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MiRNA-92a protects pancreatic B-cell function by targeting KLF2 in diabetes mellitus

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ABSTRACT

Aims: diabetes mellitus is one of the most common metabolic diseases worldwide characterized by insulin resistance and pancreatic β cell dysfunction. miRNA plays an important role in DM. In previous studies, miRNA-92a could function as targets for innovative precision medicines to reduce T1D islet autoimmunity. However, the relationship between miRNA-92a and pancreatic β cell dysfunction remains unknown. The aim of the study was to investigate the role of miRNA-92a in pancreatic β cell dysfunction.
Methods: Apoptosis, proliferation, insulin secretion and cell survival rate were detected to evaluate the function of miRNA-92a.
Results: we found that miRNA-92a could inhibit apoptosis induced by high-glucose environment and increase the insulin secretion and proliferation. Moreover, we identify the KLF2 as direct target of miRNA-92a, suggesting that miRNA-92a may function through regulating KLF2.
Conclusion: Altogether, we verified the function and mechanism of miRNA-92a and provide evidence that miRNA-92a may serve a potential candidate for the clinical treatment for DM.

1. Introduction

Diabetes mellitus (DM) is characterized as high blood glucose conditions and has been identified as the most threaten disease in the world [1]. DM can not only lead to serious health problem, but also caused heavy social economic burdens. Despite significant progress has been put into the clinical treatment and scientific research, the mechanism of DM remains poorly understood [2].

Pancreatic β cell dysfunction has been proved to serve a significant role in the progress and development of DM [3]. Hyperglycemia plays an important role in inducing pancreatic β cell apoptosis and insulin secretion [4]. Pancreatic β cells are specifically sensitive to hyperglycemia and dysfunctional pancreatic β cell can contribute to serious DM symptoms [5]. In present clinical treatment, it is important to maintain the normal glucose conditions and homeostasis. Previous studies have concentrated on how to protect and improve functional β cells to regulate glucose homeostasis [6]. However, the exact mechanisms of the dysfunctional β cells remain unknown.

In recent years, new evidence estimated that approximately 95% of human genome transcripts are non-coding RNAs [7]. miRNA, a specific non coding RNA, 18–25 nucleotides in length, has been proved to associated with various disease, such as heart development [8], diabetes [9], obesity [10], tumor development [11]. miRNA could negatively regulate their target gene by directly binding to their 3′-UTR regions to induce mRNA deregulation and translational repression [12]. Previous studies have revealed that miRNA may play an important role in pancreatic function. For example, miR-375 was demonstrated to negatively regulate glucose-stimulated insulin secretion by regulating the expression of myotrophin and phosphoinositide-dependent protein kinase 1 [13]. The evidence all pointed the importance of miRNAs in disease aetiology.

In previous study, miRNA-92a could function as targets for innovative precision medicines to reduce T1D islet autoimmunity [14]. However, the relationship between miRNA-92a and pancreatic β cell dysfunction remains unknown. The aim of this study was to elucidate the biological functions and potential target of miRNA-92a in pancreatic β cells and to evaluate its role in a high-glucose environment. Our results showed that miRNA-92a could inhibit

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apoptosis induced by high-glucose environment and increase the insulin secretion and proliferation by targeting KLF2. Taken together, we verified the function and mechanism of miRNA-92a and provide evidence that miRNA-92a may serve a potential candidate for the clinical treatment for DM.

2. Materials and methods

2.1. Cell culture and transfection

Min-6 mouse pancreatic β cells were purchased from American Type Culture Collection (Manassas, VA, USA) and cultured in Dulbecco's Modification of Eagle's medium (DMEM; Gibco) containing 10% fetal bovine serum (FBS; Gibco) in 5% CO2 at 37 °C. Min-6 cells were treated with 25 mmol/L glucose for further experiment as previously described [15]. miRNA-92a mimics, miRNA-92a inhibitor and controls were constructed by Shanghai GenePharma (Shanghai, China). Min-6 cells were transfected with 50 nM miRNA-92a mimic, miRNA-92a inhibitor or NC miRNAs with Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. pcDNA-KLF2 transfection was used to perform overexpression experiment. The empty pcDNA3.1 (Invitrogen, Shanghai, China) was used as control. The protocol was same as miRNA-92 transfection.

2.2. Gene detection and western blotting

Total RNA was extracted from Min-6 cells using TRIzol reagent (Thermo Fisher Scientific). The purity of RNA was determined by measuring the absorbance ratio of 260/280 nm using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific) Reverse transcription of RNA was carried out using a PrimeScript™ RT reagent Kit with gDNA eraser (RR047A; Takara, Tokyo, Japan), and cDNA was performed by qRT-PCR using SYBR® Premix Ex Taq™(RR420A; Takara, Tokyo, Japan). The data were normalized using GAPDH levels and further analyzed by the $2^{-\Delta\Delta CT}$ method. All the primers used for qPCR are listed in the Table S1.

Total protein was isolated from Min-6 cells and solubilized using RIPA lysis buffer containing proteinase inhibitor (Sigma, USA). Concentrations of total protein were determined using a BCA assay kit (Pierce, Rockford, IL, USA). Total protein samples (30 μg) were analyzed by 10% SDS-PAGE gel (120 V, 120 mins) and transferred to polyvinylidene difluoride (PVDF) membrane (300 mA, 90 min). After 1 h blocking using milk, the membranes were incubated with primary antibodies against KLF2 (1:1000, Abcam, MA, USA), Caspase3 (1:1000, Abcam, MA, USA), Notch (1:1000, Abcam, MA, USA) at 4 °C overnight. Immunopositive bands were analyzed using a FluorChem M system (ProteinSimple, San Jose, CA, USA).

2.3. Insulin secretion

The cells were seeded in a 96-well plate and cultured for 24 h. ELISA was used to determine the insulin level. Total insulin content was measured after sonication of cells in acid ethanol (2% H_2SO_4), followed by 3 cycles, and then centrifuged for 5 min at 10,000 g. The supernatant was used to further measure the insulin level.

2.4. ROS detection

The cells were seeded in a 6-well plate and cultured for 24 h. The cells were stained with DCFH-DA (10 μmol/L, Beyotime, Shanghai, China) at 37 °C for 30 min and the intracellular ROS was detected using fluorescence microscopy (BX61, Olympus). The results were analyzed by Image J software (version 1.48, USA).

2.5. Cell survival rates assay

Cardiomyocytes were digested for 3 min and then pipetting completely. Trypan bule staining was used to assess the cell survival rates. Cell proliferation was detected using a CCK8 assay kit (Dojindo, Japan). Cells were seeded (1000 cells per well) into 96-well plates, and CCK8 was added 0, 24, 48, 72 and 96 h later for 1 h, after which the OD values were recorded.

2.6. Caspase 3 activity assay

Total protein was isolated from Min-6 cells and solubilized using RIPA lysis buffer containing proteinase inhibitor (Sigma, USA). Concentrations of total protein were determined using a BCA assay kit (Pierce, Rockford, IL, USA). The supernatant were mixed with reaction buffers using Caspase-3 activity kits (caspase-3, Ac-DEVD-pNA) and incubated at 37 °C for 2 h. The Caspase-3 activity was detected by the fluorescence level.

2.7. Luciferase reporter assay

The 3′-UTR of KLF2, with wild-type or mutant (mut) binding sites for miRNA-92a, was amplified and cloned into the pGL3 vector (Promega, Madison, WI, USA) to generate the plasmid pGL3-wt-KLF2-3′-UTR or pGL3-mut-KLF2-3′-UTR. HEK 293 cells were used to perform the luciferase reported assay, and the miRNA-92a mimics or inhibitor and the KLF2 vector were transfected using Lipofectamine 2000 reagent. Luciferase activity was analyzed using Dual-Luciferase system following the manufacture's protocol.

2.8. Statistical analysis

SPSS 13.0 was used to calculate all the values (means ± standard error of the mean (SEM)). Statistical analyses were analyzed with Student's *t*-test. The statistical significance was $P < 0.05$.

3. Results

3.1. Bioinformatics information of miRNA-92a

High-glucose induced dysfunctional pancreatic β cell model is one most commonly used diabetes model. Firstly, we examined the expression level of miRNA-92a in HG conditions. miRNA-92a was decreased under HG conditions in a time dependent manner (Fig. 1A). To evaluate the role of miRNA-92a in pancreatic β cell, we constructed the miRNA-92a mimics to verify the function of miRNA-92a. The expression of miRNA-92a was significantly increased compared with control (Fig. 1B). Furthermore, transcripts of miRNA-92a could be detected in multiple tissues in adult mice (Fig. 1C). Assessment of the distribution indicated that miRNA-92a is mainly expressed in pancreas and lungs, suggesting that miRNA-92a may have an important function in pancreas.

3.2. MiRNA-92a attenuated HG-induced apoptosis in Min-6 cells

To investigate the role of miRNA-92a in HG conditions, caspase3 activity was measured to assess the apoptosis effect. We found that caspase3 activity was increased in HG group, whereas miRNA-92a group showed opposite effect. miRNA-92a could inhibit the HG-induced caspase3 activity (Fig. 1D). In addition, we also detected the protein level of caspase3 through western blotting. Interestingly, the cleaved caspase3 band was significantly decreased compared with control group (Fig. 1E). All the evidence emphasizes the anti-apoptosis effect of miRNA-92a.