Journal of Molecular and Cellular Cardiology 105 (2017) 70–76

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

miR-146a mediates inflammatory changes and fibrosis in the heart in diabetes

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ARTICLE INFO

Article history:
Received 18 October 2016
Received in revised form 2 March 2017
Accepted 3 March 2017
Available online 6 March 2017

Keywords:
NF-κB
Diabetic cardiomyopathy
Inflammatory
Fibrosis

ABSTRACT

Hyperglycemia induced endothelial injury is a key pathogenetic factor in diabetic cardiomyopathy. In diabetes, changes in pro-inflammatory cytokines are a key mechanism leading to cardiac fibrosis. We have previously demonstrated alteration of miR-146a in chronic diabetic complications. Here, we investigated the role of endothelial miR-146a in mediating inflammation and fibrosis in diabetic cardiomyopathy.

To examine the effects of miR-146a on the inflammatory mediators, an endothelial specific miR-146a overexpressing transgenic mice (TG) using tie-2 promoter, was generated. We examined these mice and wild type littermate controls with or without STZ induced diabetes. Transthoracic echocardiography was performed. Cardiac tissues were examined for inflammatory cytokine mRNAs and proteins by real time RT-PCR or ELISA. Cardiac fibrosis was examined by histology staining. Human cardiac microvascular endothelial cells (HCMECs) and primary endothelial cells isolated from mice were used following incubation with various levels of glucose with or without miR-146a mimics or antagomir transfection.

In hearts of wild type mice with diabetes, increased expression of inflammatory markers and extracellular matrix proteins (IL6, TNFα, IL-1β, MCP-1, NF-κB, Col1α1, Col4α1) were seen compared to wild type controls. These changes were prevented in the diabetic TG mice. In addition, WT diabetic mice showed cardiac functional abnormalities, which were improved in the diabetic TG mice. In vitro studies showed glucose induced increase the expressions of the above inflammatory cytokines and specific NF-κB regulators (IRAK1 &TRAF6). Such changes were corrected in the HCMECs following miR-146a mimic transfection.

These data indicate that in diabetes, increased inflammatory cytokine and extracellular matrix protein productions and associated cardiac functional alterations are regulated by endothelial miR-146a. Identification of such mechanisms may potentially lead to the development of novel RNA based therapeutics.

1. Introduction

Diabetic cardiomyopathy is functionally characterized by defects in cardiac contractility. The condition is structurally manifested as cardiomyocyte hypertrophy and eventual apoptosis, focal scarring etc. [1]. Cardiomyopathy eventually leads to cardiac failure [2]. Microvascular affection primarily causing endothelial cell damage is possibly a major initiating factor in all diabetic complications including diabetic cardiomyopathy [3]. As a result of hyperglycemia induced oxidative stress, significant transcriptional changes occur in the cells resulting in augmented production of multiple growth factors and cytokines, including several inflammatory mediators [3–5].

In hyperglycemia and in other inflammatory conditions, activation of NF-κB has been demonstrated to act as a key mediator of such altered synthetic phenotype [5]. However, in addition to transcription factors, we and others have demonstrated that alterations of additional epigenetic factors, e.g. transcription co-activator p300, which regulates such gene expression at the transcriptional level through histone acetylation in diabetic complications are also important [6]. Post-transcriptionally microRNAs (miRNA) also play a significant role in such process [7,8].

miRNAs are small (~22 nucleotide) RNA molecules that are major regulators of gene expression at the post-transcriptional level. They mostly bind to specific 3′UTR of the mRNA and in most cases negatively regulate gene expression by mRNA degradation or translational repression [9]. We have previously demonstrated alteration of multiple miRNAs in several chronic diabetic complications [7,10,11]. To this extent, we have previously shown downregulation of miR-146a in association of upregulation of extracellular matrix protein transcripts in the hearts, kidneys and retinas both in type 1 and type 2 diabetes [8]. Other investigators in diabetic and in other non-diabetic models of inflammation have demonstrated alteration of miR-146a and regulatory relationships of miR-146a with components of NF-κB pathway [12–14]. Interestingly miR-146a polymorphism has been associated with

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http://dx.doi.org/10.1016/j.yjmcc.2017.03.002
0022-2828/

diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, coronary artery disease and inflammatory bowel disease [15–19]. We have demonstrated glucose-induced reduced miR-146a expression in endothelial cells and it's regulatory relationship with histone acetylator p300. These pathways may reflect early transcriptional changes in diabetes [8]. Similar to our study reduced miR-146a expression has been shown in the hippocampus of diabetic rats and in the skin wounds of diabetic animals [20,21]. In contrast, expression of miR-146a was found to be increased in the heart in a rat model of type 1 diabetes [22]. On the other hand, examination of rat aorta showed reduced miR-146a level in diabetes [13]. Furthermore, miR-146a was found to have a protective effect on myocardial ischemia reperfusion injury [12].

miR-146a has been shown to be of importance in innate immunity, sepsis and inflammatory diseases [23,24]. It targets Interleukin-1 associated Kinase 1 (IRAK1) and tumour necrosis factor receptor associated factor 6 (TRAF6); through which it regulates NF-κB activity [25]. One of the key features of diabetic cardiomyopathy is occurrence of low grade inflammation [26]. We have shown that activation of NF-κB in the endothelial cells and in the major organs affected by chronic diabetic complications including heart [5,27].

The purpose of this study was to examine pathogenetic role of miR-146a in diabetic cardiomyopathy with respect to specific inflammatory mediators. Furthermore, as mentioned previously, endothelial damage is possibly an initiating factor in this process. Hence, we focused on the cardiac endothelial cells as a primary site of such alterations. To this extent, we studied human cardiac endothelial cells to perform mechanistic studies. We further used a unique tool. We generated a transgenic mouse with endothelial specific miR-146a overexpression. We used these animals with chemically induced diabetes and examined them for biochemical, functional and structural changes.

2. Materials and methods

Details regarding the reagents, antibodies, cell culture, Western blot analyses, animal models, and statistical analysis are provided in the Supplementary Online Data.

3. Results

3.1. miR-146 is reduced in the endothelial cells and in the hearts of diabetic animals

We initially confirmed our previous findings that miR-146a levels are reduced in the hearts of diabetic mice. We used Quantitative RT-PCR to measure miR-146a levels. Two months of poorly controlled diabetes led to significant reduction of miR-146a in the heart of wild-type B6 mice with STZ induced diabetes (Fig. 1A). We further isolated cardiomyocytes and endothelial cells from these heart and assessed individual cell types for miR-146a levels. The main cell type with reduced miR-146a was endothelial cells. Myocytes showed no significant alteration of miR-146a content in diabetes (Fig. 1B/C). Furthermore, to confirm that in our transgenic mice, miR-146a is cell specific, we isolated endothelial cells, cardiomyocytes and fibroblasts from these mice and compared miR-146a expression levels with corresponding cells isolated from wild-type B6 mice. Transgenic mice sowed similar levels of expression in the myocytes and fibroblasts, whereas in the endothelial cells they showed a 3.5 fold increased level of miR-146a compared to wild type controls (Fig. 1D). In parallel, incubation of HCMECs in a medium containing 25 mM glucose (HG) caused significant downregulation of miR-146 compared to the cells incubated in 5 mM glucose (NG) (Fig. 1E). Such effects were not seen when the cells were incubated in

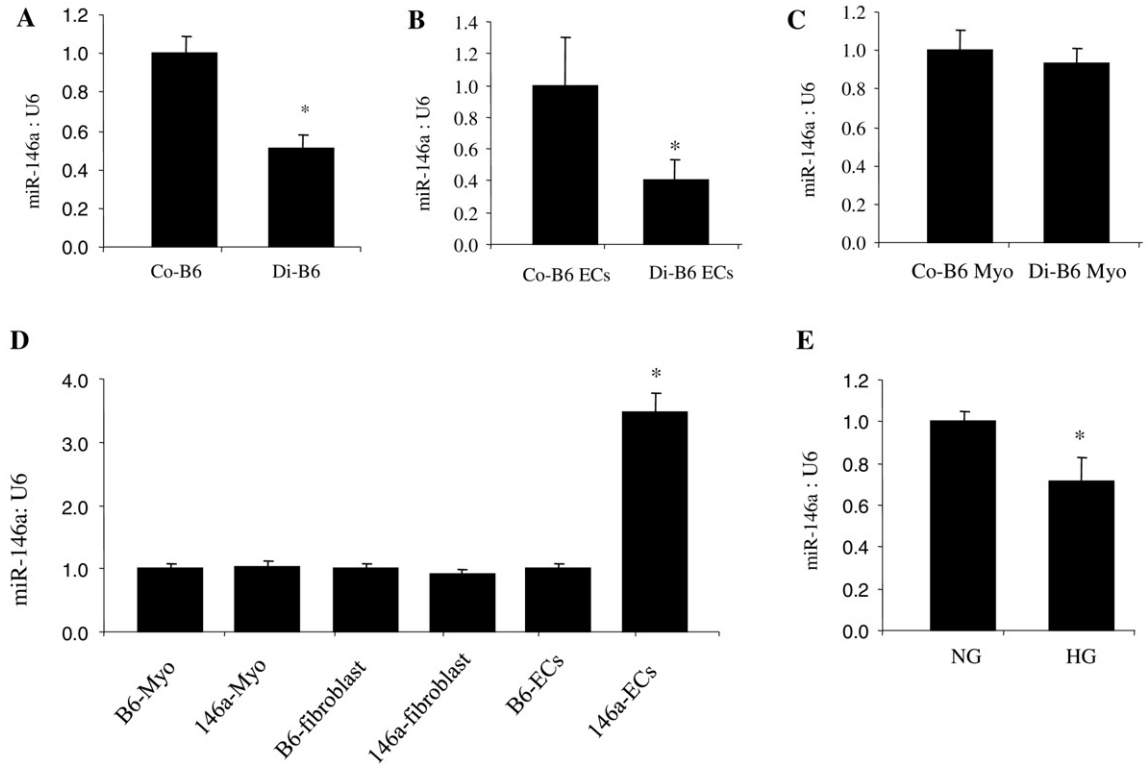

Fig. 1. miR-146a expression analyses showed A) reduced miR-146a in the hearts of wild type animals with diabetes (Di-B6) compared to controls (Co-B6). Such reduction occurred B) primarily in the endothelial cells (isolated from the hearts), but C) not in the isolated cardiomyocytes (Myo). D) Analyses of isolated cells from the heart of wild type mice and miR-146a transgenic mice (146a) showed that miR-146a expression is increased in endothelial cells (ECs), but not in the myocytes and fibroblasts compared to, those in the wild type mice (B6). E) miR-146a levels were decreased in HCMECs treated with HG. [miRNA levels are expressed mean ± SE, as a ratio of U6 snRNA (U6) and normalized to Co where applicable, * = significantly different from Co-B6, B6 EC or NG group, for cells, n = 3–6, for animals, n = 7–8/group].