cause abnormal gene expression and alter signal transduction, which may activate pathways leading to cardiac hypertrophy and myocytes apoptosis. Hyperglycemia may also directly induce myocyte necrosis, which results in increased collagen deposition, fibrosis and cardiac remodeling in the heart. Dysregulation of extracellular matrix degradation due to remodeling of matrix metalloproteinases (MMPs), in particular reduced expression of MMP-2, contribute to increased connective tissue content in diabetic hearts (Diabetes. 2007;56(3):641-646, Basic Res Cardiol. 2008;103(4):319-327).

Signal pathways: A number of signaling pathways have been implicated as important contributors to the development of diabetic cardiomyopathy including AMPK, PPARs, O-GlcNAc, PKC pathways (Circ Res. 2018;122(4):624-638). For example, AMPK pathway was impaired in DCM. AMPK enhances expression of GLUT4 and thus insulin-induced glucose uptake. Therefore, AMPK activation plays a beneficial role in preventing the progression of diabetic cardiomyopathy (Autophagy. 2013;9(4):624-625). PPAR-γ plays important roles in cardiac anti-hypertrophic and anti-inflammatory effects. PPAR-γ agonists enhance cardiomyocyte insulin sensitivity and improve cardiomyocyte glucose uptake (Nat Rev Endocrinol. 2016;12(3):144-153). Sustained O-GlcNAc signaling exists in the diabetic heart and can exert detrimental effects on mitochondrial function, energy generation cardiac function (Pharmacol Ther. 2014;142(1):62-71). PKC signaling pathways are activated in diabetic cardiomyopathy in response to hyperglycemia and insulin resistance. PKCα, β, ε, θ, and δ isoforms have been proposed to be involved in the development of diabetic cardiac hypertrophy (Br J Pharmacol. 2014;171(11):2913-2924.). PKC β2 has been shown to induce diastolic cardiac dysfunction in diabetic rats through caveolin-3 and insulin metabolic Akt/eNOS signaling (Diabetes. 2013l;62(7):2318-2328). In addition, other signaling pathways such as SGLT2, MAPK, NFκB, nuclear factor erythroid 2-related factor 2 (Nrf2), cyclic

adenosine 5′-monophosphate-responsive element modulator (CREM) were also suggested to participate in diabetic cardiomyopathy (Circ Res. 2018;122(4):624-638).

We have added these new information (in red) in the revised manuscript (Page 5, line 11-32 and Page 6, line 1-6).

Reviewer B

In this review, the authors explain the pathogenesis of diabetic cardiomyopathy and show several hypotheses which have been proposed the mechanisms responsible for the development of diabetic cardiomyopathy. Next, review some newly discovered subcellular miRNAs such as mitochondria- and nucleus-localized miRNAs, are dysregulated in diabetic cardiomyopathy. Then, they mainly summarized and analyzed these mechanisms about diabetic cardiomyopathy.

The content is abundant in this review and extensive references were quoted, but it is not coherent in the logic and the overall structure is very loose.

1. The authors should focus on the title and subject *Subcellular microRNAs in diabetic cardiomyopathy,* I suggest they should decreased the details on other several hypotheses which have been proposed the mechanisms responsible for the development of diabetic cardiomyopathy except *microRNAs*.

Response: Thank you so much for pointing out this crucial issue. Indeed, it seemed quite irrelevant to show the details on other hypotheses (such as autophagy, apoptosis/necrosis pathway) which have been proposed the mechanisms responsible for the development of DCM. However, after careful deliberation, these mechanisms were still presented in this manuscript, because miRNAs are part of these signaling networks (such as autophagy, apoptosis/necrosis) listed in this review. MiRNA frequently served as a mediator of crosstalk between signaling pathways, coordinating their activity. Dysregulation of miRNAs caused aberrant signaling pathways. Some of the connections between signaling networks and miRNA biogenesis are starting to be revealed at the molecular level. For example, nuclear miR-320, a potent lipid metabolism mediator, induced expression of CD36 is responsible for increased fatty acid uptake and cardiac lipid accumulation, thereby causing cardiac lipotoxicity and increased cardiomyocyte apoptosis (Circ Res. 2019;125(12):1106-1120). Noteworthy,

lipotoxicity and apoptosis are the crucial mechanisms responsible for the development of DCM. Therefore, these hypotheses/mechanisms might seem not coherent with the main topic in this review - miRNAs, in fact, they are closely related and miRNAs served as nodes of signaling networks that regulate progression of DCM (Nat Rev Mol Cell Biol. 2010;11(4):252-263).

However, as you pointed out, in the initial manuscript, the overall structure was very loose and the connection between mechanisms/signaling pathways and miRNAs was not clearly elaborated. In the revised manuscript, we have modified the sentences in the Conclusions and Perspectives section to clarify the inner collection between miRNAs and other mechanisms involved in DCM: It is a common view that miRNAs may serve as fine-tuning tools and may play regulatory roles in virtually all diabetic cardiomyopathy mechanisms. In particular, an accumulation of lipids is frequently observed in type 1 and 2 diabetic hearts. This negatively influences myocardial performance through the excessive production of toxic intermediates. However, how glucose toxicity leads to lipotoxicity is not well-defined. Our recent work reveals that nuclear miR-320 might be one of the "missing links" between glucose toxicity and lipotoxicity in diabetic hearts. In terms of the relationship between miRNAs and the signaling pathways involved in DCM such as inflammation, autophagy and apoptosis/necrosis. MiRNAs could act as nodes of signaling networks that regulate progression of DCM. Signal pathways are prime candidates for miRNA-mediated regulation. 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 relationship, future studies need to reveal the complicated crosstalk between miRNAs and DCM related signaling pathways using unambiguous and pathway-specific readouts in cultured cells or other model systems (Page 10, line 13-30).

2. I suggest they can divide into 2 parts to illustrate the mechanism of miRNAs in diabetic cardiomyopathy ---Non-canonical mechanism and Canonical mechanism. Also, they can provide some explanations the particular roles miRNAs in different

organelles (nuclear, mitochondrial). Such as: 1. Canonical mechanism: …….2. Non-canonical mechanism: nuclear miRNAs, mitochondrial miRNA……

Response: Thank you for your highly valued suggestion. We have taken your advice to divide the mechanism of miRNAs in diabetic cardiomyopathy into Non-canonical mechanism and Canonical mechanism. Moreover, as suggested, we provided more mechanistic insights into the particular roles of miRNAs in different organelles.

1. Canonical mechanism: For decades, miRNAs were reported as posttranscriptional regulators in multicellular organisms in the cytoplasm. MiRNAs typically elicited their inhibitory effects by base pairing with the 3′ untranslated regions (3′-UTR) of target mRNAs through their seed sequence (J Cell Physiol. 2019;234(5):5451-5465, Cell. 2018;174(4):1038-1038.e1), which represented the canonical mechanism of miRNAs functioning.

2. Non-canonical mechanism: In contrast to typical miRNA functions in the cytoplasm, some miRNAs are localized in the nucleus or mitochondrial (Mol Cell. 2004;(15):185-197, Cell. 2014;158(3):607-19). Functional experiments in mammalian cells provide strong evidence that nuclear miRNAs regulate the transcription of target genes by binding to reverse complementary sequences in promoter regions (Sci Rep. 2019;(9):9320, Cells. 2019;8(11):1465). In mitochondria, instead of negatively regulate gene transcription by binding the 3′-UTR of target mRNAs, mitochondria localized miRNAs stimulated, rather than repressed, the translation of specific mitochondrial genome-encoded transcripts. These subcellular localized miRNAs usually function through non-canonical mechanisms.

We have added these sentences (in red) in the revised manuscript (Page 7, line 3-6 and Page 8, line 24-32)

3. They just listed lots of miRNAs in the different studies lack of induction. Such as from different aspects (clinical, cellular, animal) …….or different roles of miRNAs (transcription, post-transcription, chromosomal reconstruction……)

Response: Thank you. Indeed, we should have presented detailed induction of these studies. In fact, the assessment of cardiac dysfunction in diabetes require the echocardiography or catheterization experiment. Therefore, in vivo animal studies were

primarily included in this review, and most of these studies contained evidence from cell experiment. Isolated in vitro studies were not included in this review because of the absent of cardiac function indicators. As for clinical studies, though dozens of miRNAs were suggested to dysregulated in plasma or heart of diabetic patients (Sci Rep. 2017;7(1):13514, Circ Res. 2019;125(12):1106-1120), their functions in human were unavailable and therefore were not listed in this review.

For the different roles of miRNAs, cytoplasm localized miRNAs usually regulate genes expression through post-transcriptional manner, which fell into the canonical mechanism of miRNAs. Nuclear miRNAs regulated gene transcription or chromosomal reconstruction through the non-canonical mechanism (Circ Res. 2019;125(12):1106-1120, Proc Natl Acad Sci U S A. 2008;105(5):1608-1613). Mitochondrial miRNAs stimulated, rather than repressed, the translation of specific mitochondrial genome-encoded transcripts by targeting the coding sequence (CDS), but not the 3′-UTR of target mRNAs, which also belonged to the non-canonical mechanism of miRNAs (Cell. 2014;158(3):607-619).

To make the logic and structure of this review more clearly, we have added these introductions in the revised manuscript. (Page 11, line 4-10)