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Original Article

miR-29a promotes myocardial cell apoptosis induced by high glucose through down-regulating IGF-1

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Abstract: This study was aimed to investigate the role of miR-29a in myocardial cell apoptosis induced by high glucose. Myocardial cells were cultured in normal (5.6 mmol/l) or high glucose medium (30 mmol/l). The apoptotic rate of myocardial cells was evaluated using flow cytometry. The mRNA levels of *Bax*, *Bcl-2*, *miR-29a*, and *IGF-1* were determined using real-time quantitative PCR (RT-qPCR). The level of IGF-1 in the culture medium was analyzed using enzyme-linked immunosorbent assay (ELISA). The interaction sites between miR-29a and IGF-1 was analyzed using the the Targetscan program. The regulatory effect of miR-29a on the expression of *IGF-1* was investigated using dual luciferase reporter system. The results showed that the expression of *miR-29a* and the *Bax/Bcl-2* ratio in myocardial cells were significantly increased after the cells were cultured in high glucose medium for 72 h, which was consistent with increased apoptosis of myocardial cells. The expression of *IGF-1* in myocardial cells was significantly decreased after the cells were cultured in high glucose medium for 72 and 96 h. Targetscan identified a potential binding site on the 3'-UTR of IGF-1 for miR-29a. We also observed that miR-29a mimic and miR-29a inhibitor reduced and increased the expression of *IGF-1* in myocardial cells cultured in high glucose medium, respectively. Dual luciferase reporter analysis showed that miR-29a significantly reduced the fluorescence intensity of wild-type psichek2-IGF-1-3'UTR-WT but the fluorescence intensity of mutant psichek2-IGF-1-3'UTR-MT was not significantly affected. In conclusions, the expression of miR-29a in myocardial cells cultured in high glucose medium was significantly increased, which down-regulated IGF-1 and increased myocardial cell apoptosis.

Keywords: miR-29a, myocardial cells, IGF-1, myocardial cell apoptosis, high glucose

Introduction

With increasing incidence and prevalence of diabetes mellitus (DM), research on the pathogenesis of DM and its complications has been highlighted [1]. Chronic complications of DM, especially cardiovascular complications, are the major reasons causing disability in DM patients. Diabetic cardiomyopathy (DCM) is one of the common complications of cardiovascular system in DM patients. DCM, a myocardial metabolic disorder first described by Hamby in 1974 [2], is characterized by extensive histopathological changes of myocardial microvascular and myocardial fibrosis, leading to left ventricular hypertrophy and systolic and/or diastolic dysfunction.

DCM is a complex pathological process in which numerous factors are involved. In DM, elevated blood glucose, insulin deficiency, insulin resistance, and direct toxic effects of hyperinsulinemia on myocardial cells may cause myocardial metabolic disorder, oxidative stress, abnormal activation of the neuroendocrine system, accumulation of non-enzymatic glycosylation products, and calcium signaling dysfunction, leading to myocardial apoptosis, cardiac remodeling, myocardial fibrosis, abnormal cardiac contraction, and heart failure [3]. Myocardial apoptosis is a central event in the development of DCM. Elevated level of blood glucose induces myocardial apoptosis. In addition, extensive myocardial apoptosis can aggravate cardiac remodeling and dysfunction in the development of DCM [4, 5].

It has been shown that microRNA (miRNA) plays an important role in the development of diabetes and its complications [6-9]. Previous stud-

Table 1. Primers used in RT-qPCR assay in the present study

Gene	Primer	Primer sequence (5'->')
miR-29a	Forward primer	CGGCGGTAGCACCATCTGAAAT
	Reverse primer	CCAGTGCAGGGTCCGAGGTA
U6	Forward primer	CTCGCTTCGGCAGCACA
	Reverse primer	AACGCTTCACGAATTTGCGT
Bcl-2	Forward primer	CTGGTGGACAACATCGCTCT
	Reverse primer	GCATGCTGGGGCCATATAGT
Bax	Forward primer	CGAGAGGTCTTCTTCCGTGT
	Reverse primer	GATCAGCTCGGGCACTTTAG
IGF-1	Forward primer	GGACCAAGGGGCTTTTACTT
	Reverse primer	GCAACACTCATCCACAATGC
GAPDH	Forward primer	GCTCTCTGCTCCTCCCTGTTCT
	Reverse primer	GCCAAATCCGTTCACACCGACCT

ies have reported that the miR-29 family members are involved the development of cardiovascular complications of DM [10-13]. In addition, miR-29 is also associated with tumor cell proliferation and apoptosis [14, 15]. Therefore, we speculate that miR-29a, a representative of the miR-29 family, is involved in myocardial apoptosis in DCM.

Insulin signal transduction is considered to be one of the protective factors against the diabetic vascular complications. Insulin resistance in adipocyte caused by the elevating miR-29a expression level in the muscles, adipocyte and liver of typeII diabetes mellitus mice could be due to the inactivated Akt in the insulin signal pathway [16]. Insulin-like growth factor-1 (IGF-1), which is widespread in various tissues, can regulate the proliferation and growth of many types of cells, and play important roles in embryonic heart development, and postnatal cardiac morphology and function regulation [17]. Based on bioinformatic prediction, we found that insulin-like growth factor-1 (IGF-1) was a potential target gene of miR-29a. Therefore, we speculate miR-29a and IGF-1 may interact with each other to regulate myocardial apoptosis induced by high glucose.

In the present study, we investigated the expression of miR-29a in myocardial cells cultured in high glucose medium. The potential target genes of miR-29a were predicted using bioinformatic programs and the regulation of miR-29a on the expression of its target genes were confirmed using the *dual-luciferase reporter* (DLR™) assay system. Our results are useful for understanding the role and mechanism of miR-29a in the apoptosis of myocardial cells in DCM, and may provide new therapeutic targets for the prevention and treatment of DCM.

Materials and methods

Cell culture

All animal experiments were approved and supervised by the Animal Care and Use Committee of Sun Yat-sen Universitythe and it conforms to the provisions of the 1964 Declaration of Helsinki and its later amendments. Sprague Dawley (SD) rats (1-3 days of age) were obtained from the Department of Experimental Animal Research Center, Sun Yat-sen University. The SD rat heart was surgically dissected and digested using double enzymes. The myocardial cells were cultured in DMEM medium in an incubator containing 5% CO_2 at 37°C.

Flow cytometry

Myocardial cells were collected and inoculated onto a 6-well plate at a density of 2×10^5 cells/well. After incubated in 0.1 mM BrdU (5-Bromo-2-deoxyUridine, SIGMA, St. Louis, MO, USA) for 48 hours, the medium was removed and cells were washed using phosphate buffered saline (PBS). Then, the cells were treated with low-glucose DMEM (5.6 mmol/l) or high glucose DMEM (30 mmol/l) in 10% fetal bovine serum (FBS) for 24-96 hours. Appropriate amount of fresh trypsin (0.125%) was added to harvested cells for digestion. Equal amount of FBS (10%) was added to terminate trypsin digestion when cells started to be round. The suspension was centrifuged at 4°C and 500 rpm/min for 5 min. Then 1 mL pre-cooled PBS was added to suspend cell pellet, and the suspension was centrifuged at 4°C and 500 rpm/min for 5 min. After three times of centrifugation and suspension, myocardial cells were suspended in binding buffer (500 μl), AnnexinV-FITC (5 μl) (Biosea, Beijing, China), and propidium iodide (5 μl). After the cell suspension was placed at room temperature for 15 min, binding buffer was added into the cell suspension for flow cytometry analysis (BD Biosciences, Franklin Lakes, NJ, USA) within an hour.

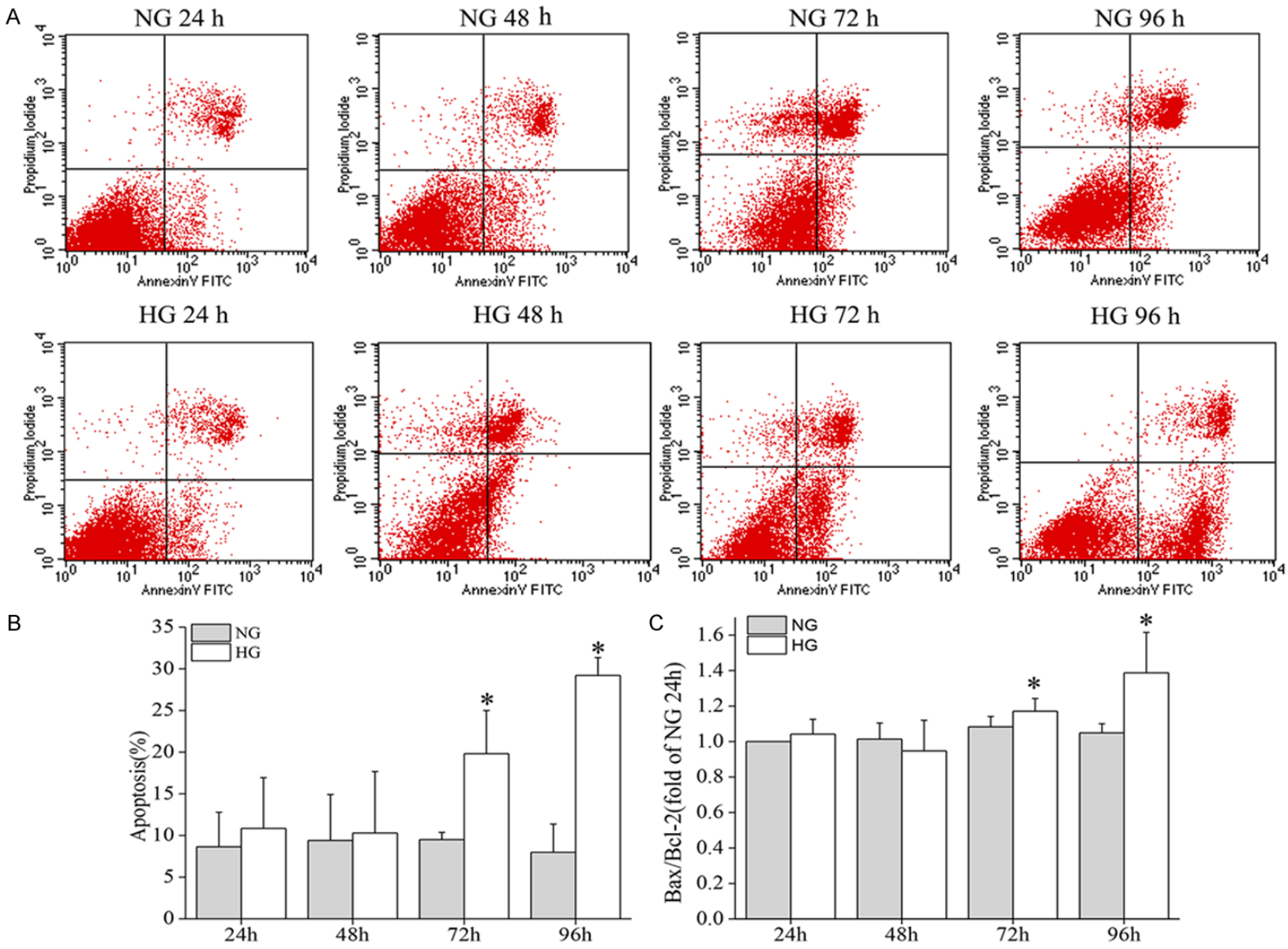

Figure 1. Influence of high glucose medium on the apoptosis of myocardial cells. A, B. The rate of apoptotic myocardial cells cultured in normal (NG) or high glucose (HG) medium for 24, 48, 72, and 96 hours. C. The ratio of the expression of *Bax* and *Bcl-2* in myocardial cells cultured in normal (NG) or high glucose (HG) medium for 24, 48, 72, and 96 hours. The expression of *Bax* and *Bcl-2* was evaluated using RT-qPCR and GAPDH was used as a loading control. **P*<0.05 vs NG 24 h.

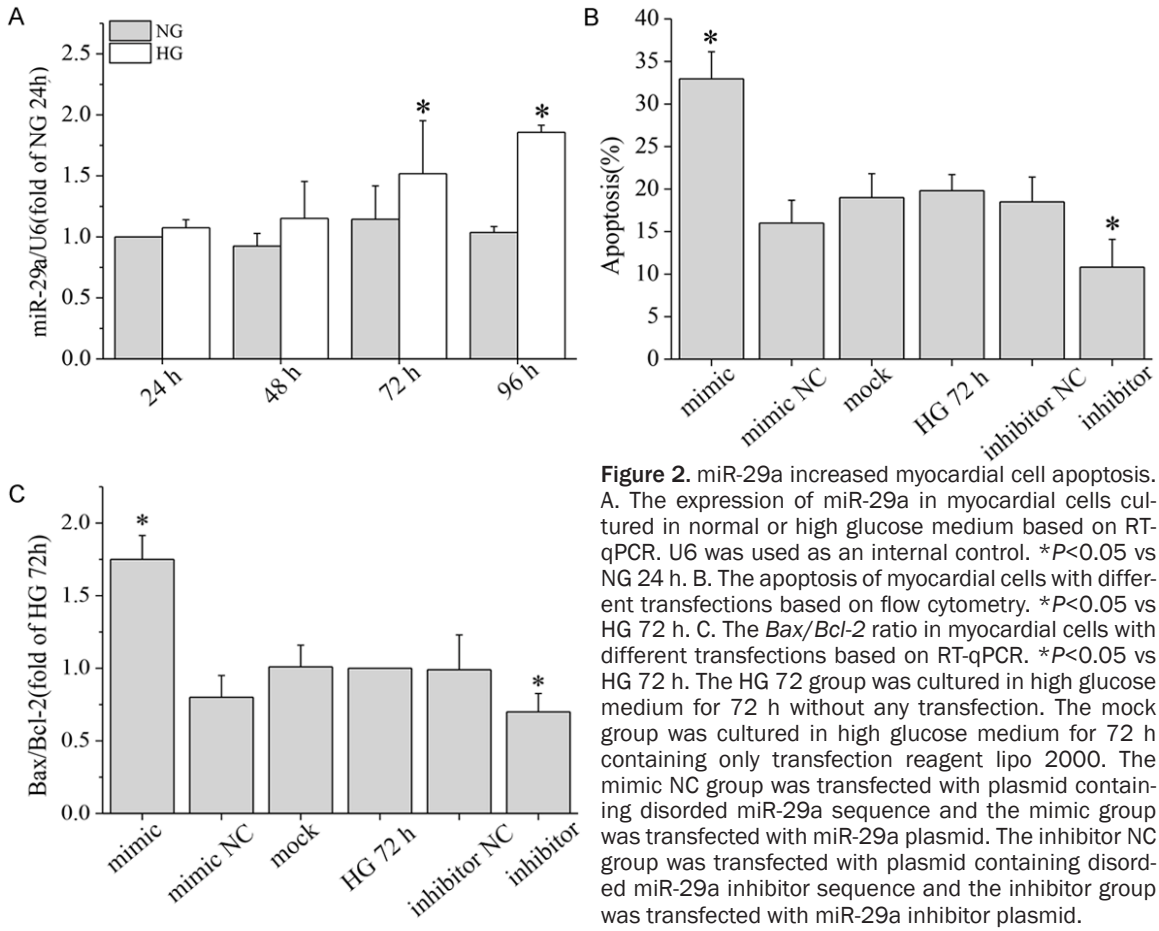

Figure 2. miR-29a increased myocardial cell apoptosis. A. The expression of miR-29a in myocardial cells cultured in normal or high glucose medium based on RT-qPCR. U6 was used as an internal control. **P*<0.05 vs NG 24 h. B. The apoptosis of myocardial cells with different transfections based on flow cytometry. **P*<0.05 vs HG 72 h. C. The *Bax/Bcl-2* ratio in myocardial cells with different transfections based on RT-qPCR. **P*<0.05 vs HG 72 h. The HG 72 group was cultured in high glucose medium for 72 h without any transfection. The mock group was cultured in high glucose medium for 72 h containing only transfection reagent lipo 2000. The mimic NC group was transfected with plasmid containing disorded miR-29a sequence and the mimic group was transfected with miR-29a plasmid. The inhibitor NC group was transfected with plasmid containing disorded miR-29a inhibitor sequence and the inhibitor group was transfected with miR-29a inhibitor plasmid.

Real-time quantitative PCR (RT-qPCR)

Total RNA was isolated from myocardial cells using the Trizol according to the protocol previously described (Invitrogen, Life Technology, Carlsbad, CA). Complementary DNA (cDNA) was synthesized from the isolated RNA (0.5 μg) using a reverse transcription kit (Invitrogen, Carlsbad, CA, USA). The primers used in RT-qPCR were listed in **Table 1**. RT-qPCR of triplicates was conducted in a Roche 96-well PCR plate using a Roche LightCycler480 Real-time PCR instrument (Roche, USA). RT-qPCR program for miR-29a, U6, Bcl-2 and Bax genes were a total of 40 cycles of denaturation at 95°C for 20 s, degeneration at 95°C for 10 s, annealing at 60°C for 20 s, and extension at 70°C for 5 s, and the program for IGF-1 gene was a total of 40 cycles of denaturation at 95°C for 30 seconds, degeneration at 95°C for 5 s, and annealing at 60°C for 20 s. The expression of miR-29a was normalized with the mRNA level of U6, and the expression of *Bcl-2*, *Bax*, and IGF-1 genes was normalized by the mRNA level of GAPDH. The expression changes of these genes in the experimental group compared to the control group was calculated using the $2^{-\Delta\Delta Ct}$ formula.

Cell transfection

Primary myocardial cells were inoculated into a six-well plate at a density of 2×10^5/well and cultured in BrdU medium for 48 h. Then, the BrdU medium was removed and cells were washed for 2-3 times using PBS. The myocardial cells were transfected with miR-29 mimic or miR-29 inhibitor using the ribo FECT™ CP Transfection Kit (Ribobio, Guangzhou, China). The transfection experiment was conducted for six groups. The HG 72 group was cultured in high glucose medium for 72 h without any transfection. The mock group was cultured in high glucose medium for 72 h containing only transfection reagent lipo 2000. The mimic NC group was transfected with plasmid containing disorded miR-29a sequence and the mimic group was transfected with miR-29a plasmid. The inhibitor NC group

	predicted consequential pairing of target region (top) and miRNA (bottom)	seed match	site-type contri-bution	3' pairing contri-bution	local AU contri-bution	position contri-bution	TA contribution	SPS contribution	context+ score	context+ score percentile	conserved branch length
Position 970-977 of IGF1 3' UTR rno-miR-29b	5' ...UUGUUUUUAGUACAAUGGUGCUA... 3' UUGUGACUAAAGUUUACCACGAU	8mer	-0.247	-0.008	-0.083	0.131	-0.003	-0.085	-0.30	89	2.708
Position 970-977 of IGF1 3' UTR rno-miR-29a	5' ...UUGUUUUUAGUACAAUGGUGCUA... 3' AUUGGCUAAAGUCUACCACGAU	8mer	-0.247	-0.008	-0.083	0.131	-0.003	-0.085	-0.30	89	2.708
Position 970-977 of IGF1 3' UTR rno-miR-29c	5' ...UUGUUUUUAGUACAAUGGUGCUA... 3' AUUGGCUAAAGUUUACCACGAU	8mer	-0.247	-0.008	-0.083	0.131	-0.003	-0.085	-0.30	89	2.708
Position 3560-3567 of IGF1 3' UTR rno-miR-29c	5' ...CCAGCUACGCCAAUGUGGUGCUA... 3' AUUGGCUAAAGUUUACCACGAU	8mer	-0.247	0.013	0.003	0.265	-0.003	-0.085	-0.05	16	2.963
Position 3560-3567 of IGF1 3' UTR rno-miR-29b	5' ...CCAGCUACGCCAAUGUGGUGCUA... 3' UUGUGACUAAAGUUUACCACGAU	8mer	-0.247	0.013	0.003	0.265	-0.003	-0.085	-0.05	15	2.963
Position 3560-3567 of IGF1 3' UTR rno-miR-29a	5' ...CCAGCUACGCCAAUGUGGUGCUA... 3' AUUGGCUAAAGUCUACCACGAU	8mer	-0.247	0.024	0.003	0.265	-0.003	-0.085	-0.04	12	2.963

Figure 3. Predicted gene targets of miR-29a using the Targetscan program.

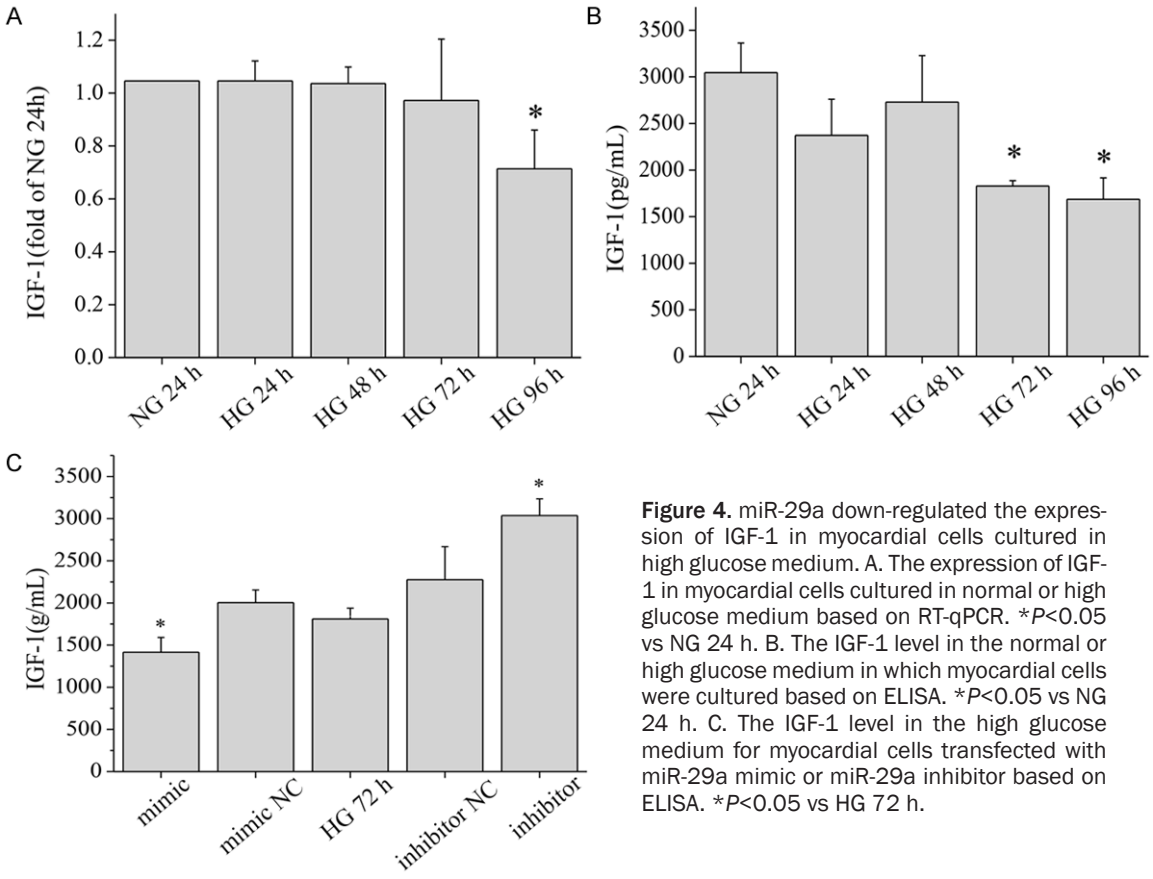

Figure 4. miR-29a down-regulated the expression of IGF-1 in myocardial cells cultured in high glucose medium. A. The expression of IGF-1 in myocardial cells cultured in normal or high glucose medium based on RT-qPCR. **P*<0.05 vs NG 24 h. B. The IGF-1 level in the normal or high glucose medium in which myocardial cells were cultured based on ELISA. **P*<0.05 vs NG 24 h. C. The IGF-1 level in the high glucose medium for myocardial cells transfected with miR-29a mimic or miR-29a inhibitor based on ELISA. **P*<0.05 vs HG 72 h.

was transfected with plasmid containing disorded miR-29a inhibitor sequence and the inhibitor group was transfected with miR-29a inhibitor plasmid.

Determination of the IGF-1 level in the medium of myocardial cells using enzyme-linked immunosorbent assay (ELISA)

Determination of the IGF-1 concentration in the myocardial cells culture was conducted using the rat IGF-1 ELISA kit (R&D System, McKinley Place NE, Minneapolis, MN, USA) according to the manufacture's instruction. The OD450 value was detected in a Wellscan MK3 microplate reader (Labsystem Dragon, USA). The standard curve was established according to the absorbance value of the standard and the concentration of IGF-1 in each sample was determined according to the relative position of each sample in the standard curve.

Dual luciferase assay

The DNA sequence of *IGF-1* gene (GenBank accession number: AH002176.1) was retrieved from NCBI nucleotide database. The 3'-UTR of *IGF-1* was cloned into plasmid psichek2 (Promega, Madison, WI, USA) between the *XhoI* and *NotI* sites to construct the psichek2-IGF-3'UTR-WT plasmid. The "TGGTGCT" in the 3'-UTR of *IGF-1* in the psichek2-IGF-3'UTR-WT plasmid was mutated to "GTTGCTG" to generate the plasmid psichek2-IGF-3'UTR-MT. To investigate whether miR-29a interacts with the 3'-UTR of *IGF-1*, 293T cells were transfected with plasmids psichek2-IGF-3'UTR-WT or psichek2-IGF-3'UTR-MT with miR-29a mimic, miR-29a inhibitor, or their controls. Therefore, there were a total of eight transfection groups including the mimics NC+WT, NC mimics+MT, miR-29a mimics+WT, miR-29a mimics+MT, NC inhibitor+WT, NC inhibitor+MT, miR-29a inhibitor+WT, and miR-29a inhibitor+MT.

The 293T cells at logarithmic growth phase were inoculated into a 96-well plate (4×10^3 cells per well) and cultured in an incubator containing 5% CO_2 at 37°C for 24 h. Cell transfection was conducted using the DharmaFECT Duo Transfection Reagent according the manufac-

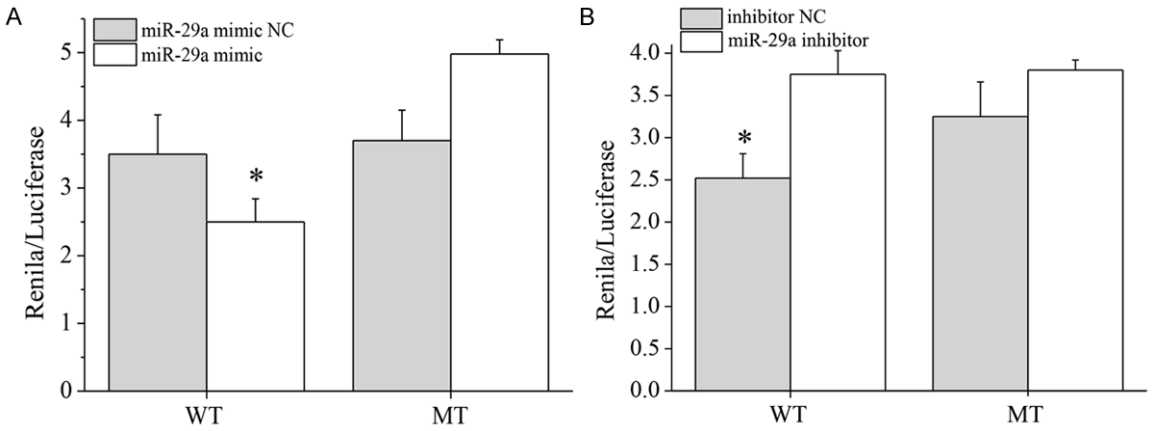

Figure 5. miR-29a down-regulated the expression of IGF-1 in myocardial cells in high glucose medium. A. The luciferase activity in myocardial cells transfected with psichek2-IGF-1-3'UTR-WT and miR-29a mimic was significantly lower than that in myocardial cells transfected with psichek2-IGF-1-3'UTR-WT and miR-29a mimic NC ($P<0.05$). B. The luciferase activity in myocardial cells transfected with psichek2-IGF-1-3'UTR-WT and miR-29a inhibitor NC was significantly lower than that in myocardial cells transfected with psichek2-IGF-1-3'UTR-WT and miR-29a inhibitor ($P<0.05$).

ture's introduction. The luciferase enzyme activity was detected 48 h after transfection using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA).

Results

High glucose increased myocardial cell apoptosis

Flow cytometry and RT-qPCR results showed that the apoptosis of myocardial cells was not significantly affected by prolonged incubation in the medium containing normal concentration of glucose (5.6 mmol/L). No significant changes in myocardial cell apoptosis and the Bax/Bcl-2 ratio were observed when myocardial cells were cultured in high glucose medium (30 mmol/L) for 24 h and 48 h compared to myocardial cells cultured in normal glucose medium (5.6 mmol/L). However, the apoptosis of myocardial cells and the Bax/Bcl-2 ratio were significantly increased in myocardial cells cultured in high glucose medium for 72 and 96 h compared with that in myocardial cells cultured in normal glucose medium for 24 h (**Figure 1**), suggesting that high glucose increased myocardial cell apoptosis.

Increased expression of miR-29a in myocardial cells cultured in high glucose medium promoted myocardial cell apoptosis

The expression of miR-29a in myocardial cells cultured in normal (5.6 mmol/L) or high (30 mmol/L) glucose medium was evaluated using RT-qPCR. Our results showed that the expression of miR-29a in myocardial cells cultured in normal glucose medium was not significantly affected by the culture time (24-96 h) (**Figure 2A**). No significant changes in the expression of miR-29a in myocardial cells cultured in high glucose medium for 24 and 48 h compared to that in myocardial cells cultured in normal glucose medium for 24 h. However, the expression of miR-29a in myocardial cells cultured in high glucose medium for 72 and 96 h was significantly higher than that in myocardial cells cultured in normal glucose medium for 24 h, suggesting that high glucose medium increased the expression of miR-29a in myocardial cells.

To investigate the effects of increased expression of miR-29a on myocardial cell apoptosis, myocardial cells were transfected with different plasmids and cultured in distinct media. The transfection experiment was conducted for six groups. The apoptosis of myocardial cells of different groups was analyzed 72 h after transfection and compared with that of the HG 72 h group using flow cytometry. We found that miR-29a mimic transfection significantly increase myocardial cell apoptosis and miR-29a inhibitor significantly reduced myocardial cell apoptosis (**Figure 2B**). In addition, RT-qPCR assay showed that the Bax/Bcl-2 ratio in the miR-29a mimic and miR-29a inhibitor groups were significantly higher and lower than that of the HG 72 h group (**Figure 2C**), respectively, suggesting that miR-29a increased myocardial cell apoptosis.

miR-29a promoted myocardial cell apoptosis through down-regulating IGF-1

Over 800 potential targets of miR-29a have been identified based on bioinformatics prediction. Through preliminary function screening, we selected IGF-1 to further study its interaction with miR-29a and its role in myocardial cell apoptosis. Based Targetscan analysis, we found that the eight nucleotides (5'-UGGUGCUA-3)', 970-977nt and 3560-3567nt on the 3'-UTR of IGF-1, were completely complementary with the 5' seed sequence of miR-29a, suggesting that IGF-1 is the target gene of miR-29a (**Figure 3**).

Next, we evaluated the expression of IGF-1 in myocardial cells using RT-qPCR. No significant difference in the expression of IGF-1 was observed between myocardial cells cultured in high glucose medium for 24, 48, or 72 h and myocardial cells cultured in normal glucose medium for 24 h. However, the level of IGF-1 mRNA in myocardial cells cultured in high glucose medium for 96 h was significantly lower than that in myocardial cells cultured in normal glucose medium for 24 h (**Figure 4A**). The ELISA results were consistent with the RT-qPCR results. Briefly, no significant difference in the IGF-1 level was detected between the high glucose medium in which myocardial cells were cultured for 24 or 48 h and the normal glucose medium in which myocardial cells were cultured for 24 h. However, the level of IGF-1 in the high glucose medium in which myocardial cells were cultured for 72 or 96 h was significantly lower than that in the normal glucose medium in which myocardial cells were cultured for 24 h (**Figure 4B**).

To verify the interaction between miR-29a and IGF-1, ELISA was used to detection IGF-1 level in the high glucose medium in which myocardial cells transfected with miR-29a mimic or miR-29a inhibitor were cultured. Our results showed that the IGF-1 level in the medium in which myocardial cells transfected with miR-29a mimic were cultured was significantly lower than that in the high glucose medium in which myocardial cells were cultured for 72 h. In addition, the IGF-1 level in the medium in which myocardial cells transfected with miR-29a inhibitor were cultured was significantly higher than that in the high glucose medium in which myocardial cells were cultured for 72 h (**Figure 4C**). No significant difference in the IGF-1 level was observed between the media for myocardial cells transfected with mimic NC and inhibitor NC.

To further investigate the interaction between miR-29a and IGF-1, we constructed two plasmids, psichek2-IGF-3'UTR-WT and psichek2-IGF-3'UTR-MT, which contain the wide-type and mutant of the 3'-UTR of IGF-1, respectively. Each plasmid was co-transfected with miR-29a mimic or miR-29a inhibitor into myocardial cells. Our results showed that the luciferase activity in myocardial cells transfected with psichek2-IGF-1-3'UTR-WT and miR-29a mimic was significantly lower than that in myocardial cells transfected with psichek2-IGF-1-3'UTR-WT and miR-29a mimic NC (**Figure 5A**). No significant difference in the luciferase activity was detected between myocardial cells transfected with psichek2- IGF-1-3'UTR-MT and miR-29a mimic and myocardial cells transfected with miR-29a mimic NC. In addition, the luciferase activity in myocardial cells transfected with psichek2-IGF-1-3'UTR-WT and miR-29a inhibitor NC was significantly lower than that in myocardial cells transfected with psichek2-IGF-1-3'UTR-WT and miR-29a inhibitor (**Figure 5B**). No significant difference in the luciferase activity was detected between myocardial cells transfected with psichek2-IGF-1-3'UTR-MT and miR-29a inhibitor and myocardial cells transfected with negative control (**Figure 5B**). Taken together, these result suggest that miR-29a down-regulated the expression of IGF-1 in myocardial cells.

Discussion

Myocardial cell apoptosis plays an important role in ventricular remodeling and cardiac function failure in DCM. High blood glucose is the initial factor leading to DCM, therefore, myocardial cell apoptosis induced by high blood glucose is an essential event in the development of DCM. Shizukuda et al. [13] found that the apoptosis of myocardial cells isolated from adult rats increased three times after cultured in high glucose medium for 24 h. Increased apoptosis of myocardial cells has also been observed in diabetes patients and animal model of diabetes, and myocardial cell apoptosis could be significantly inhibited by antioxidants or specific inhibitors against apoptosis signaling pathways [18, 19]. In the present study, we found that the apoptosis of myocar-

dial cells cultured in high glucose medium increased with the extension of culture time. After 72 h of culturing in high glucose medium, the apoptosis of myocardial cells was significantly higher than that cultured in normal glucose medium. Our results are consistent with previous reports.

The Bcl-2 family members, which are closely involved in apoptosis, can be divided into two categories. One category inhibits apoptosis, such as Bcl-2, Bcl-xl, and Mcl-1. Another category promotes apoptosis, including Bax, Bak, and Bok. Previous studies have shown that the survival or death of cells was mainly determined by the ratio of Bax/Bcl-2. Apoptosis occurs when the Bcl-2/Bax ratio is over 1 and cells survive when the Bcl-2/Bax ratio is less than 1 [20, 21]. In the present study, we found that the Bcl-2/Bax ratio and apoptosis of myocardial cells increased when the cells were cultured in high glucose medium, which is consistent with the flow cytometry results.

The miR-29 family is widely involved in the development of diabetes and its complications, differentiation and apoptosis of tumor cells, and fibrosis of heart, kidney, and liver. The miR-29 family has three isomers, miR-29a, miR-29b, and miR-29c, which share identical seed sequence and similar biological functions. In the present study, we investigated the role of miR-29a in the development of DCM. We found that the expression of miR-29a was increased in myocardial cells cultured in high glucose medium, which was accompanied by increased apoptosis of myocardial cells, suggesting that miR-29a is involved in myocardial cell apoptosis induced by high glucose.

IGF-1 regulate the proliferation of many types of cells, which is essential for embryonic heart development and postnatal cardiac morphology and function. The myocardial protection effect of IGF-1 has been previously confirmed [22]. In addition, recent studies have demonstrated that IGF-1 inhibited apoptosis through activating PI3, MEK-1, and Raf-1 signaling pathways and BAD phosphorylation [22-25]. In the present study, IGF-1 was focused in the apoptosis of myocardial cells based on bioinformatics analysis of the target genes of miR-29a. We found that the levels of IGF-1 mRNA and protein in myocardial cells were significantly reduced after the myocardial cells were cultured in high glucose medium for 72 h. We then transfected myocardial cells with miR-29a mimic or miR-29a inhibitor to investigate the interaction between miR-29a and IGF-1. Our results showed that miR-29a mimic and miR-29a inhibitor transfection significantly reduced and increased the expression of IGF-1, respectively. In addition, increased miR-29 expression and reduced IGF-1 expression were consistent with promoted apoptosis of myocardial cells, suggesting that miR-29a increased myocardial cell apoptosis through down-regulating IGF-1. Down-regulation of IGF-1 by miR-29a was further confirmed by the dual luciferase reporter analysis. Taken together, our results suggest that miR-29a promoted myocardial cell apoptosis by down-regulating IGF-1.

In summary, we studied the role of miR-29a and IGF-1 in the apoptosis of myocardial cells induced by high glucose. We found increased expression of miR-29a in myocardial cells cultured in high glucose medium. In addition, we observed increased apoptosis and reduced expression of IGF-1 of myocardial cells cultured in high glucose medium. Bioinformatics analysis, over-expression and inhibition of miR-29a, and mutation analysis of the 3'-UTR of IGF-1 suggest that IGF-1 was the target gene of miR-29a and played an important role in myocardial cell apoptosis.

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Disclosure of conflict of interest

None.

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