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MiR-129-5p protects against myocardial ischemia-reperfusion injury *via* targeting HMGB1

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Abstract. – OBJECTIVE: To investigate the protective effect of miR-129-5p on ischemia-reperfusion (I/R) injury *via* targeting high mobility group box-1 (HMGB1).

MATERIALS AND METHODS: Rat models of myocardial I/R and hypoxia/reoxygenation (H/R) cardiomyocytes were established, and the miR-129-5p and HMGB1 expression levels in myocardium of I/R rats and in cardiomyocytes of H/R rats were quantified by RT-PCR. The over-expression of miR-129-5p was performed on I/R rats, and the over-expression of miR-129-5p and down-regulation of HMGB1 were performed on cardiomyocytes of H/R rats. Triphenyltetrazolium chloride (TTC) staining was used to measure myocardial infarct size (IS). TUNEL (TdT-mediated dUTP end nick labeling) staining was employed to observe cardiomyocyte apoptosis in the myocardium of rats, and flow cytometry to observe cardiomyocyte apoptosis of I/R and H/R rats respectively. Dual-Luciferase reporter assay was used to verify the target relation between miR-129-5p and HMGB1.

RESULTS: MiR-129-5p was lowly expressed and HMGB1 was highly expressed in myocardial I/R injury rats and cardiomyocytes of H/R rats. Over-expression of miR-129-5p effectively reduced myocardial IS and cardiomyocyte apoptosis in rats with myocardial I/R injury, and significantly down-regulated the pro-apoptotic protein Bax, as well as significantly up-regulated the anti-apoptotic protein Bcl-2. Either over-expression of miR-129-5p or low-expression of HMGB1 in cardiomyocytes of H/R rats also achieved the same effects as described above. Dual-Luciferase reporter assay determined that miR-129-5p was a target for HMGB1.

CONCLUSIONS: MiR-129-5p plays a protective role on myocardial I/R injury by regulating HMGB1 expression. Besides, it inhibits cardiomyocyte apoptosis and is expected to become a novel molecular marker or therapeutic target for myocardial I/R injury.

Key Words:
MiR-129-5p, HMGB1, Ischemia reperfusion injury, Cardiac protection.

Introduction

As a clinically common cardiovascular disease, acute myocardial infarction (AMI) is caused by acute occlusion of coronary artery, resulting in reduction or interruption of coronary blood flow, thus leading to myocardium damage and cardiomyocyte necrosis[1,2]. At present, the recommended treatment for AMI is to restore the cardiac perfusion with thrombolytic agents. Although reperfusion therapy is effective in myocardial infarction, it can induce myocardial necrosis as well, known as ischemia-reperfusion (I/R) injury[3]. Therefore, finding a treatment for myocardial I/R injury is one of the clinical issues that need to be solved urgently.

MicroRNAs (miRNA), a class of non-coding small RNAs, are factors that regulate genes by pairing with 3'-untranslated region (UTR) of target mRNAs[4]. An increasing number of miRNAs have been found to be closely related to the development and progression of ischemic cardiomyopathy, heart failure, and other cardiovascular diseases[5]. MiR-129-5p is a miRNA that acts as a tumor suppressor gene in various tumors and plays a role in cardiovascular diseases[6]. Geng et al[7] revealed that miR-129-5p inhibited the autophagy of endothelial cells in atherosclerosis by regulating Beclin-1. In addition, Zhou et al[8] reported that miR-129-5p had a neuroprotective effect by alleviating cerebral I/R injury. Therefore, we speculated that miR-129-5p could play a cardioprotective role in myocardial I/R injury.

High mobility group box-1 (HMGB1) is a multifunctional DNA-binding protein that has

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been proved to be closely related to I/R injury[9]. It can worsen myocardial I/R injury by stimulating the release of inflammatory factors[10]. We found targeted binding sites between miR-129-5p and HMGB1 with an online miR target prediction tool (Targetscan). We speculated whether miR-129-5p could affect myocardial I/R injury through the regulation of HMGB1, but no studies have been conducted yet. Therefore, we explored the effects and molecular mechanism of miR-129-5p in myocardial I/R injury by constructing I/R model and hypoxia/reoxygenation (H/R) model in rats.

Materials and Methods

Animals and Materials

Fifty clean-grade Sprague-Dawley (SD) rats (Laboratory Animal Center of Sun Yat-sen University) with a body mass of 245-300 g were raised at 20-25°C and 45%-65% relative humidity, with normal 12 h circadian rhythm and free access to food and drink. H9c2 cardiomyocytes (Cell Bank of Chinese Academy of Sciences, Shanghai, China, CBP60588); rabbit anti-human HMGB1 monoclonal antibody (Cell Signaling Technology; Danvers, MA, USA); rabbit anti-human β-actin monoclonal antibody (Proteintech Group, Inc, Wuhan, China); bicinchoninic acid (BCA) kit for protein concentration determination (Well Biotechnology Co., Ltd., Shanghai , China); *in situ* cell death detection kit (Roche Diagnostics, Basel, Switzerland); real-time quantitative PCR instrument (BioRad, Hercules, CA, USA); flow cytometer CytoFLEX LX (Beckman, Brea, CA, USA); Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Rockville, MD, USA); fetal bovine serum (FBS) and trypsin (Hyclone, South Logan, UT, USA); TRIzol (Invitrogen, Carlsbad, CA, USA), qPCR kit and reverse transcription kit (TransGen Biotech, Beijing, China, AQ201-01, AQ202-01); Dual-Luciferase reporter gene detection kit (Solarbio, Beijing, China); miR-129-5p, miRNA NC, internal reference U6 and β-actin primers (GenePharma Co.,Ltd., Shanghai, China); CAnnexin V-FITC/PI apoptosis kit (KeyGEN Biotech Co., Ltd., Jiangsu, China).

Rat Modeling

The rats were randomly divided into blank control group, sham operation group, I/R group, I/R+miR-129-5p-agomir group, and miR-NC group with 10 rats each. The rats in blank control group were normally fed without any treatment. They were anesthetized with 4% isoflurane, fixed on the operation table in the supine position, and connected with an electrocardiogram (ECG) detector. During the experiment, a rodent ventilator was used for respiratory support, and 2% isoflurane was delivered to maintain anesthesia. Skin preparation, disinfection, and thoracotomy were carried out in a sterile environment. Afterwards, the pericardium was opened to expose the heart. Five marks in left ventricular anterior wall selected in rats in the I/R and miR-129-5p-agomir groups were injected with lentiviral vector carrying miR-129-5p-agomir (100 μL). In miR-NC group, another five marks were injected with lentiviral vector carrying miR-NC. The thoracic cavity was closed after the injection to allow the rats to recover. Three days later, the rats were anesthetized again, and the thoracic cavity was opened to expose the heart. Anterior descending branch of the coronary artery with the same direction of the great cardiac vein was ligatured with a suture at 2 mm below the left atrial appendage. The darkening of the corresponding areas on the surface of the heart indicated successful AMI modeling. After 60 min, the ligature was loosened to restore blood supply, and reperfusion was carried out for 4 h. The thoracic cavity was clamped during the reperfusion. Except that the left anterior descending branch of the coronary artery of the rats in sham operation group was not ligatured, the other operations were the same as those mentioned above. After modeling, the myocardium was collected and the myocardial infarct size (IS) was measured by Evans blue/TTC staining. Meanwhile, rat serum was collected to detect lactate dehydrogenase (LDH) and creatine kinase (CK) by ELISA. The study was conducted according to the recommendations in the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health. This experiment has passed the animal experiment ethics declaration

Detection of Cardiomyocyte Apoptosis by TUNEL Staining

Heart tissue was fixed with 4% formaldehyde at room temperature for 24 h, then washed, dehydrated, paraffin-embedded, and cut into 4-μm slices. Afterwards, the cardiomyocyte apoptosis was detected in strict accordance with the instructions of the *in situ* cell death detection kit. Cells with brown-stained nuclei showed apoptosis, and 5 fields were selected for cell

count under a fluorescence microscope. The ratio of TdT-mediated dUTP end nick labeling (TUNEL)-positive cells to total cardiomyocytes was calculated.

Cell Modeling and Transfection

H9c2 cardiomyocytes were added into DMEM containing 10% FBS and cultured in an incubator at 37°C and 5% CO_2. When reaching 80% confluence, the cells were collected and divided into blank control group, H/R group, miR-129-5p-agomir group, miR-NC group, Si-HMGB1 group, and Si-NC group. Cells in blank control group were normally cultured, while others were placed in serum-free and sugar-free medium and cultured in a hypoxia incubator at 37°C, 94% N_2, and 5% CO_2. After 24 h, the cells were transferred to a medium containing 10% FBS, and incubated for 3 hours at 37°C, 5% CO_2 to establish an H/R rat model. After modeling, cells in the miR-129-5p-agomir group and miR-NC group were transfected with miR-129-5p-agomir, miR-NC, Si-HMGB1, Si-NC respectively for 24 h by Lipofectamine 2000, while H/R group cells were not transfected.

Detection of MiR-129-5p Expression by RT-PCR

The total RNAs in tissues and cells were extracted with TRIzol, and the purity and concentration were detected by an ultraviolet spectrophotometer. Then, 5 μg of total RNA from each group was reversely transcribed to cDNA in accordance with the kit's instructions. The reaction parameters used were as follows: 37°C for 15 min, 42°C for 35 min, and 70°C for 5 min. MiR-129-5p amplification system: 1 μL of cDNA, 0.4 μL of each upstream and downstream primer, 10 μL of 2×TransTaq® Tip Green qPCR SuperMix, 0.4 μL of Passive Reference Dye (50X), and finally made up to 20 μL with ddH_2O. Amplification conditions: PCR reaction conditions: pre-denaturation at 94°C for 45 s, denaturation at 94°C for 10 s, annealing, and extension at 60°C for 45 s, with a total of 40 cycles. HMGB1 amplification system: 1 μL of cDNA, 0.4 μL of each upstream and downstream primer, 10 μL of 2x TransTaq® Tip Green qPCR SuperMix, 0.4 μL of Passive Reference Dye (50X), and finally made up to 20 μL with Nuclease-free water. Amplification conditions: pre-denaturation at 95°C for 30 s, denaturation at 95°C for 10 s, annealing and extension at 60°C for 35 s, for a total of 40 cycles. Each sample was tested in 3 repeated wells, and the experiment was carried out 3 times. U6 was used as an internal reference for miR-143-3p and β-Actin was used as an internal reference for HMGB1. $2^{-\Delta\Delta ct}$ was used to analyze the data in this study. Primer sequences were as follows: miR-129-5p: upstream sequence: 5'-CUUUUUGCGGUCUGGGCUUGC-3', downstream sequence: 5'-AAGCCCAGACCGCAAAAAGUU-3'; U6: upstream sequence: 5'-CTCGCTTCGGCAGCACA-3', downstream sequence: 5'-AACGCTTCACGAATTTGCGT--3'; HMGB1: upstream sequence: 5'-CTC GCT TCG GCAGCACA-3', downstream sequence: 5'-AAC GCTTCACGAATTTGCGT-3'; β-Actin: upstream sequence: 5'-ACACTGTGCCCATCTACG-3', downstream sequence: 5'-TGTCACGCACGATTTCC-3'.

Western Blot

Myocardium and cultured cardiomyocytes were lysed with RIPA to obtain the total protein. The protein concentration was measured by the bicinchoninic acid method and adjusted to 4 μg/μL. After separation with 10% of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), the proteins were transferred to a polyvinylidene difluoride membrane (PVDF). The membrane was stained with Ponceau S working solution, immersed in Phosphate-Buffered Saline and Tween-20 (PBST) for 5min and then washed, blocked with 5% skimmed milk powder for 2 h, and finally incubated overnight at 4°C with primary antibodies against Bax (1:500), Bcl-2 (1:500), HMGB1 (1:500), and β-Actin (1:500). Following washing to remove the primary antibodies, the horseradish peroxidase (HRP)-labeled goat anti-rabbit secondary antibody (1:1000) was added to the membrane for a 1h incubation at 37°C. Next, the membrane was rinsed 3 times with Tris Buffered Saline and Tween-20 (TBST), for 5 min each time. Excess liquid on the membrane was absorbed with a filter paper. The protein bands were developed in a dark room using the enhanced chemiluminescence (ECL) reagent. Then, luminescent bands were scanned, and the gray value was analyzed using Quantity One software. The relative expression level of each protein = the gray value of the target protein band / the gray value of the β-actin protein band.

Detection of Cardiomyocyte Apoptosis by Flow Cytometry

After being digested with 0.25% trypsin, the transfected cells were washed twice with PBS, then added with 100 μL of binding buffer, and

prepared into $1x10^6$/mL suspension. Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) were added sequentially, and the cells were incubated in the dark at room temperature for 5 min to detect apoptosis with FC500MCL flow cytometer system. The experiment was repeated for 3 times to obtain the average.

Dual-Luciferase Reporter Assay

TargetScan 7.2 was employed to predict downstream target genes of miR-129-5p. H9c2 cells were transfected with HMGB1-3'UTR wild type (Wt), HMGB1-3'UTR mutant (Mut), miR-129-5p-agomir, miR-129-5p-antagomir, and miR-NC using a Lipofectamine™ 2000 kit. 48 h after transfection, luciferase activity was measured with a Dual-Luciferase reporter assay kit (Promega, Madison, WI, USA).

Statistical Analysis

SPSS 20.0 software package (IBM, Armonk, NY, USA) was used to carry out statistical analysis on the collected data, and the GraphPad 7 software package was used for building graphs. Inter-group comparison was conducted with independent *t*-test, multi-group comparison with one-way analysis of variance (ANOVA), and following pairwise comparison with LSD-*t*-test. A value of $p<0.05$ was considered statistically significant.

Results

Expression of MiR-129-5p and HMGB1

Compared with blank control group and sham operation group, the I/R group and miR-NC group showed significantly down-regulated miR-129-5p expression, as well as up-regulated HMGB1 expression in rats, while I/R+miR-129-5p-agomir group showed significantly up-regulated miR-129-5p expression, as well as down-regulated HMGB1 expression in myocardium of rats (all $p<0.05$). Compared with H9c2 cells in blank control group, those in the H/R group and miR-NC showed significantly down-regulated miR-129-5p expression, as well as up-regulated HMGB1 expression, while cardiomyocytes in miR-129-5p-agomir group showed significantly up-regulated miR-129-5p expression, as well as down-regulated HMGB1 expression (all $p<0.05$), miR-129-5p and HMGB1 expression levels were negatively correlated in both myocardium and cardiomyocytes (Figure 1).

Over-Expression of MiR-129-5p Reduces Myocardial IS in I/R Rats and Mitigate Oxidative Stress Reaction

The myocardial IS in I/R group and miR-NC group was significantly increased compared to that in blank control group and sham operation group ($p<0.05$), but the myocardial infarct size (IS) in I/R+miR-129-5p-agomir group was significantly decreased compared to that in I/R group ($p<0.05$). Meanwhile, the activities of CK and LDH in I/R group and miR-NC group increased significantly compared with those in blank control group and sham operation group ($p<0.05$), but their activities in I/R+miR-129-5p-agomir group decreased significantly compared with those in I/R group ($p<0.05$; Figure 2).

Over-Expression of MiR-129-5p Reduces Cardiomyocyte Apoptosis in I/R Rats

The apoptotic rate of cardiomyocytes in the I/R group and miR-NC group was significantly increased compared with that in blank control group and sham operation group, pro-apoptotic protein Bax was significantly up-regulated and and anti-apoptotic protein Bcl-2 was significantly down-regulated ($p<0.05$). However, the apoptotic rate of cardiomyocytes in I/R+miR-129-5p-agomir group was significantly reduced compared with that in I/R group, Bax was significantly down-regulated and Bcl-2 was significantly up-regulated ($p<0.05$; Figure 3).

Effects of MiR-129-5p Over-Expression on Cardiomyocytes of H/R Rats

To further assess the protective effect of miR-129-5p on the heart, an H/R model was established in H9c2 cells to simulate myocardial ischemia reperfusion. The results showed that compared with blank control group, H/R group and miR-NC group showed significantly increased apoptotic rate, up-regulated Bax, and down-regulated Bcl-2 ($p<0.05$). Moreover, the apoptotic rate in the miR-129-5p-agomir group was decreased significantly compared with that in H/R group and miR-NC group, and the Bax was significantly down-regulated, and Bcl-2 was significantly up-regulated ($p<0.05$; Figure 4)

Inhibition of HMGB1 Expression on Cardiomyocytes in H/R Rats

We found that the expression levels of HMGB1 and miR-129-5p were negatively correlated in myocardium and cardiomyocytes. The cardiomyocytes in H/R rats were transfect-

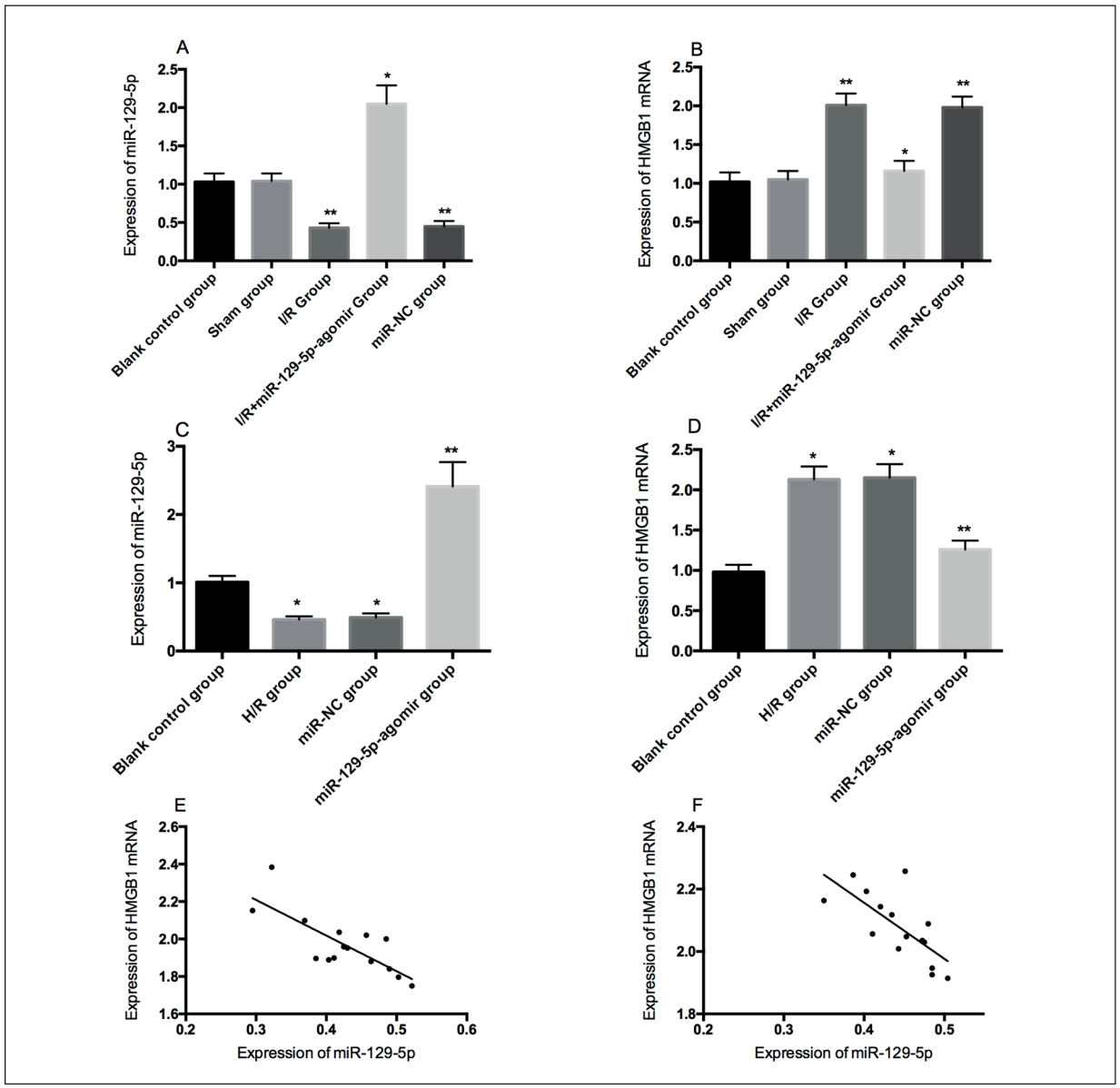

Figure 1. Expression of miR-129-5p and HMGB1. **A,** MiR-129-5p expression was down-regulated in myocardium of I/R rats. **B,** HMGB1 expression was up-regulated in myocardium of I/R rats. **C,** MiR-129-5p expression was down-regulated in cardiomyocytes of H/R rats. **D,** HMGB1 expression was up-regulated in cardiomyocytes of I/R and H/R rats. **E,** Expression of mir-129-5p and HMGB1 were negatively correlated in myocardium. **F,** Expression levels of mir-129-5p and HMGB1 were negatively correlated in cardiomyocytes. *Indicated that $p<0.05$.

ed with Si-HMGB1, and the results showed that compared with blank control group, the apoptotic rate in H/R group and Si-NC group was significantly increased, Bax was significantly up-regulated, and Bcl-2 was significantly down-regulated ($p<0.05$). Moreover, compared with H/R group and Si-NC group, the apoptotic rate in Si-HMGB1 group was significantly decreased, Bax was significantly down-regulated, and Bcl-2 was significantly up-regulated ($p<0.05$; Figure 5).

Target Relation Between MiR-129-5p and HMGB1

TargetScan 7.2 was applied to predict downstream target genes of miR-129-5p so as to verify the target relation between miR-129-5p and HMGB1, and the results showed that there were

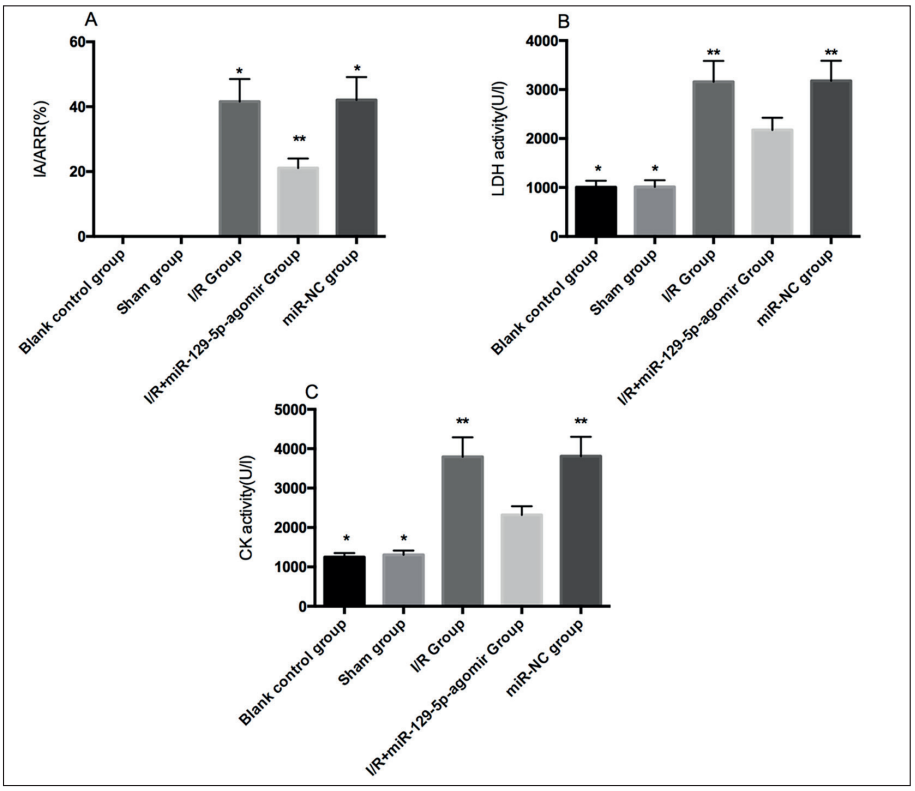

Figure 2. Over-expression of miR-129-5p reduces myocardial IS in I/R rats. **A,** Over-expression of miR-129-5p reduced myocardial IS in I/R rats. **B, C,** Over-expression of miR-129-5p reduced the activities of CK and LDH in I/R rats. *Indicated that $p<0.05$.

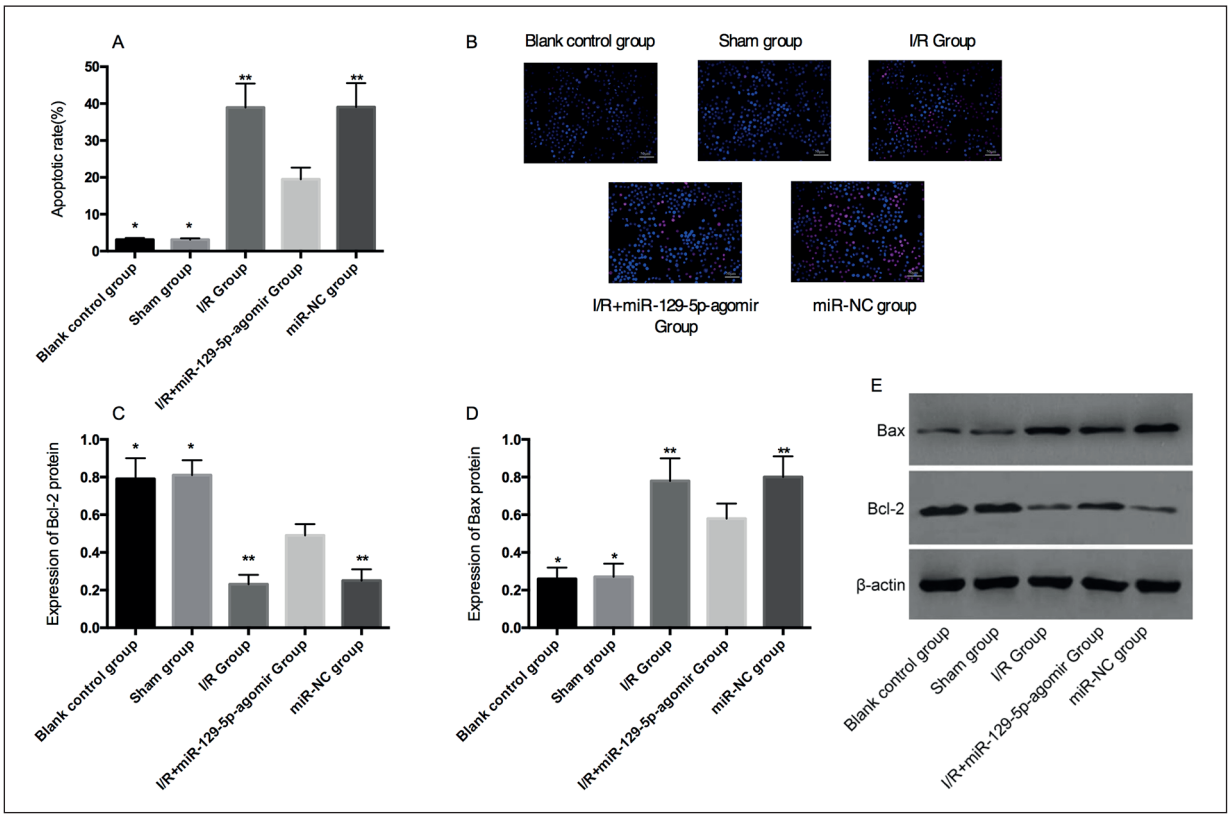

Figure 3. Over-expression of miR-129-5p reduces cardiomyocyte apoptosis in I/R rats. **A,** Over-expression of miR-129-5p reduced the apoptotic rate of cardiomyocytes in I/R group. **B,** TUNEL staining. **C, D,** Over-expression of miR-129-5p down-regulated pro-apoptotic protein Bax and up-regulated anti-apoptotic protein Bcl-2 in myocardium. **E,** Western blot. *Indicated that $p<0.05$.

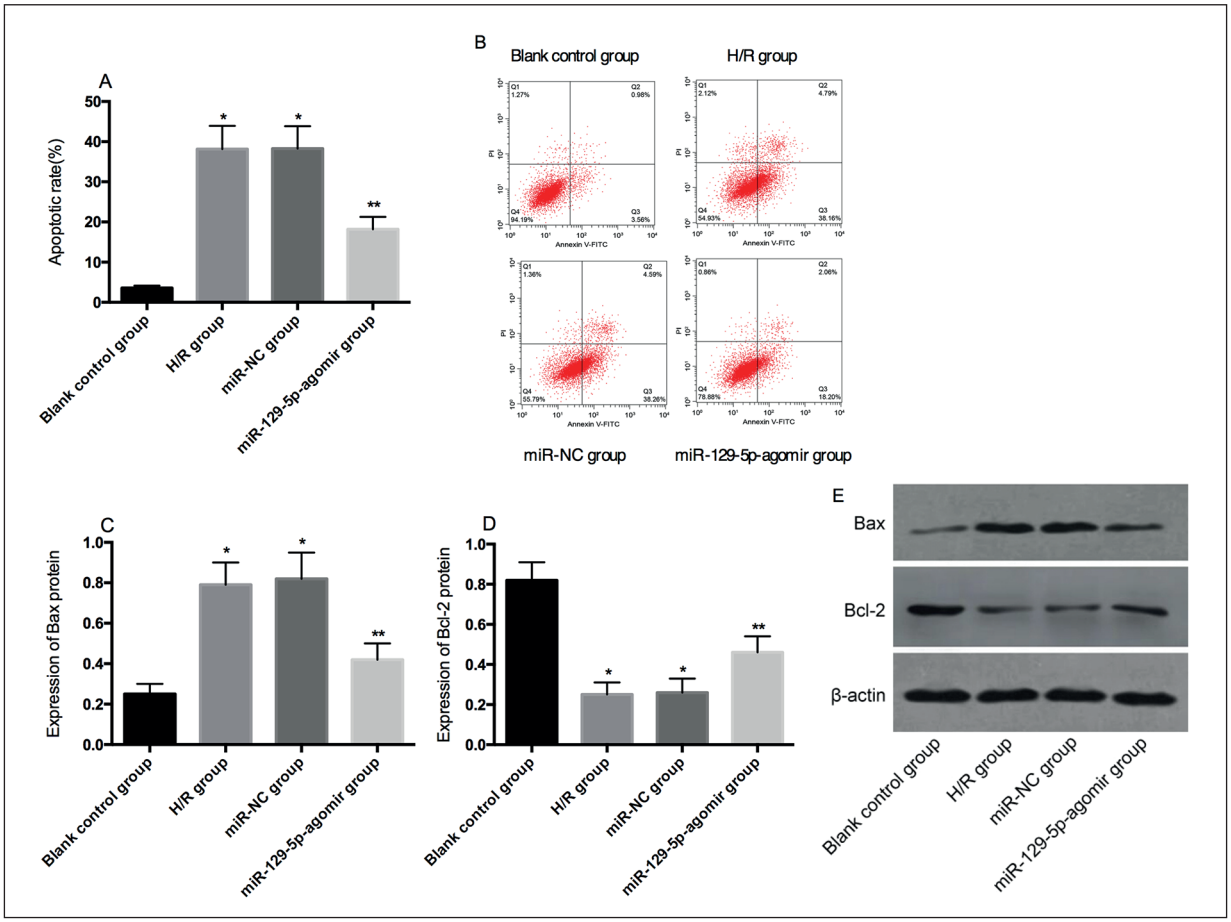

Figure 4. Over-expression of miR-129-5p reduces apoptosis of cardiomyocytes in H/R rats. **A,** Over-expression of miR-129-5p reduced the apoptotic rate of cardiomyocytes in H/R rats. **B,** Flow cytometry. **C, D,** Over-expression of miR-129-5p down-regulated Bax and up-regulated Bcl-2 in cardiomyocytes of H/R rats. **E,** Western blot. *Indicated that $p<0.05$.

targeted binding sites between HMGB1 and miR-129-5p. Therefore, we conducted Dual-Luciferase reporter detection and found that the over-expression of miR-129-5p significantly reduced the luciferase activity of HMGB1-3'UT Wt ($p<0.05$), but it had no effect on the luciferase activity of HMGB1-3'UTR Mut ($p>0.05$). Western blot found that HMGB1 protein expression in H9c2 was significantly reduced after transfected with miR-129-5p-agomir, while HMGB1 protein expression was significantly increased ($p<0.05$; Figure 6).

Discussion

AMI is one of the cardio-cerebrovascular diseases posing a great threat to human life and health. The myocardial reperfusion restored by thrombolytic therapy may lead to necrosis of myocardium and stimulate more serious inflammatory reactions in patients with myocardial ischemia[11,12]. Myocardial I/R injury is one of the main causes of death in patients with AMI, so the treatment is one of the important measures to improve the prognosis[13].

Previous studies have focused on the role of microRNA-129-5p in tumors. The up-regulation of miR-129-5p expression accelerated apoptosis of medullary thyroid cancer cells[14]. Gao et al[15] pointed out that miR-129-5p inhibited the proliferation of prostate cancer cells by targeting ETV1. However, in recent years, it has been found that miR-129-5p plays an important role in cardiovascular diseases. Zhang et al[16] revealed that miR-129-5p inhibited autophagy and apoptosis of H9c2 cells induced by hydrogen peroxide *via* the PI3K/AKT/mTOR signaling pathway by targeting ATG14, indicating the potential cardioprotective effect of miR-129-5p in myocar-

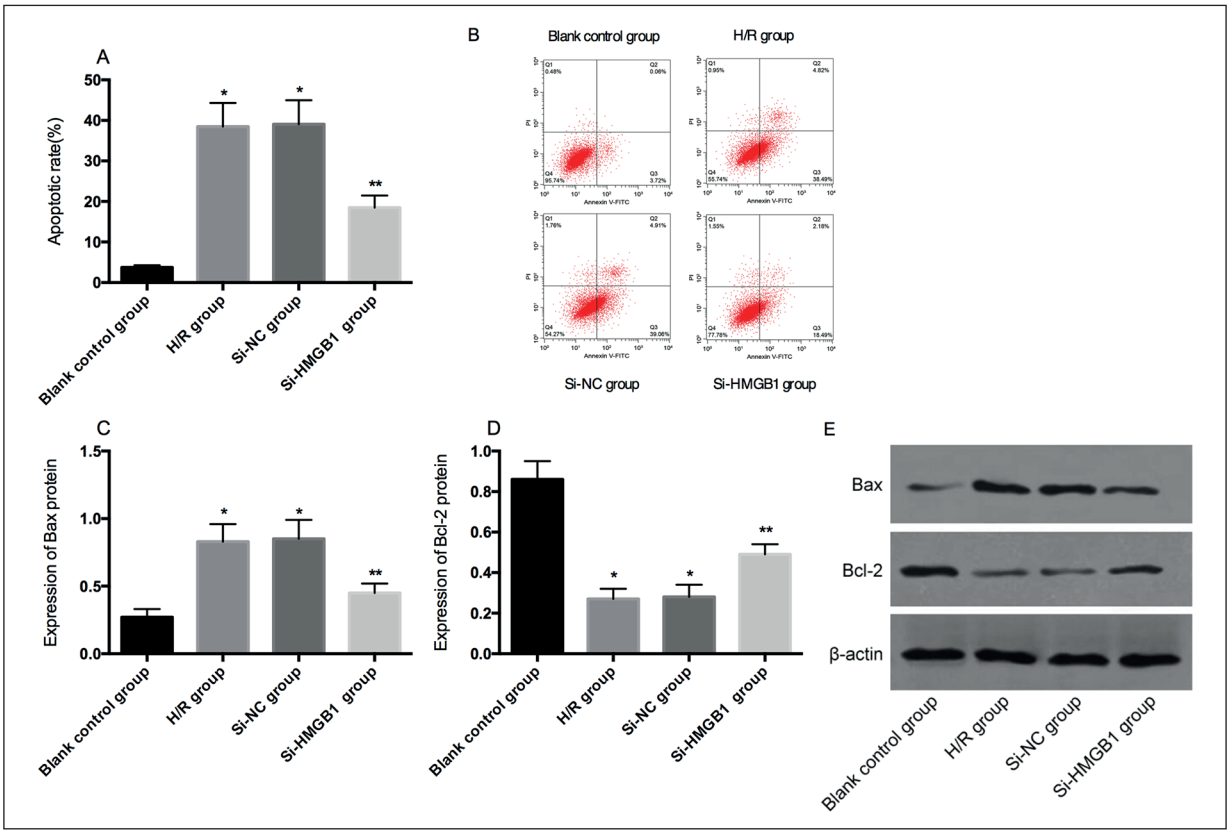

Figure 5. Effects of inhibition of HMGB1 expression on cardiomyocytes in H/R rats. **A,** Inhibition of HMGB1 expression reduced the apoptotic rate of cardiomyocytes in H/R rats. **B,** Flow cytometry. **C, D,** Inhibition of HMGB1 expression down-regulated Bax and up-regulated Bcl-2 in cardiomyocytes of H/R rats. **E,** Western blot. *Indicated that $p<0.05$.

dial I/R injury. In the present study, we found that miR-129-5p expression was significantly down-regulated in both I/R rats and cardiomyocytes of H/R rats, while serum LDH and CK were significantly up-regulated. Ramachandran et al[17] showed that miR-129-5p was down-regulated in cardiomyocytes derived from human embryonic stem cells under oxidative stress. We injected lentivirus transfected with miR-129-5p-agomir into the myocardium of the I/R rat model, and found that the over-expression of miR-129-5p significantly reduced the myocardial IS and cardiomyocyte apoptosis, as well as down-regulated the serum LDH and CK. The results suggested that miR-129-5p was likely to have a protective effect on cardiomyocytes and a mitigative effect on oxidative stress reaction in patients with I/R injury. In the case of myocardial I/R, cardiomyocyte apoptosis is an important pathological basis for cardiac injury, so an H/R injury model was used to simulate cardiomyocytes in I/R injury, and the cardioprotective effect of miR-129-5p on myocardial I/R was further verified with *in vitro* experiments. Our research showed that over-expression of miR-129-5p significantly reduced the apoptotic rate of the cardiomyocytes in H/R rats. The detection of apoptosis-related proteins also showed that the pro-apoptotic protein Bax was significantly down-regulated and the anti-apoptotic protein Bcl-2 was significantly up-regulated, which indicated that miR-129-5p could effectively inhibit the cardiomyocyte apoptosis, consistent with the results of our *in vivo* experiments.

At present, it is well-known that miRNA exerts its function by regulating its target genes. HMGB1 is a multifunctional binding protein with high expression in myocardium of patients with I/R injury[18]. When myocardial I/R occurs, HMGB1 can activate the inflammatory signaling pathway by binding to receptor of advanced glycation end products, thus causing myocardial injury[19]. Oozawa et al[20] reported that the myocardial IS in myocardial I/R injury rats was significantly reduced and the injury was effectively relieved

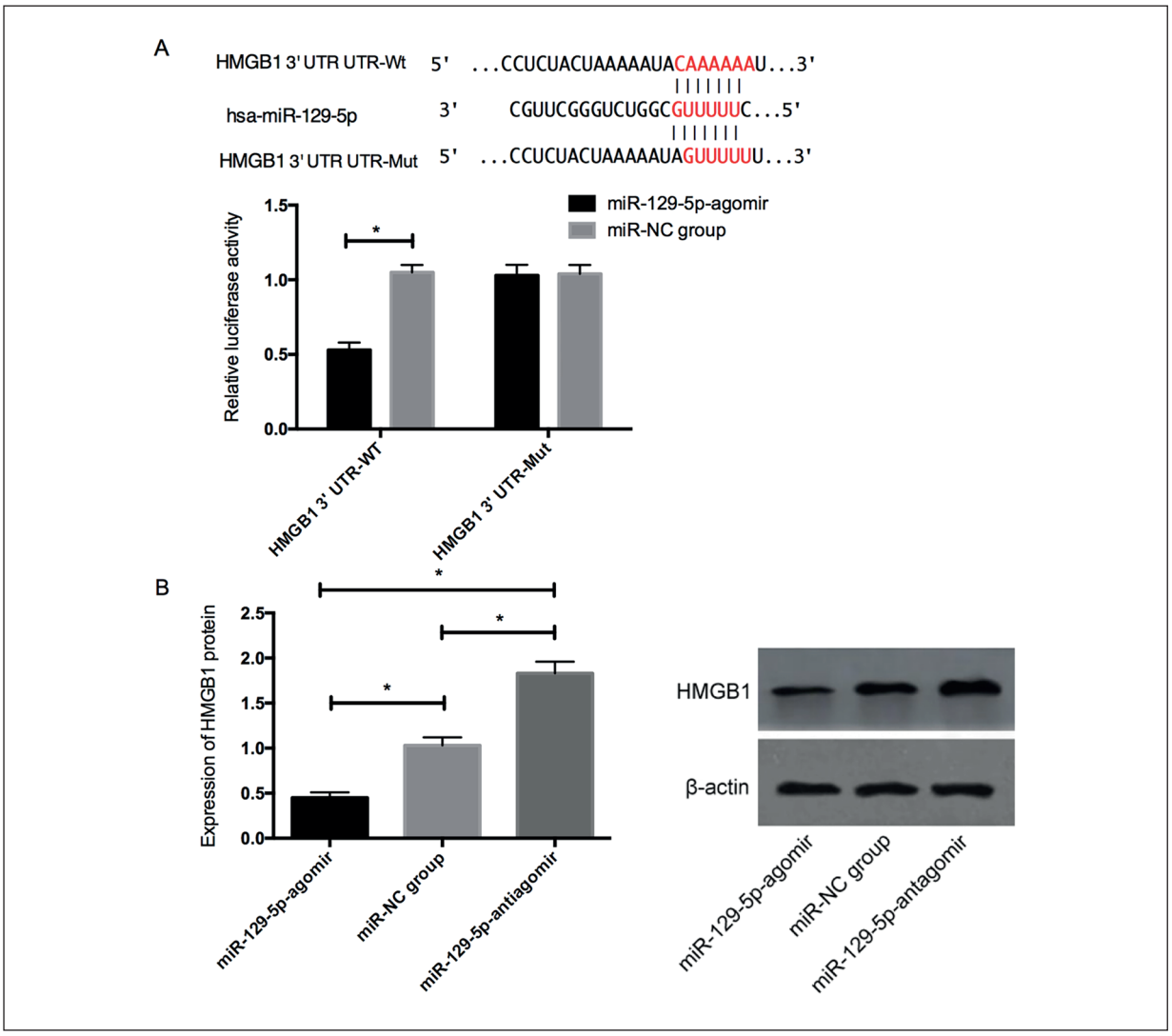

Figure 6. Target relation between miR-129-5p and HMGB1. **A,** There were binding sites between mir-129-5ph and HMGB1. **B,** HMGB1 protein expression in H9c2 was significantly reduced after transfected with miR-129-5p-agomir, while HMGB1 protein expression was significantly increased.

after treatment of anti-HMGB1 antibody. In our study, it was found that HMGB1 expression was significantly up-regulated in I/R rat model and cardiomyocyte of H/R rat model, which was consistent with previous studies. In addition, we found a target relation between miR-129-5p and HMGB1 with TargetScan database. Subsequently, to further verify their relation, Dual-Luciferase detection was performed. The results showed that the over-expressed miR-129-5p inhibited the Luciferase activity of HMGB1, which also indicated that there was indeed a regulatory relationship between miR-129-5p and HMGB1. Therefore, we speculated that miR-129-5p played a protective role on cardiomyocytes in I/R injury by regulating HMGB1. This study showed that the apoptotic rate of cardiomyocytes in H/R rats decreased significantly after inhibiting HMGB1 expression, which suggested the importance of HMGB1 in myocardial I/R injury. HMGB1, a protein secreted by inflammatory cells, can also regulate inflammatory response in I/R. Moreover, anti-HMGB1 intervention effectively improved the expression of serum inflammatory factors in rats with myocardial I/R injury, as well as alleviated myocardial injury[21,22]. We suspected that miR-129-5p inhibited inflammatory reactions by regulating HMGB1, so as to exert cardioprotec-

tive effect on myocardial I/R injury in rats. This suspicion has not been further explored, so more in-depth researches will be conducted.

Conclusions

All together, these data showed that miR-129-5p is down-regulated in both myocardium of I/R rats and cardiomyocytes of H/R rats and can inhibit cardiomyocyte apoptosis *via* targeting HMGB1. Therefore, miR-129-5p may be a promising target for relieving myocardial I/R injury.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

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