



MiR-138-5p exacerbates hypoxia/reperfusion-induced heart injury through the inactivation of SIRT1-PGC-1 α

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Abstract

Objectives A drastic reduction in myocardial cell apoptosis plays a crucial role in the treatment/management of myocardial infarction, a major cardiovascular health challenge confronting the world, especially the Western world. Accumulating evidence indicates that the cardiotoxicity caused by the apoptotic machinery is partly regulated by miRNAs. The aim of this research is to investigate the role of miR-138-5p on hypoxia/reperfusion-induced heart injury.

Methods The expression of miR-138-5p was determined in heart tissue from myocardial infarction patients and rats. Rats were transfected with a miR-138-5p inhibitor to silence miR-138-5p. The cardiac function of rats was detected via echocardiography. SIRT1 and PGC-1 α expression in cardiac infarction was detected via quantitative Real-time PCR (qPCR) and Western blot analysis, while the TUNEL assay was used to determine myocardial apoptosis.

Results Our observations showed that miR-138-5p expression was upregulated after the induction of myocardial infarction. The miR-138-5p inhibitor significantly improved cardiac function, increased the expression of SIRT1 and PGC-1 α , and decreased the rate of myocardial apoptosis, whereas siRNA-SIRT1 reversed these protective effects.

Conclusions In conclusion, our study demonstrated that miR-138-5p could promote cardiac ischemia injury via inhibition of the silent information regulator 1 and peroxisome proliferator-activated receptor gamma and coactivator 1 alpha (SIRT1-PGC-1 α) axis.

Keywords miR-138-5p · Cardiac · Hypoxia/ischemia · SIRT1 · PGC-1 α

Introduction

Increasing evidence has shown that the myocardium does not suffer sudden and complete permanent damage; rather, initiation and progression of cardiotoxicity takes time. To improve myocardial infarction patients' treatment/management outcomes, the myocardium must be salvaged within this time frame. MicroRNAs (miRNAs) are endogenous,

non-coding, 20- to 23-nucleotide RNAs that regulate a variety of target genes. Increasing evidence has shown that the cardiotoxicity caused by the apoptotic machinery is partly regulated by miRNAs that participate in hypoxia/reperfusion-induced heart injury. However, the biochemical functions of all the newly discovered miRNAs and their molecular mechanisms/signaling pathways that are involved in hypoxia/reperfusion-induced cardiotoxicity have not been fully elucidated.

Currently, our in-depth knowledge of the biochemical activities of silent information regulator 1 (SIRT1) in both normal and pathological cardiomyocytes is rapidly expanding. SIRT1 is a nicotinamide adenine dinucleotide (NAD⁺)-dependent histone deacetylase involved in the regulation of metabolism, cell survival, differentiation, and longevity. SIRT1 has been linked to control of longevity, gene silencing, cell-cycle progression, apoptosis, and energy homeostasis [1–3]. SIRT1 interacts with nuclear steroid hormone receptor coactivator proteins, such as p300, PPAR γ , and PGC-1 α , in the differentiation of muscle cells,

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adipogenesis, fat storage, and metabolism in the liver [4, 5]. MiR-34a functions via negatively regulating SIRT1 expression [6], and the SIRT1/nuclear factor erythroid 2-related factor 2 (Nrf2) pathway plays a critical role in oxidative stress that is responsible for ER stress-induced apoptosis [6–8]. Studies have indicated that myocardial I/R injury is related to the abnormal expression of miRNAs that are associated with the pathogenesis of I/R injury [9]. Recently, Yu et al. [10] discovered that miR-138-5p regulates pancreatic cancerous growth through targeting FOXC1. In addition, suppression of SIRT1 results in increased expression of Bax, activated caspase-3 and decreased expression of Bcl-2 contributes to hypoxia/reperfusion-induced apoptosis of cardiomyocytes. A recent study demonstrates that NF- κ B signal pathway mediates the upregulation of miR-138-5p in cardiomyocytes exposed to hypoxia/reperfusion-induced cardiomyocyte H9c2 injury.

In this study, we investigated the cardioprotective effects of SIRT1–PGC-1 α activation via upregulation of miR-138-5p in hypoxia/reperfusion-induced heart injury.

Materials and methods

Animals and ethics statement

All the animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996). The animal experiments were approved by Jining Medical University and performed according to the Guide for the Care and Use of Laboratory Animals according to the regulation of People's Republic of China.

In this study, healthy adult Sprague–Dawley rats (6 weeks old) weighing between 150 and 200 g were obtained from the experimental animal center. The experiment was divided into two phases. In the first phase, mice were randomly divided into three groups of five animals each: sham-operation group (Sham-group), myocardial ischemia–reperfusion group (IR-group) and miR-inhibitor group (IR + miR-inhibitor group). In the second phase, the mice were randomly divided into four groups of five animals each: myocardial ischemia–reperfusion group (IR-group), miR-inhibitor group (IR + miR-inhibitor group) and inhibitor + siRNA-SIRT1 group (IR + inhibitor + siRNA-SIRT1 group).

Dual luciferase assay for SIRT1 target identification

The potential targets of miR-138-5p were assessed in three publicly available algorithms: TargetScan, miRDB and microRNA. The recombinant luciferase reporter plasmids containing sequences of potential miR-138-5p-binding sites

in the 3' UTR located between positions 35–42 of the SIRT1 gene were constructed. Using a site-directed mutagenesis kit (TransGen, Beijing, China), the miR-138-5p complementary binding sequence GUGGUCGA was substituted with ACA ACAA to construct recombinant luciferase reporter plasmids with mutant miR-138-5p binding sequences. Human embryonic kidney (HEK) 293 cells (3×10^5 cells per well in 12-well plate) were cotransfected with 200 ng of recombinant luciferase reporter plasmid, 50 nM miR-138-5p mimic, and 20 ng of pRL-TK as an internal control (Promega, Madison, WI). Luciferase activities were examined after 24 h using a luciferase reporter assay system (Promega, USA) following the manufacturer's protocol. The experiments were performed in triplicate.

Myocardial ischemia–reperfusion injury model

Myocardial I–R injury was induced according to a previously described procedure. In brief, rats were anesthetized with 1% sodium pentobarbital (1 ml/kg, i.p.) and their left anterior descending coronary artery was temporarily occluded for 30 min, followed by 3 h of reperfusion. The same procedures were performed on the sham mice without ligation and reperfusion (Wang et al. 2016). In the second phase, siRNA-SIRT1 (Biogen Biotechnology, Beijing, China) was transfected to rats in the IR + inhibitor + siRNA-SIRT1 group. Briefly, the aorta and pulmonary artery were identified. A 23-G catheter containing 300 μ L of the lentivirus was inserted from the left ventricle into the aortic root. Three rats chosen randomly from each group were killed to detect miR-138-5p expression 28 days after ischemia–reperfusion. The remaining rats were fed until the 4th week after an operation for further study.

Echocardiographic study

Left ventricular (LV) function variables were assessed by transthoracic echocardiography. After the induction of light general anesthesia, the rats underwent transthoracic two-dimensional (2D)-guided M-mode echocardiography with an 8.5 MHz transducer (Acuson, Mountain View, CA). From the cardiac short axis (papillary level), the LV anterior wall end-diastolic thickness (LVAW.d), the systolic LV anterior wall thickness (LVAWs), the LV internal dimension at end-diastole (LVIDd), the LV internal dimension at end-systole (LVIDs), the LV posterior wall end-diastolic thickness (LVPW.d), the LV posterior wall end-systolic thickness (LVPWs), the ejection fraction (EF) and fractional shortening (FS) were determined. Echocardiographic measurements were reported as the average of three separate cardiac cycles.

A Millar catheter was implanted into the left ventricular cavity via the right common carotid artery, and changes in the left ventricular posterior wall thickness (LVPW.d)

and left ventricular ejection fraction (LVEF) as well as the maximal slope of systolic pressure increase (LV dp/dt max) and diastolic decrease (LV dp/dt min) were detected and recorded using a Transonic Scisense Pressure Measurement System (SP200, Transonic Scisense Inc., Ontario, Canada). After ultrasound examination, rats were executed, and the hearts were removed for histological examination.

Determination of myocardial infarct size

Myocardial infarct sizes were measured by the double-staining technique using Evans blue and 2,3,5-triphenyl tetrazolium chloride (TTC; Sigma-Aldrich, St Louis, MO, United States) (Bohl et al. 2009). Infarcted tissue slices were scanned, and tissue weights were evaluated by distinguishing the normal myocardium (stained blue) from the area at risk and infarct area (unstained) in a TTC staining assay.

Quantitative-real-time PCR (qPCR)

Total RNA concentrations were determined by using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies) at 260 and 280 nm (A₂₆₀/280) and examined using an Agilent 2100 Bioanalyzer (Agilent Technologies). Quantitative real-time PCR (qPCR) assays were performed using a TaqMan miRNA assay according to the manufacturer's protocol (Applied Biosystems). The amplification conditions were 40 cycles of 15 s at 95 °C and 1 min at 60 °C. The expression levels of miR-138-5p, PGC-1 α and SIRT1 were normalized by the expression level of the housekeeping β -actin gene.

Protein extraction and Western blot analysis

At the end of the experiment, myocardial tissue samples from rats and myocardial cells after transfection were homogenized with a tissue protein extraction reagent (Thermo Scientific, United States) containing a protease inhibitor cocktail (Sigma-Aldrich, St Louis, MO, United States). The homogenates were then centrifuged at 12,000g for 10 min at 4 °C. The protein level was determined using the bicinchoninic acid (BCA) method. The supernatant and the loading buffer in a ratio of 1:1 were heated at 95 °C for 10 min. The protein samples were subjected to SDS-PAGE and electrophoretically transferred onto PVDF protein sequencing membranes for 90 min. The membrane was sealed with 5% non-fat milk in PBS with 0.1% (v/v) Tween-20 (PBST) at 25 °C for 1 h. The membranes were washed and blotted with the antibodies against SIRT1, PGC-1 α (Santa Cruz, United States), and β -actin (Novus, United States). The membrane was incubated with HRP-conjugated secondary antibody (Jackson ImmunoResearch Laboratories, United States) prior to chemiluminescence

detection (Pierce, United States). Western blot analyses of protein expression were performed using two different samples from each treatment group in triplicate (using the same samples). Six values were obtained for each study group, while β -actin was used as the internal reference.

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay

Apoptotic myocardial cells were detected by TUNEL assay. This assay is based on the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) method. As per the protocol, cultured cardiomyocyte cells on coverslips were fixed in 4% paraformaldehyde after experimental treatments. Permeabilization was performed with 0.2% Triton X-100. Cy5-dUTP (Amersham, Piscataway, NJ, USA) was used to label DNA fragments in apoptotic cells. The level of TUNEL-positive cells was detected by fluorescence microscopy, and approximately 200 cells per field in five different visual fields were counted in this study.

Histological analysis

Rats were killed with an intraperitoneal injection of 2 mL of pentobarbital. The heart was excised, and the LV myocardium was fixed overnight in 10% formalin. Samples were embedded in paraffin and cut into 4- μ m-thick sections. Sections were mounted on normal glass slides and stained with Masson trichrome for histological examination. For the collagen volume fraction (CVF) analysis, eight separate views (magnification = original \times 400) were selected, and CVF was assessed using the following formula: CVF = collagen area/total area.

Statistical analysis

The data are presented as the mean \pm SEM. In each experiment, all determinations were performed at least in triplicate. Statistical significance between two measurements was determined by the two-tailed unpaired Student's *t* test, and significance among groups was determined by one-way ANOVA. A value of $p < 0.05$ was considered to be significant.

Results

The expression level of miR-138-5p in cardiac infarction patients

The level of miR-138-5p expression in cardiac tissues was evaluated to determine its role in myocardial infarction. Compared with the normal healthy cardiac tissues,

the expression level of miR-138-5p in cardiac tissues was significantly increased in myocardial infarction patients. However, plasma miR-138-5p levels in myocardial infarction patients remained slightly unchanged compared with plasma miR-138-3p levels in normal healthy person (Fig. 1a, b).

The effect of miR-138-5p inhibition on the morphology of hypoxia/reperfusion-induced heart injury in rats

Increased levels of miR-138-5p in human infarcted heart tissue indicated that miR-138-5p might act as a promoter of IR

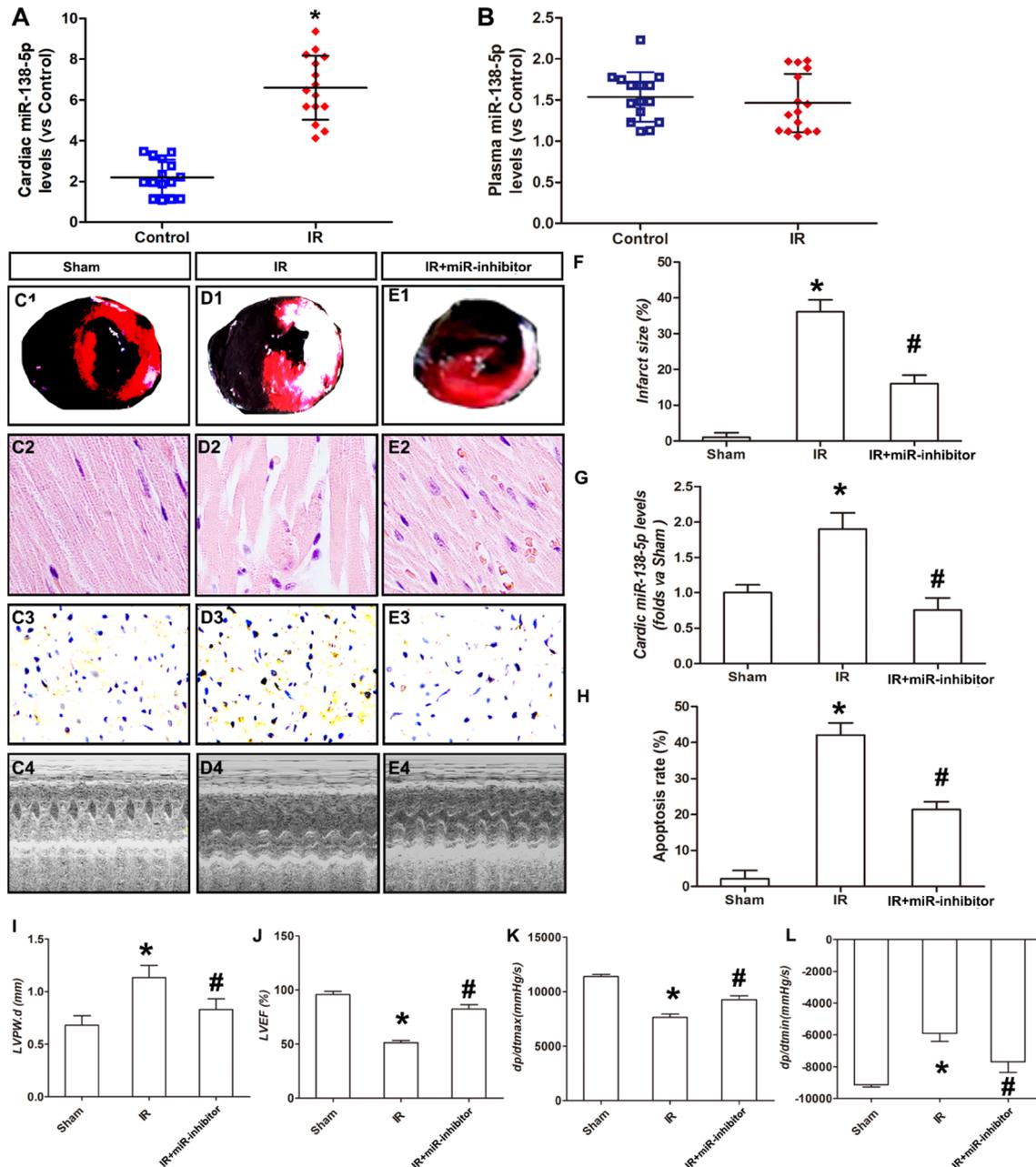


Fig. 1 Expression of miR-138-5p in myocardial infarction. **a, b** miR-138-5p levels in human myocardial tissue and human plasma. **c1–e1** Morphology (heart size) of rat hearts in each group. **c2–e2, c3–e3** H&E and TUNEL staining of left ventricular tissue. **c4–e4** Representative M-mode echocardiography recordings for the three different groups. **f** Quantitative estimation of percentage infarct size. **g** miR-138-5p levels in rats. **h** The effect of miR-138-5p on the percentage

of apoptotic cells. **i** Represents diastolic left ventricular posterior wall thickness. **j** Percentage left ventricular ejection fraction (LVEF%). **k** Peak instantaneous rate of left ventricular pressure increase (LVdp/dt_{max}). **l** Peak instantaneous rate of left ventricular pressure decline (LVdp/dt_{min}). Data are presented as the mean ± standard error of the mean (**p* < 0.001 versus Sham-group, #*p* < 0.05 versus IR-group)

injury. Thus, heart morphology and function were evaluated in rats after IR. We observed a significant increase in heart infarct size in the IR-group compared with the sham group. Our data further revealed that miR-138-5p plays a functional role in myocardial infarction. A miR-138-5p inhibitor significantly decreased the heart infarct size in the IR + miR-inhibitor group compared with the sham group (Fig. 1c–f).

The expression level of miR-138-5p in hypoxia/reperfusion-induced heart injury in rats

The level of miR-138-5p in cardiac tissues was significantly increased in the IR-group compared with the sham group, whereas the IR + miR-inhibitor group exhibited a significant decrease in miR-138-5p expression levels in rat cardiac tissue. Our data indicated that miR-138-5p expression is deeply involved in the pathogenesis of (hypoxia/reperfusion-induced heart injury in rats)/myocardial infarction (Fig. 1g).

A miR-138-5p inhibitor ameliorated cell apoptosis after IR induction

To determine the effects of miR-138-5p on apoptosis induced by hypoxia/reperfusion in rat hearts, we first examined apoptosis using the TUNEL assay method. Hypoxia/reperfusion-induced injury resulted in an alteration in cell morphology, including shell shrinkage, non-fullness, pyknosis, DNA fragmentation and necrosis, which are characteristics of apoptotic cells. The apoptosis rate was significantly increased in the IR-group (43%) compared with the sham group (2%). By contrast, visible apoptotic bodies were significantly reduced in the IR + miR-inhibitor group when compared with IR-group (Fig. 1h).

An miR-138-5p inhibitor improves cardiac function after hypoxia/reperfusion-induced heart injury

Using echocardiographic analysis, we revealed that inhibition of miR-138-5p exerts protective effects on cardiac function of hypoxia/reperfusion-induced heart injury. Hypoxia/reperfusion induces pressure overload characterized by an increase in wall thickness. Pressure overload characterized by an increase in the left ventricular posterior wall thickness (LVPW.d) was significantly increased in the IR-group ($p < 0.05$) compared with the sham group. However, the IR + miR-inhibitor group exhibited a significant decrease in (LVPW.d) compared with the IR-group (Fig. 1i). As shown in Fig. 1j, the percentage left ventricular ejection fraction (LVEF %) (oxygen-rich blood pumped out of the left ventricle of the heart with each contraction into a network of arteries/blood vessels) was significantly decreased in the IR-group (50%) compared with the sham group (98%). Compared with the sham group, the IR-group exhibited a

significant decrease in the maximal rate of increasing left ventricular pressure, whereas inhibition of miR-138-5p caused a significant increase in the maximal rate of increasing left ventricular pressure compared with the IR-group (Fig. 1k, l).

Identification of SIRT1 as a target of miR-138-5p

To identify the potential target of miR-138-5p, we analyzed three publicly available algorithms, including TargetScan, miRDB and microRNA, to elucidate the putative targets of miR-138-5p. Finally, we found that SIRT1 was a putative target of miR-138-5p. Our data showed that IR dramatically decreased the binding mRNA levels of SIRT1, and miR-138-5p transfection further decreased the binding mRNA levels of SIRT1 in rats subject to IR injury (Fig. 2a). We further assessed the level of SIRT1 protein expression. The results showed that SIRT1 expression was reduced in the IR-group and IR + miR-138-5p group (Fig. 2b). Furthermore, our results showed that the relative luciferase activity of the SIRT1 3' UTR reporter treated with miR-138-5p was significantly suppressed compared with transfection with random mimics or the mutant reporter or empty vector transfected with miR-138-5p (Fig. 2c, d).

SiRNA-SIRT1 reversed the effect of miR-138-5p inhibition against myocardial ischemia

Our current data indicated that SIRT1 is a target gene of miR-138-5p. We explored the role of siRNA-SIRT1 to elucidate the biochemical mechanism of miR-138-5p in myocardial infarction via the SIRT1–PGC-1 α pathway. Rat heart size and weight were remarkably reduced in the IR + miR-inhibitor group compared with the IR-group, whereas siRNA-SIRT1 reversed the effect of the miR-inhibitor. The IR + inhibitor + siRNA-SIRT1 group exhibited significant increase in percentage infarct size compared with the IR-group. While IR + miR-inhibitor group caused marked reduction in percentage infarct size, we observed a significant reduction in percentage of apoptosis in the IR + miR-inhibitor group but a significant increase in the IR + inhibitor + siRNA-SIRT1 group. This finding implies that the percent reduction in the rate of apoptosis by the miR-inhibitor was remarkably inhibited by siRNA-SIRT1. The pressure overload characterized by an increase in (LVPW.d) was significantly decreased in the IR + miR-inhibitor group but significantly increased in the IR + miR-138-5p-NAM group compared with the IR-group. Using echocardiographic analysis, we observed that the IR + miR-inhibitor group exhibited a significant increase, while the IR + inhibitor + siRNA-SIRT1 group exhibited a significant decrease in the percentage (% LVPF) (Fig. 3).

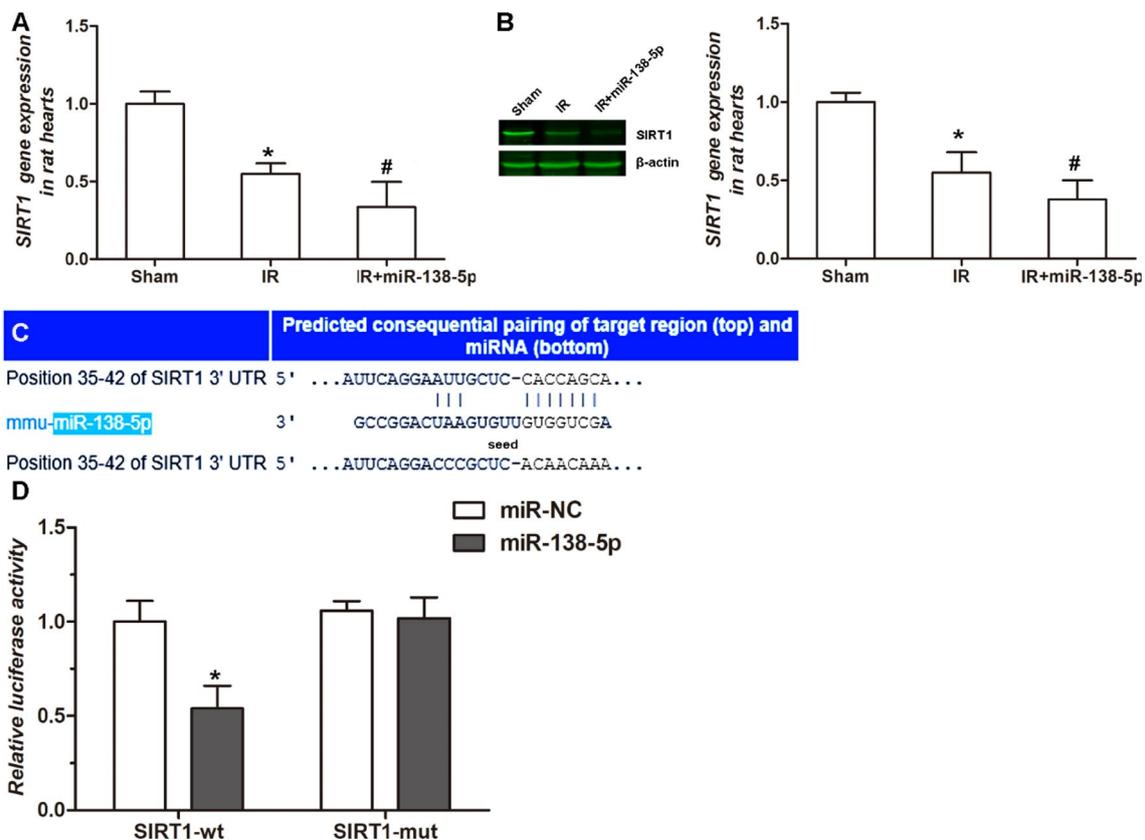


Fig. 2 miR-138-5p directly targets SIRT1. **a** SIRT1 gene expression in rat heart. **b** SIRT1 protein expression levels in rat hearts. **c** Predicted binding site of miR-138-5p in 3'-UTR of SIRT1. **d** Cotransfection of SIRT1 and miR-138-5p decreased luciferase activity, while

cotransfection of SIRT1-mut and miR-138-5p did not change luciferase activity. Data are presented as the mean \pm standard error of the mean (* $p < 0.001$ versus Sham-group, # $p < 0.05$ versus IR-group)

The SIRT1–PGC-1 α pathway mediates the antiapoptotic effect of miR-138-5p inhibition in cardiomyocytes

However, to determine the ameliorative/protective effect of miR-138-5p expression on apoptosis induced by hypoxia/reperfusion in rat heart, we employed the TUNEL assay method. Hypoxia/reperfusion-induced heart injury resulted in an alteration in cell morphology, including shell shrinkage, non-fullness, and pyknosis/necrosis, which are characteristics of apoptotic cells. The IR + miR-inhibitor group exhibited a significant reduction in the percentage of apoptotic cells compared with the IR-group. However, this significant reduction in apoptosis by the miR-inhibitor was remarkably inhibited by siRNA-SIRT1, which silences SIRT1 (Fig. 3e).

To further elucidate whether the antiapoptotic effect of miR-138-5p was mediated via the SIRT1 signaling pathway, both SIRT1 gene expression and protein expression were measured. Our results revealed that the miR-inhibitor could upregulate the level of SIRT1 mRNA and protein levels in cardiac tissues exposed to IR injury. Moreover, we

explored the effect of siRNA-SIRT1 on SIRT1 and PGC-1 α mRNA expression to elucidate the biochemical mechanism of the cardioprotective properties of miR-138-5p. Furthermore, SIRT1 and PGC-1 α mRNA expression levels in the IR + inhibitor + siRNA-SIRT1 group were significantly reduced compared with the IR + miR-138-5p group (Fig. 4a, b). These protein expression results are consistent with mRNA expression levels. Thus, our western blot assay results indicate that SIRT1 and PGC-1 α expression was significantly increased in the IR + miR-inhibitor group compared with the IR-group but were significantly decreased in the IR + inhibitor + siRNA-SIRT1 group compared with the IR + miR-inhibitor group (Fig. 4c, d).

Discussion

In the present study, we revealed the role of miR-138-5p in the pathogenesis of cardiac IR injury. First, we found that miR-138-5p expression was enhanced in both heart tissue from human and rats. Second, a miR-138-5p inhibitor significantly reduced IR-induced cardiac morphology

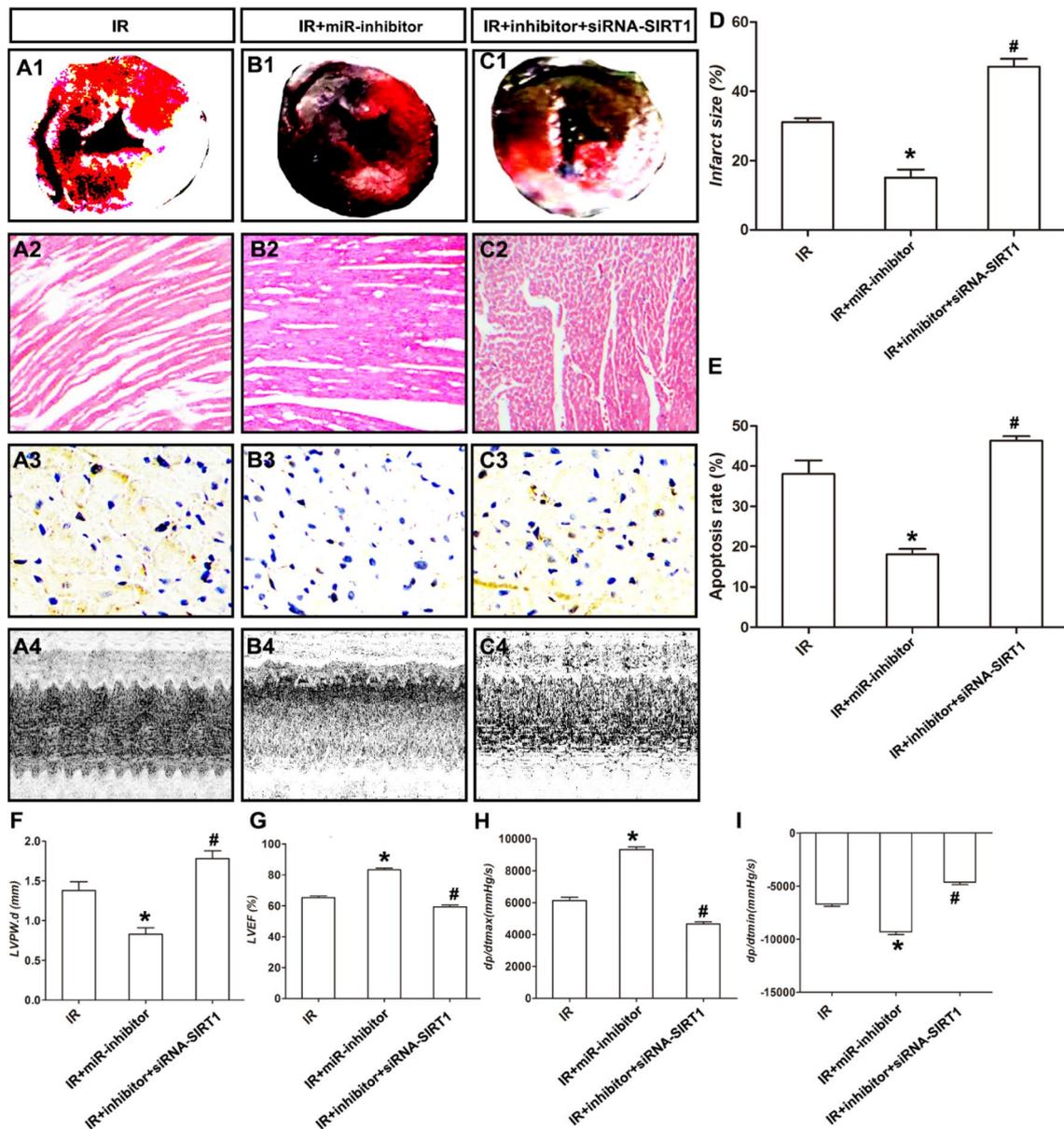


Fig. 3 siRNA-SIRT1 inhibits the ameliorative effects of the miR-138-5p inhibitor on myocardial infarction. **a1–c1** Representative images of the morphology (heart size) of rat hearts in different groups. **a2–c2**, **a3–c3** Representative images of left ventricular tissue stained with hematoxylin–eosin and TUNEL, respectively. **a4–c4** M-mode echocardiography recordings in different treatment groups. **d** Quantitative estimation of percentage infarct size. **e** The percentage of apoptotic cells. Echocardiography analysis of cardiac function

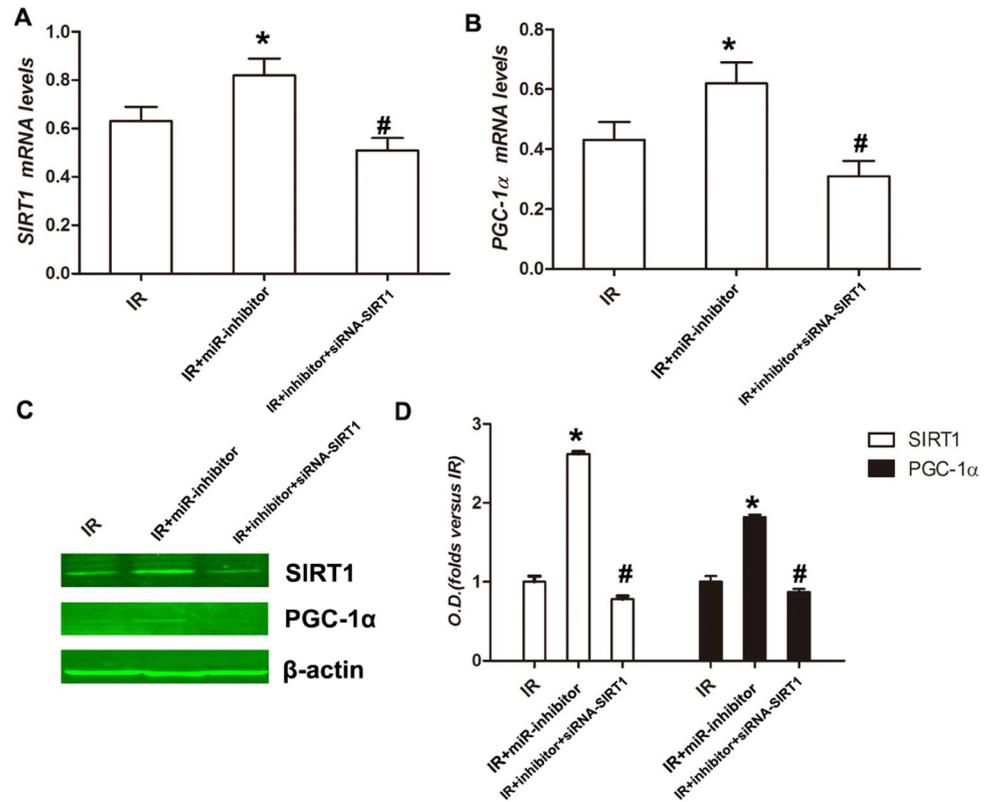
in IR-induced heart injury in mice. **f** Diastolic left ventricular posterior wall thickness. **g** Percentage left ventricular ejection fraction (LVEF%). Hemodynamic parameters were measured with the Millar cardiac catheter system in IR mice. **h** Peak instantaneous rate of left ventricular pressure increase (LVdp/dt_{max}). **i** Peak instantaneous rate of left ventricular pressure decline (LVdp/dt_{min}). Data are presented as the mean ± standard error of the mean (**p* < 0.001 versus IR-group, #*p* < 0.05 versus IR + miR-138-5p-group)

and function. Third, we proved that SIRT1 is a direct target for miR-138-5p binding. Finally, we found that SIRT1 and PGC-1α levels were both upregulated with miR-138-5p inhibitor treatment against IR, while silencing SIRT1 using siRNA can reverse the protective effect of a miR-138-5p inhibitor. Thus, we elucidated that miR-138-5p was involved in promoting the cardiac injury after IR, whereas inhibition

of miR-138-5p could exert protective effects through the SIRT1–PGC-1α signaling pathway.

Cardiac energy synthesis relies mostly on oxidative metabolism and is, therefore, highly sensitive to changes in intracellular oxygen levels. Therefore, necrosis of cardiac cells due to reduced oxygen supply as a consequence of occlusion of coronary arteries leads to myocardial infarction.

Fig. 4 miR-138-5p exacerbates myocardial Infarction by directly targeting SIRT1. **a** SIRT1 gene expression in rat heart was detected by qPCR. **b** The efficiency of PGC-1 α expression levels was detected by qPCR. **c** PGC-1 α and SIRT1 expression levels were detected by western blot. **d** The quantification of data from western blot of PGC-1 α and SIRT1 expression levels. Data are presented as the mean \pm standard error of the mean (* p < 0.001 versus IR-group, # p < 0.05 versus IR + miR-138-5p-group)



MiRNAs are important regulators of many biological processes in various diseases, including cardiovascular disease [11]. Recently, it has been proven that several miRNAs participate in regulating the activities of myocardial cells in acute myocardial infarction and affect the prognosis of acute myocardial infarction [9]. In this present research, we determined that miR-138-5p, a paracrine hormone, is expressed in the myocardial tissue/cells after cardiac ischemia, and its downregulation alleviates hypoxia/reperfusion-induced heart injury. Our observation is consistent with a previous study which shows that the majority of the observed increase in miR-138 under hypoxia-induced endothelial cell dysfunction is attributable to the endothelial cell compartment given that both skeletal muscle cells and vascular smooth muscle cells do not demonstrate a significant increase in miR-138 when subjected to hypoxia [12].

Mediators of apoptosis and necrosis via death receptor pathways and mitochondrial pathways as well as autophagy play important roles in the progression of maladaptive remodeling to heart failure. Previous studies reported that stem cell-derived exosomal microRNAs, including miR-19a, miR-21, miR-22, miR-26a, miR-125b-5p and miR-210, showed cardioprotective effects by enhancing cardiomyocyte survival and function [13]. However, there are other miRNAs that could enhance apoptosis and aggravate IR-induced cardiac injury, such as miR-143, which could promote cardiac ischemia-mediated mitochondrial impairment [14], and

miR-145-5p, which induces apoptosis after IR [15]. Inhibition of these miRNAs could exert protective effects, which further indicated that miRNAs regulate cardiomyocyte apoptosis to exert their function. Loss of myocardial cells is the most important determinant affecting prognosis after myocardial infarction; thus, a reduction in myocardial cell loss is a key factor in the treatment/management of myocardial infarction [16, 17]. In our study, miR-138-5p enhanced cell apoptosis, and inhibition of miR-138 exerted cardioprotective effects, which is also consistent with previous studies that miR-138-5p promotes cell apoptosis [18, 19]. To further understand the role of miR138-5p in cardiac ischemia, we further investigated the underlying mechanism. As indicated in our results, we found that SIRT1 could be a target in miR138-5p regulation. This result is also parallel with a previous study demonstrating that miR-138-5p could suppress autophagy in pancreatic cancer by targeting SIRT1 and reducing SIRT1 levels [20]. Previous studies have illustrated the important role of SIRT1 in the regulation of IR. Reduced SIRT1 levels could lead to hepatic IR, whereas enhancing SIRT1 levels attenuated cell damage in liver transplant IR [21]. However, whether SIRT1 could be the target of miR138-5p in the model of IR injury remains unclear. Our study revealed that the miR-138-5p inhibitor upregulated and the SIRT1-inhibitor reversed SIRT1 gene and protein levels in cardiac tissues exposed to IR injury. The literature suggests that SIRT1–PGC-1 α signaling plays an important

role in neuronal survival and mitochondrial oxidative stress [22]. PGC-1 α regulates cell metabolism, oxidative stress and mitochondrial biogenesis [23]. PGC-1 α also acts as a regulator to manipulate IR injury in different animal models. Downregulation of PGC-1 α enhanced IR-induced injury and impaired ischemic preconditioning [24]. Another study suggested that activation of PGC-1 α ameliorated ischemia-induced neuronal injury [25]. Moreover, nuclear SIRT1 may increase its activity by deacetylating PGC-1 α ; this effect can be cardioprotective given that deacetylation of PGC-1 α increases its activity [26–28]. SIRT1 suppression decreases PGC-1 α in ischemic heart disease [29]. Previous research suggests that mitochondrial apoptosis is reduced after PGC-1 α overexpression in human sarcoma cell lines [30], indicating that the SIRT1–PGC-1 α pathway may be protective during mitochondrial-dependent apoptosis. Western blot assay results revealed that both SIRT1 and PGC-1 α exhibited significant increases in the IR + miR-inhibitor group compared with the IR-group. These significant increases were reversed by siRNA-SIRT1. Collectively, we demonstrated that miR-138-5p promotes hypoxia/reperfusion-induced cardiotoxicity via the SIRT1–PGC-1 α pathway. Currently, alterations in SIRT1 expression and localization have been linked to cardiac/hepatic-ischemia/reperfusion damage and hepatic lipid homeostasis [29, 31, 32]. In addition, reduced electron transport chain proteins may have resulted from decreased deacetylated PGC-1 α .

In conclusion, as briefly described in Fig. 5, our data indicate that downregulation of miR-138-5p may play a crucial role in regulating hypoxia/reperfusion-induced cardiomyocytes apoptosis possibly through targeting SIRT1, which deacetylates PGC-1 α to enhance its antiapoptotic activity. Overexpression of miR-138-5p may serve as a novel approach for the treatment of patients with myocardial infarction.

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Author contributions AC designed research and wrote the paper; CW and XS performed research and analyzed data; ZQ helped with the qPCR experiments and data analysis.

Compliance with ethical standards

Conflict of interest We declare no competing financial interests.

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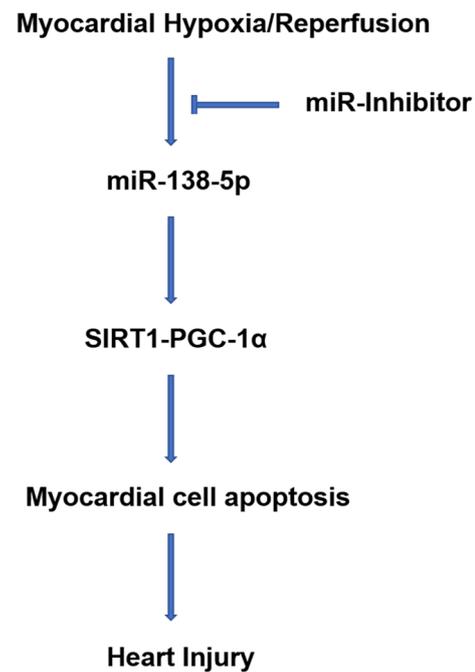


Fig. 5 Graphical data to illustrate the role of miR138-5p in myocardial hypoxia/reperfusion. In brief, myocardial hypoxia/reperfusion enhanced the level of miR138-5p, which could directly bind to SIRT1 and downregulate SIRT1 expression. Next, downregulated SIRT1 will poorly deacetylate PGC-1 α and results in myocardial cell apoptosis, which further induces heart injury. However, miR-138-5p inhibitor could enhance the activity of SIRT1–PGC-1 α signaling pathway and reverse the myocardial cell apoptosis and heart injury

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