European Review for Medical and Pharmacological Sciences 2020; 24: 6961-6970

MicroRNA-323-3p inhibits oxidative stress and apoptosis after myocardial infarction by targeting TGF-β2/JNK pathway

C.-C. SHI[1], L.-Y. PAN[2], Y.-Q. ZHAO[3], Q. LI[3], J.-G. LI[3]

[1]Department of Intensive Care Unit, Henan Provincial People's Hospital, Zhengzhou, China
[2]Department of Physiology, Henan Health Cadre College, Zhengzhou, China
[3]Department of Intensive Care Unit, Zhongnan Hospital of Wuhan University, Wuhan, China

Abstract. – OBJECTIVE: Myocardial infarction (MI), which causes irreversible damage and loss of cardiomyocytes, is the most important cause of death in the world. MicroRNA is an important regulator of physiological and pathological activities of cardiovascular system. The aim of this research was to study the effect of microRNA-323-3p (miR-323-3p) on MI and its underlying mechanisms of action.

MATERIALS AND METHODS: A rat model of MI was established to measure the expression of miR-323-3p, Bax, Bcl-2, SOD1, and SOD2 in ischemic myocardial tissue, and the cardiac function of rats were tested at seventh day after MI. H9c2 cells were divided into control group, miRNA negative control (NC) transfection group, miR-323-3p mimic (miR-323-3p min) transfection group, and then, treated with H2O2. Oxidative stress and apoptosis of H9c2 cells were observed by Western blot, Real Time-Polymerase Chain Reaction (RT-PCR), flow cytometry, SOD activity assay, TUNEL staining, DHR dye assay, etc.

RESULTS: The level of miR-323-3p was decreased in ischemic myocardium, as well as H2O2-treated H9c2 cells. MiR-323-3p overexpression greatly decreased the level of Bax and increased the levels of SOD1, SOD2, and Bcl-2. After treated with miR-323-3p mimic, TUNEL positive cells were greatly reduced, and apoptosis rate of H9c2 cells was greatly decreased. Moreover, SOD levels significantly increased, while ROS production decreased after treatment of miR-323-3p. After intravenous injection of miR-323-3p agomir in rats with MI, the cardiac function of the rats was significantly improved. Western blot and Luciferase reporter gene experiments illustrated that miR-323-3p acts by targeting TGF-β2.

CONCLUSIONS: MiR-323-3p was downregulated in ischemic myocardium and H2O2-treated H9c2 cells, and miR-323-3p overexpression reduced oxidative stress and apoptosis of cardiomyocytes. The protective function was achieved via regulation of TGF-β2/JNK pathway.

Key Words:
Myocardial infarction (MI), MiR-323-3p, TGF-β2.

Introduction

MI, as a typical representative of coronary heart disease, seriously endangers human health. In recent years, the global mortality rate of myocardial infarction has generally increased. In the United States, more than 360,000 people die of coronary heart disease each year, and 120,000 of them die from MI[1]. Drug interventions, lifestyle changes, and healthy diets can help improve symptoms in patients, and surgeries, such as coronary artery bypass grafting, can restore blood supply and reduce myocardial damage[2]. However, existing treatments still fail to fundamentally repair necrotic myocardium and improve impaired cardiac function. How to improve survival rate of myocardial cells, inhibit oxidative stress, reduce apoptosis, promote angiogenesis, reduce myocardial fibrosis and inflammation, and improve cardiac function after MI are the focus of research in myocardial infarction[3].

The mature miRNA consists of approximately 22 nucleotides and inhibits translation or promotes degradation of the target mRNA by specifically connecting to the 3'-untranslated region (3'-UTR) of the target mRNA[4]. The target genes regulated by miRNAs are not specific. Filipowicz et al[5] have shown that one miRNA can regulate multiple different mRNAs, and one mRNA could also be regulated by many miRNAs simultaneously. MiRNAs are involved in the pathophysiological processes of many diseases, such as signal transduction between various cells, cell proliferation and death, myocyte contraction and relaxation, neuronal formation, cardiovascular disease, and

Corresponding Author: Jianguo Li, MM; e-mail: lijianguo1817@163.com

regulation after viral infection[6]. Currently, lots of studies have confirmed the important role of miRNAs in the pathology and physiology of cardiovascular system. In particular, some miRNAs could regulate myocardial oxidative stress and apoptosis. Zhu et al[7] show that miR-21, miR-133, and miR-499 can prevent the oxidative stress and apoptosis of cardiomyocytes, while miR-1, miR-199a, and miR-320 can facilitate oxidative stress and apoptosis.

Many articles have studied the role of miR-323-3p in ovary, skeletal muscle, lung, and nerve. Zhao et al[8] suggested that miR-323-3p had anti-apoptotic effect on cumulus cells. Qin et al[9] suggested that miRNA-323-3p could promote myogenesis. But no one has studied its role in oxidative stress and apoptosis of cardiomyocytes.

In this paper, we studied the regulation of miR-323-3p in oxidative stress and apoptosis of myocardial cells using MI rats and H_2O_2-induced H9c2 cell injury model. Our results suggested that miR-323-3p may provide a new treatment for MI.

Materials and Methods

Rat MI Model

This study was approved by the Animal Ethics Committee of Wuhan University Animal Center. Forty Sprague Dawley (SD) rats (body weight 250-300 g, male) were purchased from Shanghai Experimental Animal Center of Chinese Academy of Sciences (Shanghai, China). All rats were divided into four groups: sham, MI, MI with NC, MI with agomiR-323-3p. SD rats were anesthetized using 10% chloral hydrate, fixed on a hard plate, connected to an electrocardiograph, shaved on the left side of the chest, disinfected, intubated, connected to the ventilator, and selected the fourth intercostal incision on the left chest. Then, we cut the skin and fascia in turn, bluntly separated the chest muscles with a vascular clamp, opened the chest, tore the pericardium with tweezers, exposed the heart, found the left coronary artery, and ligatured the left coronary artery 2-3 mm below the left auricle. The whiteness of the ligated site and distal myocardium, as well as the significant elevation of ST segment in the electrocardiogram (> 0.2 mv), were observed to determine whether the modeling was successful. The muscles, aponeurosis, and skin were then sutured layer by layer, again disinfected and penicillin was intramuscularly injected with 100,000 units. The rats in the sham group were only opened the chest without being ligated. The miR-323-3p agomir (Novogene, Nanjing, China) was the same double-stranded RNA analog as mature mmu-miR-323-3p. The agomiR-323-3p (5 mg/kg) or negative control (NC) was injected *via* the tail vein immediately after rat MI to investigate the role of miR-323-3p. The levels of miR-323-3p in rat myocardium on the first day, the fourth day, and the seventh day after injection of agomiR-323-3p were measured. On the seventh day after surgery, the cardiac function and the expression of SOD1, SOD2, Bax, and Bcl-2 in the border zones of myocardium were detected.

Echocardiographic Measurement

Rats underwent echocardiography on the seventh day after MI. We calculated left ventricular ejection fraction (EF) and fractional shortening (FS).

Cell Culture and Transfection

Rat cardiomyocytes H9c2 were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Rockville, MD, USA) containing 15% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) at 37°C and 5% CO_2. Then, we placed the cells into a 6-well plate. After 24 hours, we transfected miR-323-3p mimic and NC (RiboBio, Guangzhou, China) into H9c2 cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Transfection was performed in accordance with the protocols. After transfected for 48 hours, H9c2 cells were treated with H_2O_2 (100 μM) for 4 hours to generate H9c2 cell injury model.

Western Blot

The total protein of H9c2 cells and rat myocardial tissue was extracted with radioimmunoprecipitation assay (RIPA) buffer (Beyotime, Shanghai, China). Then, we used bicinchoninic acid (BCA) method (Beyotime, Shanghai, China) to detect the protein concentration. 50 μg of total protein was taken for electrophoresis and then transferred to the polyvinylidene difluoride (PVDF; EpiZyme, Shanghai, China) membrane. After that, 5% skim milk was used to block the non-specific antigens of the bands at room temperature for 2 hours. Then, the primary antibodies [SOD1, Abcam, Cambridge, MA, USA, Rabbit, 1:1000; SOD2, Abcam, Cambridge, MA, USA, Rabbit, 1:1000; Bcl-2, Abcam, Cam-

bridge, MA, USA, Mouse, 1:1000; Bax, Abcam, Cambridge, MA, USA, Mouse, 1:1000; glyceraldehyde 3-phosphate dehydrogenase (GAPDH), ProteinTech, Rosemont, IL, USA, 1:1000] were used to incubate the membranes overnight at 4°C. After washing 3 times, the membranes were incubated with the secondary antibody for 1.5 hours. Finally, the protein bands were exposed by Image Lab™ Software.

RNA Extraction and Real Time-Polymerase Chain Reaction (RT-PCR)

TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used to extract the total RNA of H9c2 cells. The experimental steps were consistent with the protocols. TRIzol was added to lyse the cells, and then, the protein was removed with chloroform, RNA was precipitated with isopropanol, and DNA was removed with 75% ethanol. Finally, the precipitate was dissolved in 20 μL of ribonuclease free water (Thermo Fisher Scientific, Waltham, MA, USA). RNA concentration was detected using NanoDropTM 8000. The complementary deoxyribose nucleic acid (cDNA) was obtained by reverse transcription kit (Roche, Basel, Switzerland). Prism 7900 System was used to perform RT-PCR. The expression of miR-323-3p was normalized using U6 and the expression of Bax, Bcl-2, SOD1, SOD2 mRNA were normalized using GAPDH. All the primers were listed in Table I.

Flow Cytometry

The apoptosis rate of cells was detected by the apoptosis detection kit, according to the protocols. Differently treated H9c2 cells were collected by trypsin digestion to stain with Annexin V-FITc (KeyGen, Shanghai, China) and PI (KeyGen, Shanghai, China). Finally, the apoptosis rate was detected by flow cytometry.

TUNEL Staining

The apoptosis in H9c2 cells was observed using TUNEL Apoptosis Detection Kit (Roche, Basel, Switzerland) in line with the protocols. The nucleus was stained with 4',6-diamidino-2-phenylindole (DAPI; Roche, Basel, Switzerland). Confocal Laser Scanning Microscope (CLSM) was used to observe the staining.

Luciferase Activity Assay

The plasmids (RiboBio, Guangzhou, China) which contained wild-type (WT) or mutant (MUT) 3′-UTR of TGF-β2 were constructed. The plasmids were then co-transfected with miR-323-3p mimic or NC into HEK293T cells cultured in 24-well plates. After 48 hours, Luciferase reagent was added into the cells in accordance with the protocols and the activities of Luciferase, including Firefly and Renilla, were detected using Dual-Glo® Luciferase Assay System (Promega, Madison, WI, USA).

Superoxide Dismutase (SOD) Activity Assay

H9c2 cells were collected after lysed by lysate. The SOD levels were detected by SOD Assay Kit (KeyGen, Shanghai, China) in accordance with the manufacturer's protocols.

ROS Quantification

Dihydrorhodamine 123-Reactive Oxygen Species (DHR-ROS) test kit (Bestbio, Shanghai, China) was used to detect the levels of ROS in H9c2 cells in accordance with the manufacturer's protocols.

Statistical Analysis

Measurement data were expressed as $\bar{x} \pm s$, and the measurement data were tested for normality. The differences between the two groups were analyzed by using the Student's *t*-test. Comparison

Table I. Real time PCR primers.

Gene name	Forward (5′>3′)	Reverse (5′>3′)
Bax	CAGTTGAAGTTGCCATCAGC	CAGTTGAAGTTACCATCAGC
Bcl-2	GACTGAGTACCTGAACCGGCATC	CTGAGCAGCGTCTTCAGAGACA
SOD1	GGTGAACCAGTTGTGTTGTC	CCGTCCTTTCCAGCAGTC
SOD2	CAGACCTGCCTTACGACTATGG	CTCGGTGGCGTTGAGATTGTT
miR-323-3p	CACAUUACACGGUCGACCUCU	GGCGGTCTCCCATCCAAGTA
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
GAPDH	ACAACTTTGGTATCGTGGAAGG	GCCATCACGCCACAGTTTC

RT-PCR, quantitative reverse-transcription polymerase chain reaction

between multiple groups was done using One-way ANOVA test followed by post-hoc test (Least Significant Difference). Test level α=0.05.

Results

MiR-323-3p was Downregulated in H_2O_2-Treated H9c2 Cells and Ischemic Myocardium

We treated H9c2 cells with H_2O_2 (100 μM, 4 h), and detected the level of miR-323-3p using RT-PCR. It can be seen from Figure 1A that its expression was greatly reduced after H_2O_2 treatment. The expression of miR-323-3p in myocardial tissue of rats with MI was also examined. Figure 1B showed a significant reduction of miR-323-3p in the infarcted zone and border zone compared to the sham group. In order to study the role *in vitro* and *in vivo*, miR-323-3p mimic was transfected into H9c2 cells and agomiR-323-3p was injected into the rat tail vein. From Figure 1C and Figure 1D, we can see that the levels of miR-323-3p were greatly enhanced in H9c2 cells and in rat ischemic myocardial tissue after treated with miR-323-3p mimic and agomiR-323-3p.

MiR-323-3p Overexpression Inhibited H_2O_2-Mediated Oxidative Stress in H9c2 Cells

We used Western blot to detect the expression of SOD1 and SOD2 proteins in different treatment groups. As it is possible to see from Figure 2A-2C, H_2O_2 treatment can significantly downregulate SOD1 and SOD2, but overexpression of miR-323-3p can increase the expression of these two proteins. The expression of SOD1 mRNA and SOD2 mRNA detected by RT-PCR was consistent with the protein expression (Figure 2D and 2E). The levels of SOD in H9c2 cells in different groups were also detected using the SOD activity assay. We can see from Figure 2F that the SOD level of the H_2O_2 treatment group was significantly decreased, and it was greatly increased in the miR-323-3p mimic group. Finally, DHR dye assay was used to detect the ROS production

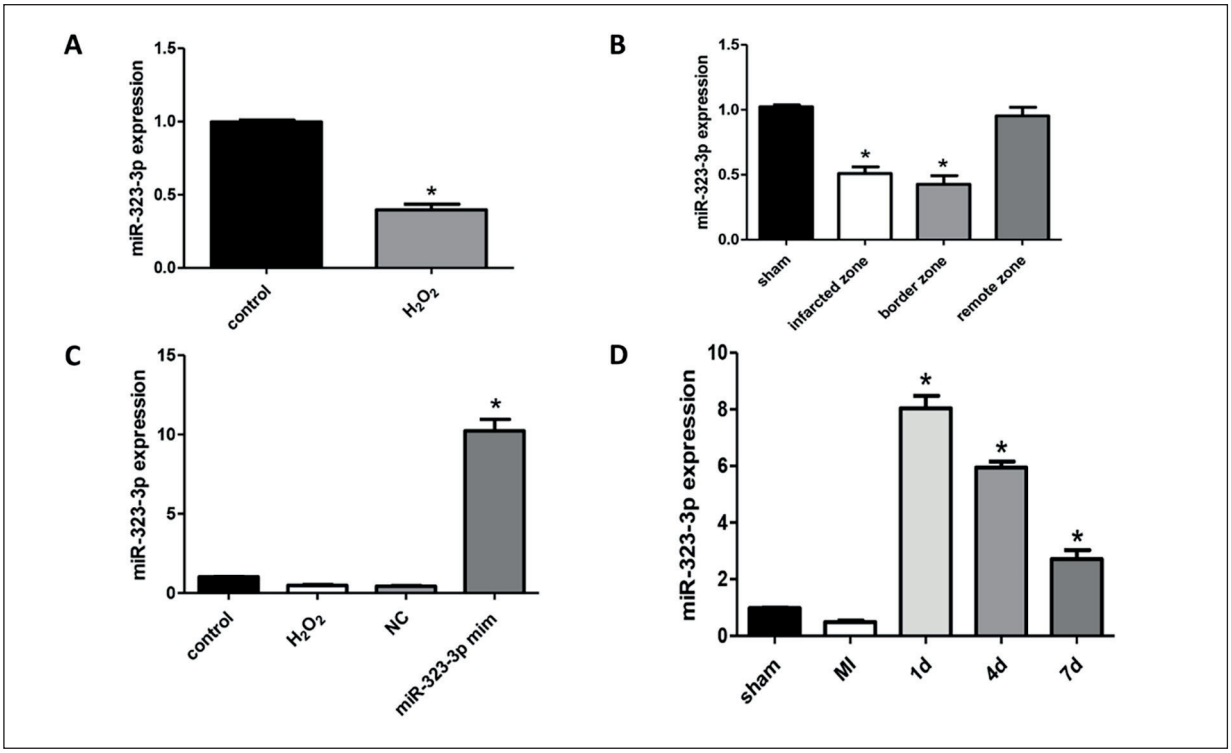

Figure 1. MiR-323-3p was downregulated in MI rat models and H_2O_2-treated H9c2 cells. **A,** RT-PCR analysis showed the downregulation of miR-323-3p expression in H9c2 cells treated with H_2O_2 ("*" p<0.05 *vs.* control, n = 3). **B,** The expression of miR-323-3p was decreased in the infarcted zone and border zone of MI rats compared with the sham group ("*" p<0.05 *vs.* sham, n = 3). **C,** MiR-323-3p mimic enhanced the expression of miR-323-3p in H9c2 cells ("*" p<0.05 *vs.* NC, n = 3). **D,** AgomiR-323-3p increased the expression of miR-323-3p in rat ischemic myocardial tissue on the first day, the fourth day and the seventh day ("*" p<0.05 *vs.* MI, n = 3).

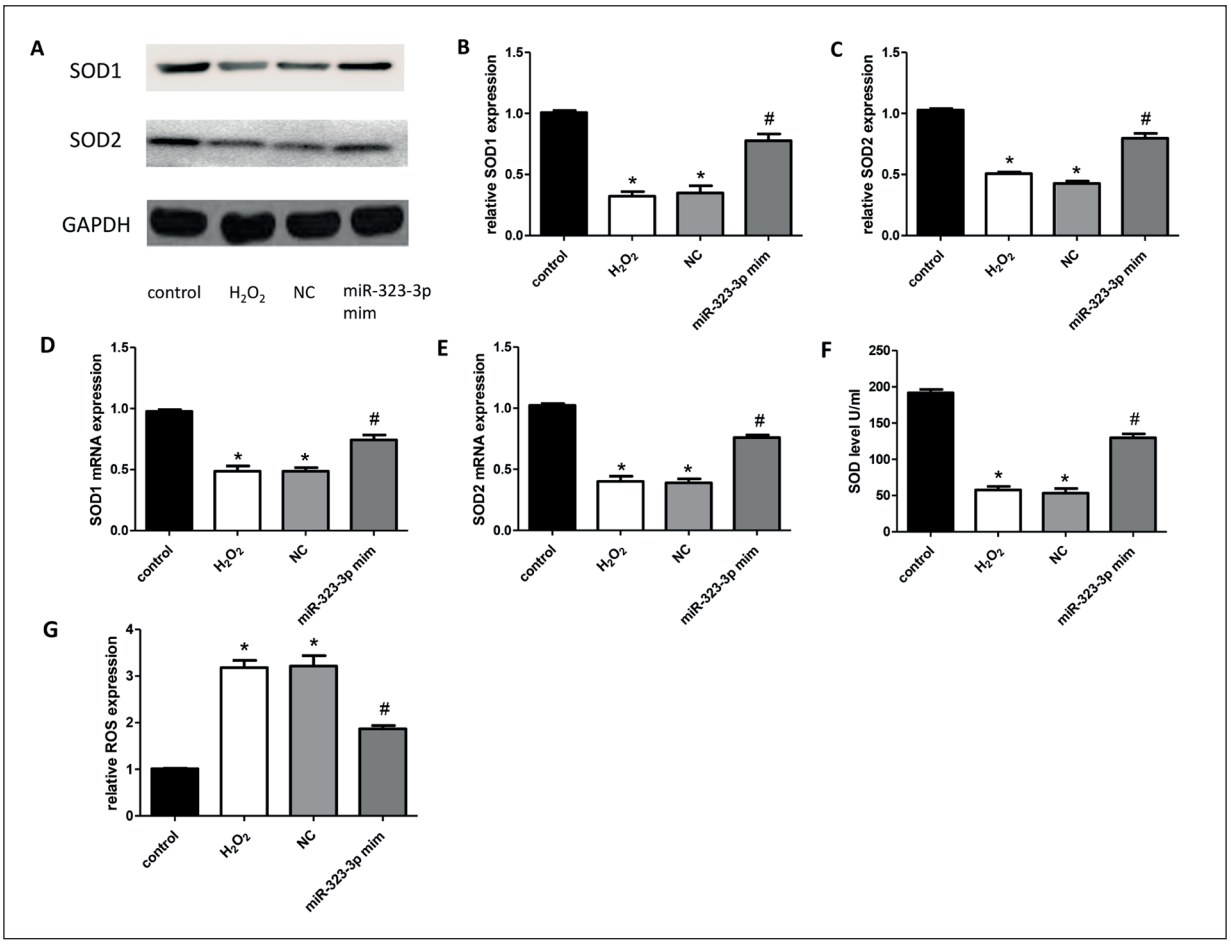

Figure 2. MiR-323-3p overexpression inhibited oxidative stress in H9c2 cells. **A,** The expression of SOD1 and SOD2 in H_2O_2 treatment group decreased significantly, and the expression of SOD1 and SOD2 increased in miR-323-3p mimic group. **B,** Statistical results of protein level of SOD1 ("*" $p<0.05$ *vs.* control, "#" $p<0.05$ *vs.* NC, n=3). **C,** Statistical results of protein level of SOD2 ("*" $p<0.05$ *vs.* control, "#" $p<0.05$ *vs.* NC, n=3). **D,** The expression of SOD1 mRNA in H_2O_2 treatment group decreased, and increased in miR-323-3p mimic group ("*" $p<0.05$ *vs.* control, "#" $p<0.05$ *vs.* NC, n=3). **E,** The expression of SOD2 mRNA in H_2O_2 treatment group decreased, and increased in miR-323-3p mimic group ("*" $p<0.05$ *vs.* control, "#" $p<0.05$ *vs.* NC, n=3). **F,** SOD activity assay showed that H_2O_2 can significantly reduce SOD levels, while overexpression of miR-323-3p could reverse SOD levels ("*" $p<0.05$ *vs.* control, "#" $p<0.05$ *vs.* NC, n=3). **G,** The expression of ROS increased in the H_2O_2 treatment group, but decreased significantly in miR-323-3p mimic group ("*" $p<0.05$ *vs.* control, "#" $p<0.05$ *vs.* NC, n=3).

(Figure 2G). MiR-323-3p can greatly reverse the increase of ROS expression in H9c2 cells treated with H_2O_2. These results illustrated that miR-323-3p overexpression can inhibit H_2O_2-mediated oxidative stress in H9c2 cells.

MiR-323-3p Overexpression Inhibited H_2O_2-Mediated Apoptosis in H9c2 Cells

The expression of Bax and Bcl-2 was detected using Western blot. Bax expression increased significantly in the H_2O_2 treatment group but decreased in the miR-323-3p mimic group (Figure 3A and B). The expression of Bcl-2 was opposite to that of Bax (Figure 3A and C). Then, we verified again with RT-PCR and the results were consistent with the results of Western blot (Figure 3D and E). In addition, the results of TUNEL staining showed that TUNEL-positive cells in the H_2O_2-treated group and the NC group were significantly increased, while the TUNEL-positive cells in the miR-323-3p mimic group were significantly reduced (Figure 3F and G). Later, we also utilized flow cytometry to prove anti-apoptotic effect of miR-323-3p. The apoptotic rate of H9c2 cells in the miR-323-3p mimic group was greatly lower than that in the NC group (Figure 3H-3I). These results demonstrated the anti-apoptotic effect of miR-323-3p in H9c2 cells.

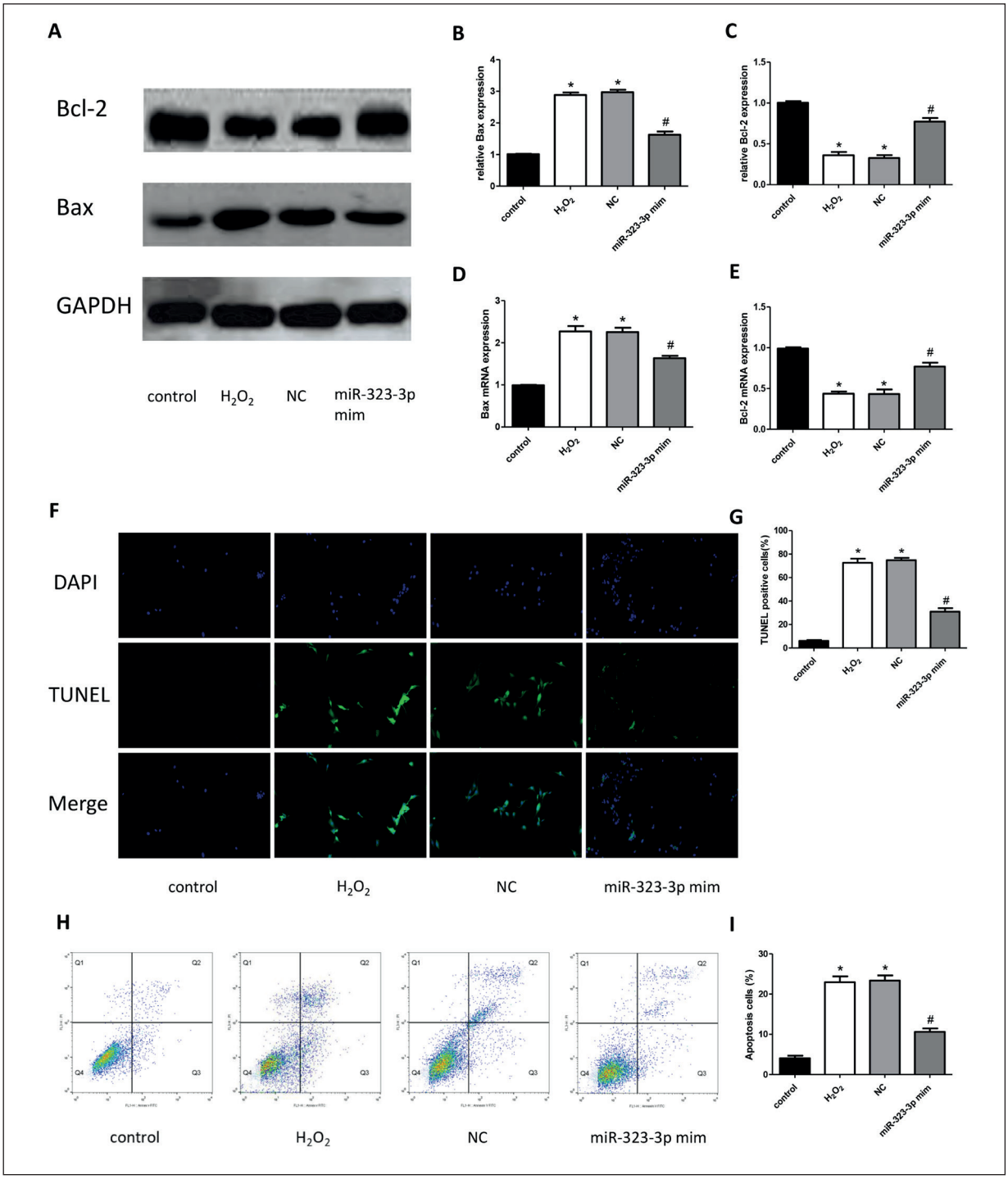

Figure 3. MiR-323-3p overexpression inhibited apoptosis in H9c2 cells. **A,** The expression of Bcl-2 in H_2O_2 treatment group decreased significantly, but increased in miR-323-3p mimic group. Bax expression was opposite to Bcl-2. **B,** Statistical results of protein level of Bax ("*" $p<0.05$ *vs.* control, "#" $p<0.05$ *vs.* NC, n=3). **C,** Statistical results of protein level of Bcl-2 ("*" $p<0.05$ *vs.* control, "#" $p<0.05$ *vs.* NC, n=3). **D,** The expression of Bax mRNA in H_2O_2 treatment group increased, and decreased in miR-323-3p mimic group ("*" $p<0.05$ *vs.* control, "#" $p<0.05$ *vs.* NC, n=3). **E,** The expression of Bcl-2 mRNA was opposite to Bax mRNA ("*" $p<0.05$ *vs.* control, "#" $p<0.05$ *vs.* NC, n=3). **F,** Representative images of TUNEL staining of H9c2 cells showing the apoptotic cells (nucleus stained in blue with DAPI and apoptotic cells stained in green) (magnification: 400×). **G,** The statistical results of TUNEL positive cells showed that miR-323-3p mimic reduced the H9c2 cells apoptosis induced by H_2O_2 ("*" $p<0.05$ *vs.* control, "#" $p<0.05$ *vs.* NC, n=3). **H,** The representative images of flow cytometry using Annexin V-FITC and PI staining. **I,** Statistical analysis of apoptosis rate detected by flow cytometry ("*" $p<0.05$ *vs.* control, "#" $p<0.05$ *vs.* NC, n=3).

Overexpression of MiR-323-3p Attenuated Oxidative Stress and Apoptosis in Cardiomyocytes of Rats with MI

On the 7th day after MI in rats, we examined the expression of SOD1, SOD2, Bax, and Bcl-2 in the border zones of myocardium. From Figure 4A-4C, it is possible to note that the expression of SOD1 and SOD2 in the MI with agomiR-323-3p group was significantly higher than that in the MI with NC group. This indicated that miR-323-3p can inhibit oxidative stress in rat myocardium *in vivo*. Compared with the MI with NC group, the Bax expression in the MI with agomiR-323-3p group was greatly decreased and the expression of Bcl-2 was significantly increased (Figure 4D-F). These results indicated that miR-323-3p overexpression can inhibit myocardial apoptosis *in vivo*.

MiR-323-3p Overexpression Improved Cardiac Function in Rats with MI

On the 7th day after MI in rats, we performed echocardiography on all rats in the four groups (Figure 5A); EF and FS were calculated (Figure 5B and 5C). Compared with the MI with NC group, the EF and FS of the rats in MI with agomiR-323-3p group were significantly improved. These results indicated that miR-323-3p overexpression could enhance cardiac function in rats with MI.

MiR-323-3p Inhibited H_2O_2-Mediated Oxidative Stress and Apoptosis in H9c2 Cells by Targeting TGF-β2

To explore the mechanism of action of miR-323-3p, we predicted the target gene using the TargetScan database. We found that TGF-β2 has a binding site with miR-323-3p (Figure 6A). Then, we used Western blot to verify that, as shown in Figure 6B-6D, H_2O_2 treatment can significantly increase the expression of TGF-β2 and JNK, but the expression of TGF-β2 and JNK was decreased after treated with miR-323-3p mimic. In addition, we performed a Luciferase activity assay. Moreover, miR-323-3p overexpression greatly reduced the Luciferase activity

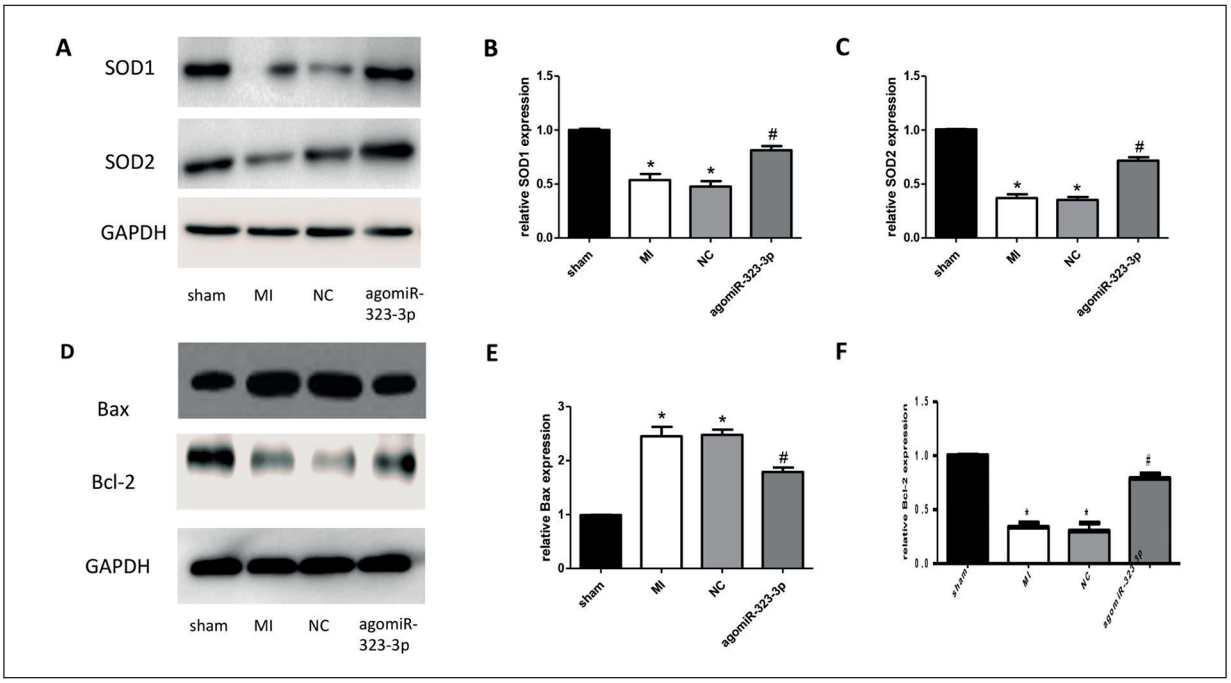

Figure 4. Overexpression of miR-323-3p attenuated oxidative stress and apoptosis of ischemic myocardium *in vivo*. **A,** The expression of SOD1 and SOD2 in MI group decreased significantly, and the expression of SOD1 and SOD2 increased in MI with agomiR-323-3p group. **B,** Statistical results of protein level of SOD1 ("*" $p<0.05$ *vs.* sham, "#" $p<0.05$ *vs.* NC, n=3). **C,** Statistical results of protein level of SOD2 ("*" $p<0.05$ *vs.* sham, "#" $p<0.05$ *vs.* NC, n=3). **D,** The expression of Bcl-2 in MI group decreased significantly, but increased in MI with agomiR-323-3p group. Bax expression was opposite to Bcl-2. **E,** Statistical results of protein level of Bax ("*" $p<0.05$ *vs.* sham, "#" $p<0.05$ *vs.* NC, n=3). **F,** Statistical results of protein level of Bcl-2 ("*" $p<0.05$ *vs.* sham, "#" $p<0.05$ *vs.* NC, n=3).

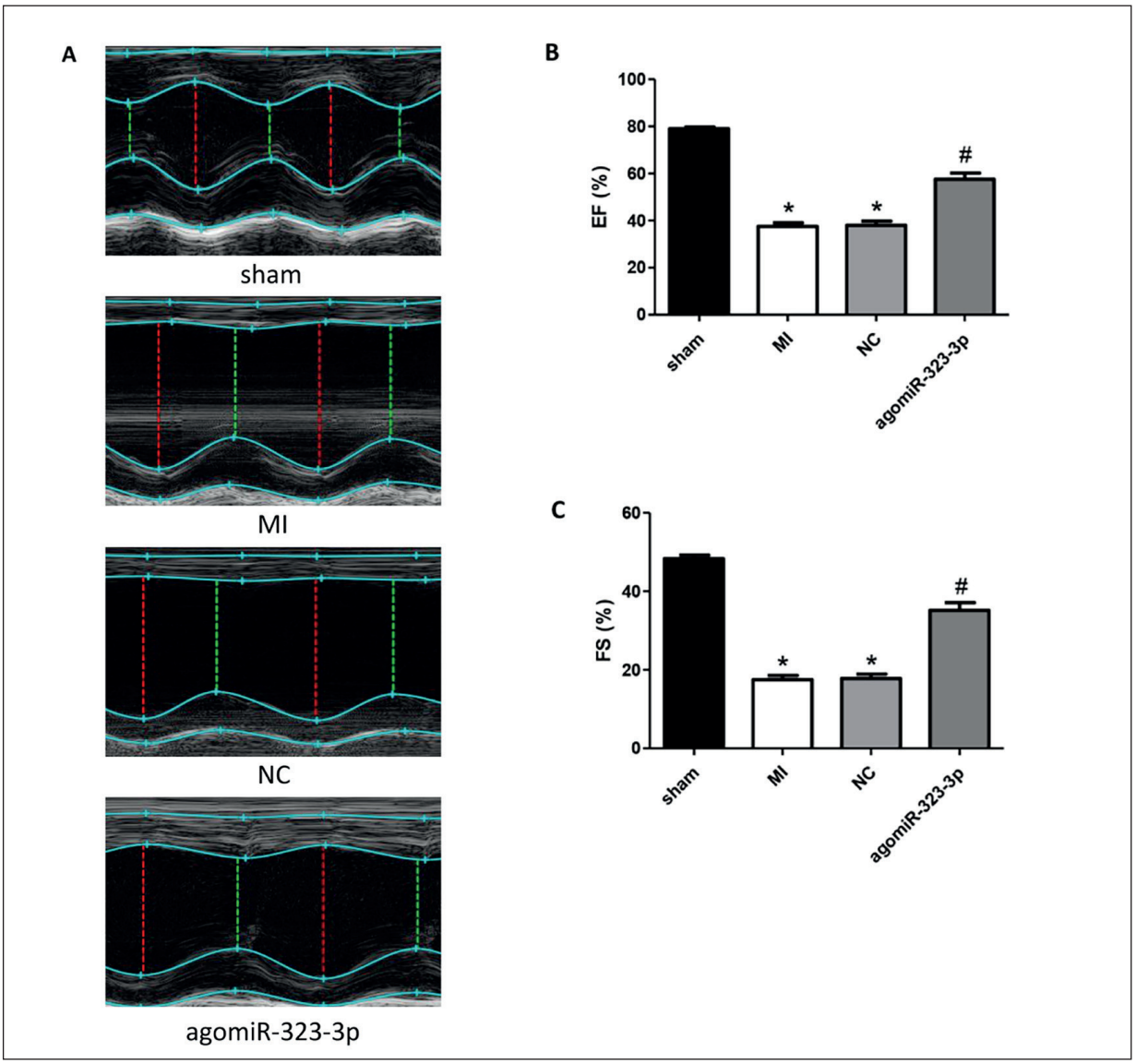

Figure 5. MiR-323-3p overexpression improved cardiac function in rats with MI. **A,** Representative photographs of rat echocardiography. **B,** Ejection fractions (EF). **C,** Fractional shortening (FS) ("*" $p<0.05$ *vs.* sham, "#" $p<0.05$ *vs.* NC, n=6).

of the WT group but had no effect on the mutant group (Figure 6E). Therefore, miR-323-3p targeted TGF-β2 and inhibited TGF-β2/JNK signaling pathway.

Discussion

The heart is an organ with high metabolism in the body. Cardiomyocytes have high requirements for blood supply and oxygen supply. Coronary heart disease causes absolute or relative ischemia and hypoxia of cardiomyocytes to promote oxidative stress and apoptosis[10]. There are several papers that have illustrated the role of miR-323-3p in regulating apoptosis[11]. However, studies on the role of miR-323-3p in ischemic myocardium are rare. Therefore, we sought to demonstrate that miR-323-3p can inhibit oxidative stress and apoptosis in cardiomyocytes in this study.

In vitro, we used H_2O_2 to induce H9c2 cell injury. The results showed that the treatment with H_2O_2 greatly decreased the expression of

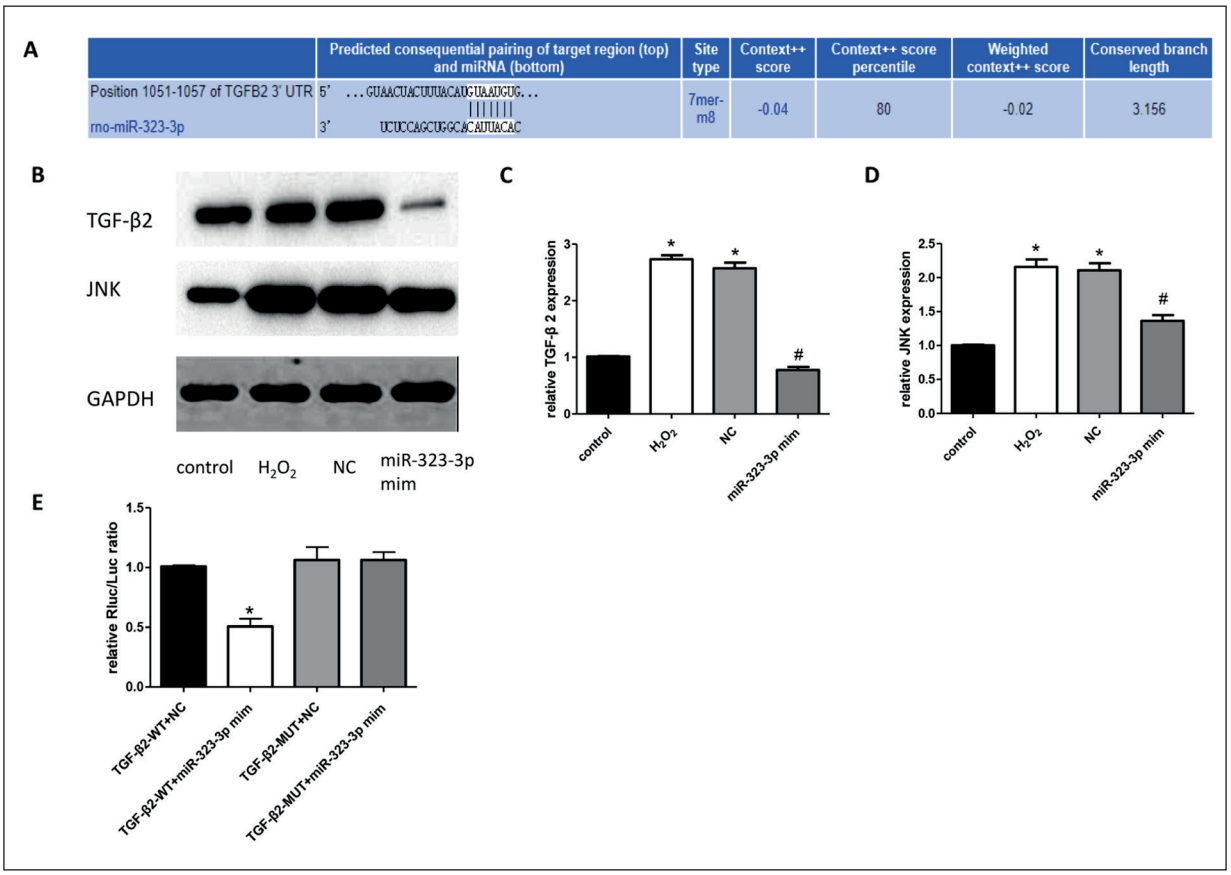

Figure 6. MiR-323-3p directly targeted TGF-β2 and inhibited TGF-β2/JNK signaling pathway. **A,** Binding site predicted by the TargetScan database. **B,** Western blot showed that the expression of TGF-β2 and JNK in H_2O_2 treatment group increased and decreased in miR-323-3p mimic group. **C,** Statistical results of protein level of TGF-β2 ("*" $p<0.05$ *vs.* control, "#" $p<0.05$ *vs.* NC, n=3). **D,** Statistical results of protein level of JNK ("*" $p<0.05$ *vs.* control, "#" $p<0.05$ *vs.* NC, n=3). **E,** MiR-323-3p overexpression significantly decreased the relative Luciferase activity in WT group but did not decrease the relative Luciferase activity in MUT group ("*" $p<0.05$ *vs.* WT+NC, n = 3).

miR-323-3p in H9c2 cells. Moreover, the expression of SOD1 and SOD2 was decreased and the expression of ROS was increased in the H_2O_2 treatment group. However, in the miR-323-3p mimic group, SOD1 and SOD2 expression increased significantly, and ROS levels decreased significantly. These illustrated the role of miR-323-3p in anti-oxidative stress in myocardial injury. Similarly, after treatment with H_2O_2, the expression of Bax increased, the expression of Bcl-2 decreased, the number of TUNEL-positive cells increased, and the apoptosis rate of cells increased. However, overexpression of miR-323-3p reversed these results, demonstrating the anti-apoptotic effect of miR-323-3p in H9c2 cells.

In vivo, we constructed a rat MI model. Oxidative stress and apoptosis of myocardium in rats with MI injected with agomiR-323-3p were significantly inhibited, and cardiac function was significantly improved. Therefore, we believed that miR-323-3p was involved in the regulation of cellular oxidative stress and apoptosis induced by myocardial ischemia.

The TGF-β superfamily plays an important role in cell growth, differentiation, tissue repair, inflammation, and other aspects[12]. TGF-β2 is a member of the superfamily. Using a database to predict target genes, we found that TGF-β2 has a binding site with miR-323-3p. Luciferase activity assay demonstrated that TGF-β2 is a target gene of miR-323-3p. Moreover, using Western blot, we found that miR-323-3p overexpression significantly inhibited the expression of TGF-β2 and JNK. This indicated that miR-323-3p may play a role in inhibiting oxidative stress and apoptosis of cardiomyocytes by regulating the TGF-β2/JNK pathway.

Conclusions

The results of this study revealed that the expression of miR-323-3p was reduced in ischemic myocardium and H_2O_2-treated H9c2 cells. Moreover, miR-323-3p overexpression can inhibit oxidative stress and cardiomyocyte apoptosis by regulating the TGF-β2/JNK pathway.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Benjamin EJ, Virani SS, Callaway CW, Chamberlain AM, Chang AR, Cheng S, Chiuve SE, Cushman M, Delling FN, Deo R, de Ferranti SD, Ferguson JF, Fornage M, Gillespie C, Isasi CR, Jimenez MC, Jordan LC, Judd SE, Lackland D, Lichtman JH, Lisabeth L, Liu S, Longenecker CT, Lutsey PL, Mackey JS, Matchar DB, Matsushita K, Mussolino ME, Nasir K, O'Flaherty M, Palaniappan LP, Pandey A, Pandey DK, Reeves MJ, Ritchey MD, Rodriguez CJ, Roth GA, Rosamond WD, Sampson U, Satou GM, Shah SH, Spartano NL, Tirschwell DL, Tsao CW, Voeks JH, Willey JZ, Wilkins JT, Wu JH, Alger HM, Wong SS, Muntner P. Heart disease and stroke statistics-2018 update: a report from the American Heart Association. Circulation 2018; 137: e67-e492.
2) Nabel EG, Braunwald E. A tale of coronary artery disease and myocardial infarction. N Engl J Med 2012; 366: 54-63.
3) Chiong M, Wang ZV, Pedrozo Z, Cao DJ, Troncoso R, Ibacache M, Criollo A, Nemchenko A, Hill JA, Lavandero S. Cardiomyocyte death: mechanisms and translational implications. Cell Death Dis 2011; 2: e244.
4) Pal AS, Kasinski AL. Animal models to study microRNA function. Adv Cancer Res 2017; 135: 53-118.
5) Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nat Rev Genet 2008; 9: 102-114.
6) Kawai S, Amano A. BRCA1 regulates microRNA biogenesis via the DROSHA microprocessor complex. J Cell Biol 2012; 197: 201-208.
7) Zhu H, Fan GC. Role of microRNAs in the reperfused myocardium towards post-infarct remodelling. Cardiovasc Res 2012; 94: 284-292.
8) Zhao Y, Tao M, Wei M, Du S, Wang H, Wang X. Mesenchymal stem cells derived exosomal miR-323-3p promotes proliferation and inhibits apoptosis of cumulus cells in polycystic ovary syndrome (PCOS). Artif Cells Nanomed Biotechnol 2019; 47: 3804-3813.
9) Qin J, Sun Y, Liu S, Zhao R, Zhang Q, Pang W. MicroRNA-323-3p promotes myogenesis by targeting Smad2. J Cell Biochem 2019; 120: 18751-18761.
10) Neri M, Fineschi V, Di Paolo M, Pomara C, Riezzo I, Turillazzi E, Cerretani D. Cardiac oxidative stress and inflammatory cytokines response after myocardial infarction. Curr Vasc Pharmacol 2015; 13: 26-36.
11) Wang T, Liu Y, Lv M, Xing Q, Zhang Z, He X, Xu Y, Wei Z, Cao Y. miR-323-3p regulates the steroidogenesis and cell apoptosis in polycystic ovary syndrome (PCOS) by targeting IGF-1. Gene 2019; 683: 87-100.
12) Morikawa M, Derynck R, Miyazono K. TGF-beta and the TGF-beta family: context-dependent roles in cell and tissue physiology. Cold Spring Harb Perspect Biol 2016; 8. pii: a021873.