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Original Article

miR-92a inhibits myocardial infarction-induced apoptosis by targeting PTEN

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Abstract: Objective: Acute myocardial infarction (AMI) is the primary cause of sudden mortality worldwide. MicroRNAs (miRs) are a class of small non-coding RNA molecules that function as modulators of cardiovascular disorders, including myocardial infarction (MI). In the present study, we investigated the role of miR-92a in MI. Methods: A MI animal model was used in this study. MTT Assay was conducted to determine the cell viability. Binding sites between miR-92a and PTEN were identified using a luciferase reporter system, whereas mRNA and protein expression of target genes was determined by RT-PCR and immunoblot, respectively. Evaluation of cell death, infarct size, and cardiac activity were also performed. Results: miR-92a prevented down-regulation of Bcl-2 expression and up-regulation of caspase-3 and Bax caused by H_2O_2. miR-92a expression prevented up-regulation of PTEN expression in H_2O_2-treated cardiomyocytes. Moreover, our findings revealed that miR-92a directly binds to the 3'-untranslated region (3'-UTR) of the PTEN gene. Furthermore, miR-92a agomir treatment considerably suppressed cell death, reduced LDH levels in circulation, and inhibited the function and expression of myocardial caspase-3 and PTEN in MI mice. Injection of miR-92a agomir in mice reduced the size of the infarcted regions and promoted cardiac activity. Conclusions: miR-92a expression was found to be down-regulated in murine cardiomyocytes and in MI cardiac tissues in response to H_2O_2 stress, while miR-92a over-expression protected against cell death via modulation of PTEN/caspase-3-associated pathways.

Keywords: miR-92a, MI, cell death, PTEN, caspase-3

Introduction

MI (myocardial infarction) is a major contributor to mortality and cardiovascular diseases worldwide [1]. Nevertheless, there is a need to elucidate the etiology of MI pathogenesis, which is associated with oxidative stress (OS), amplified inflammatory response, Ca^{2+} overload inside cells, opening of the mitochondrial permeability transition pore, and rapid recovery of physiological pH following reperfusion [2].

Cell death, which results from the imbalance between the signals that promote cell death and those that inhibit cell death, has been frequently reported in cardiac tissues in MI animals [3]. Loss of cardiomyocytes deteriorates heart function and leads to heart failure. Dead cardiomyocytes at the fringes between MI regions can lead to human death [4, 5]. Therefore, suppression of cardiomyocyte death during the primary period of MI is crucial for reducing the infarcted region and promoting cardiac recovery. However, understanding the factors involved in MI-linked cell death in cardiomyocytes is insufficient.

miRNAs represent a category of non-coding RNAs of 22 to 25 nucleotides that modulate targeted expression subsequent to transcription and subsequently induce mRNA degeneration [6]. Recent studies have demonstrated that miRNAs participate in various biological processes, including migration, proliferation, and necrosis, as well as cell death [7, 8]. Several studies have revealed that miRNAs participate in cardiac reactions mediated by MI and act by regulating crucial pathways, thereby indicating that miRNAs can serve as crucial targets for MI treatment [9, 10]. miR-92a is a member of the miR-17-92 cluster, whose expression has been observed in juvenile endotheliocytes instead of old endotheliocytes to induce aggregated OS and cell death [11].

PTEN, a tumor suppressor gene, plays an essential role in cell proliferation, apoptosis, differentiation, migration, etc [12]. Recently, increasing studies have demonstrated that PTEN is a regulator in myocardial remodeling, cardiac hypertrophy, myocardial fibrosis, and myocardial ischemia reperfusion injury [13-15].

In the present study, the effects of miR-92a on heart malfunction and MI-induced cardiomyocyte death were investigated in MI mice and H_2O_2-treated cardiomyocytes. Our findings clearly demonstrate that miR-92a targets PTEN to prevent cardiomyocyte death. Therefore, our results indicate that miR-92a is a promising target for the treatment of MI.

Material and methods

Reagents

Lactate dehydrogenase (LDH) and creatine kinase (CK) commercial kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Annexin V-FITC/propidium iodide (PI) apoptosis kit was purchased from Beyotime Institute of Biotechnology (Shanghai, China). PTEN, caspase-3, Bax, Bcl-2, and β-actin antibodies were obtained from Santa Cruz Biotechnology (USA). Dual luciferase reporter assay kit was from Promega (USA).

Animals

Normal male adult BALB/c mice with body weights ranging from 25 to 30 g were purchased from Shanghai SLAC Animal Co., Ltd. (Shanghai, China). Mice were maintained under standard animal room conditions (humidity, 55±5%; temperature, 23±1°C) and provided with water and food for one week prior to the experiments. All procedures were conducted in accordance to the Guide for the Care and Use of Laboratory Animals (see NIH Publication, 8th Edition, 2011).

MI model and miR-92a agomir supplementation

miR-92a agomir is double-stranded RNA analogs of the mature mmu-miR-92a-5p (5'-UCUGUAUGGUAUUGCACUUGUCCCGGCCUGUU-3') that have been chemically modified and conjugated to a cholesterol moiety to promote specificity and stability for additional assays.

Prior to surgery, narcosis was induced in mice using tribromoethanol (20 mg/kg) with ventilation. Thoracic surgery was performed through the fourth intercostal space. The aorta ascendens and main pulmonary artery were clamped (LAD ligation) for 10 s to produce a MI model. The animals were divided into three groups: (1) Negative control (NC) group: mice did not receive any injection or LAD ligation. (2) miR-92a group: Mice were injected with miR-92a agomir (300 nmol/kg with a final volume of 50 μl) into the left chest cavity through the cardiac apex using a thirty-gauge syringe before MI model establishment. (3) MI model group: mice received the same treatment but instead were injected 80 μl of saline [16].

Detection of infarct size

Heart samples were obtained three days after agomir treatment. TTC staining was performed to evaluate infarct size [17]. After blood washing, left ventricles were cut into 1-mm-thick slices and stained with 1% TTC at 37°C for 30 min. The infarcted region showed no staining, but the normal region stained red. The infarcted region was analyzed using Image ProPlus 5.0 software. Data were presented as the percentage of left ventricular volume per sample.

Evaluation of cardiac activity

Narcosis was induced using tribromoethanol (20 mg/kg), and mice were subjected to two weeks of ventilation after agomir supplementation. Cardiac activity was assessed using a high-resolution imaging system ultrasonic apparatus at the probe frequency of 40 MHz. Left ventricular end diastolic diameter (LVEDd), left ventricular endsystolic diameter (LVESD), and ventricular fraction shortening (LVFS) were evaluated using a horizontal section of the two-dimensional left ventricular short axis. A total of four successive cardiac cycles were obtained, and the mean value was used for subsequent analysis.

Cultivation and transfection of ventricular cardiomyocytes

Newborn murine ventricular cardiomyocyte (NMVCs) were collected from mice aged from one to three days and cultivated [18, 19]. Briefly, hearts were excised under sterile conditions, and ventricle samples were cut into pieces before digestion with trypsin (0.25%). Isolated cells were suspended in DMEM containing 10% FBS, centrifuged for 5 min at 1000 rpm, and subsequently resuspended for 2 h. Separated cells were incubated in non-coated cultivating flask. The culture medium was sup-

plemented with 0.1 mmol/l bromodeoxyuridine to eliminate non-myocytes. Cardiomyocytes were cultivated at 37°C with 5% CO_2 atmosphere. The miR-92a mimic and NC-mimic were obtained from Guangzhou RiboBio. Cardiomyocytes were starved for 24 h by incubation in medium without serum before transient transfection using the X-treme GENE siRNA transfection reagent. Cells were divided into four groups: (1) Normal group: cells did not receive any transfection or H_2O_2 treatment; (2) The H_2O_2 model group: cells treated with 100 μM hydrogen peroxide (H_2O_2) for 4 h to induce myocardial infarction; (3) miR-92a experimental groups: cells were transfected with miR-92a mimic (50 nM). Forty-eight hours after transfection, cells were subsequently treated with 100 μM H_2O_2 for 4 h. (4) NC experimental groups: cells were transfected with NC-mimic (50 nM). Forty-eight hours after transfection, cells were subsequently treated with 100 μM H_2O_2 for 4 h.

MTT assay

MTT assay was conducted to evaluate cell survival. Briefly, cells were treated with 20 μl of MTT (0.5 mg/ml), and the supernatant was discarded. DMSO was added to each well at a volume of 150 μl, after which formazan was dissolved by rotation for 10 min. An infinite M200 microplate reader was used to measure absorbance at 490 nm.

TUNEL staining

TdT-mediated dUTP nick end labeling (TUNEL) staining was employed to detect apoptosis in NMVCs using a TUNEL fluorescence FITC kit (Roche, USA) according to the manufacturer's instruction. After TUNEL staining, the cardiomyocytes were stained with DAPI (1:5000, Beyotime Biotechnology, China) solution to stain nucleus DNA. Fluorescence staining was viewed by a Laser Scanning Confocal Microscope (SP8, Leica, Japan). The apoptotic rate was calculated as TUNEL-positive cells per field.

Evaluation of cell death

Cell death in NMVCs was evaluated using an Annexin V-FITC/PI apoptosis detection kit. Briefly, NMCVs were resuspended in 20 μl of binding buffer after transfection and then incubated for 20 min with 5 μl of PI and 10 μl of Annexin V-FITC in the dark. Cell death was evaluated via flow cytometry (FC).

Protein separation and western blot analysis

RIPA buffer (50 mM Tris-HCl, 150 mM NaCl, 1% NP-40, 0.1% SDS, pH 8.0) was added with protease inhibitor cocktail (Roche Applied Science) and used to induce cell lysis. Protein quantification was conducted using BCA Protein Quantitation Kit. Proteins were separated via 10% SDS-PAGE (Bio-Rad) and subsequently transferred onto 0.45-μm PVDF membranes (Millipore). Membranes were blocked with 5% BSA for 1 h at 25°C. Membranes were incubated with the following antibodies at 4°C overnight: rabbit anti-Bcl-2 (1:1,000), rabbit anti-Bax, (1:1,000); mouse anti-Actin antibody (1:5,000); or mouse anti-caspase 3 (1:5000). Membranes were incubated with the secondary antibodies goat anti-rabbit IgG (1:10,000) or goat anti-mouse IgG (1:10,000) for 1 h at 25°C. Super Signal West Femto Maximum Sensitivity Substrate Kit (Thermo) was used to measure immunoreactivity using a C-DiGit Blot Scanner.

RNA isolation and real-time PCR

After sample preparation procedures, total RNA was extracted from cultivated NMVCs using Trizol reagent (Invitrogen, USA). miR-92a quantification was performed using SYBR Green on a Roche Light-Cycler 480 Real Time PCR system (Roche, Germany). GADPH served as internal control. The following primers were used for amplification: *miR-92a* F: 5'-GCT GAG GTA GTA AGT TGT ATTG-3'; R: 5'-CAG TGC GTG TCG TGG AGT-3'; *Caspase-3* (mouse) F: 5'-AAG AAG GTG GTG AAG CAG GC-3' and R: 5'-TCC ACC ACC CAG TTG CTGTA-3'; *GAPDH* (mouse): F: 5'-GGA AAG CTG TGG CGT GAT-3'; R: 5'-AAG GTG GAA G AA TGG GAG TT-3'. qRT-PCR was carried out with a final volume of 20 μl using SYBR Green PCR Master Mix with the following profile: 95°C for 10 min; and 40 cycles of 95°C for 15 s, 60°C for 30 s, and 72°C for 30 s on a Light Cycler 480 instrument. Target values were calculated based on the $2^{-\Delta\Delta CT}$ method via normalization to the internal reference relative to the calibrator (mean of the control samples).

Dual-luciferase reporter assay (LRA)

LRA was performed with PTEN as target. The whole PTEN 3'-UTR region was amplified and inserted into downstream of the GV126 Luci-

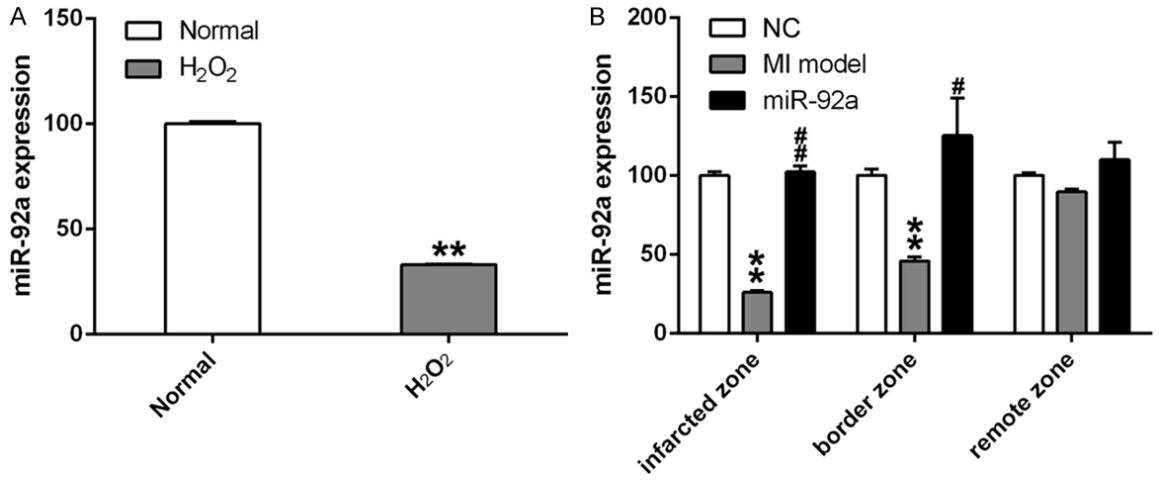

Figure 1. miR-92a expression was suppressed not only in H_2O_2-treated murine cardiomyocytes, but also in the infarcted myocardia of MI mice. A. RT-PCR revealed down-regulation of miR-92a expression in NMVCs treated with 100 μM H_2O_2. B. miR-92a expression was inhibited in MI mice compared to the normal tissue. $^*P < 0.05$, $^{**}P < 0.01$ vs. Normal/NC group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. MI group.

ferase gene. Site-directed mutagenesis was performed based on the predicted binding sites of rno-miR-320 in the *PTEN* gene to eliminate the binding sites. The mutant cells served as controls. Renilla luciferase plasmid harboring the thymidine kinase promoter (pRL-TK vector, TaKaRa, Japan) served as reporter control and used to monitor transfection efficiency. PTEN-WT, or PTEN-MUTANT was co-transfected with the luciferase reporter vector in HEK293T cells, after which LRA was carried out. HEK293T cells were purchased from Basic Research Institute of Peking Union Medical College Hospital. Cells were cultivated in DMEM containing 10% FBS and supplemented with mycillin. Cells were incubated at 37°C with 5% CO_2. Culture media were replaced every other day. Cells were digested with tryptase (0.25%). Cells were sub-cultivated upon reaching 70% to 80% confluency.

Detection of LDH and CK

Levels of two specific marker enzymes, namely, lactate dehydrogenase (LDH) and creatine kinase (CK), were evaluated by using commercial kits according to manufacturer's instructions.

Statistical analysis

Results are expressed as mean ± SD. One-way ANOVA or two-tailed Student's *t*-test was performed to evaluate differences between two groups. Statistical significance was considered at $p < 0.05$.

Results

miR-92a expression was down-regulated not only in H_2O_2-treated murine cardiomyocytes, but also in the infarcted myocardia of MI mice

H_2O_2 has been widely reported to be able to induce injury and apoptosis of cardiomyocytes [20]. Several studies revealed that H_2O_2 reduced cardiomyocyte survival in a time- and concentration-dependent manner [18]. First, miR-92a expression was evaluated in MI heart samples and H_2O_2-treated cardiomyocytes. Cells treated with H_2O_2 showed considerable down-regulation of miR-92 expression compared to the controls (**Figure 1A**).

miR-92a expression was further evaluated in murine cardiac samples at 3 days after generating the MI models. miRNAs were isolated from infarcted regions, fringe regions, and distant regions to determine the changes in miR-92a expression in various regions of infarcted hearts. RT-PCR analysis revealed that miR-92a expression in the infarcted and fringe regions of murine hearts was significantly down-regulated compared to that in the controls (**Figure 1B**). However, miR-92a expression in the distant regions showed no significant differences compared to the controls.

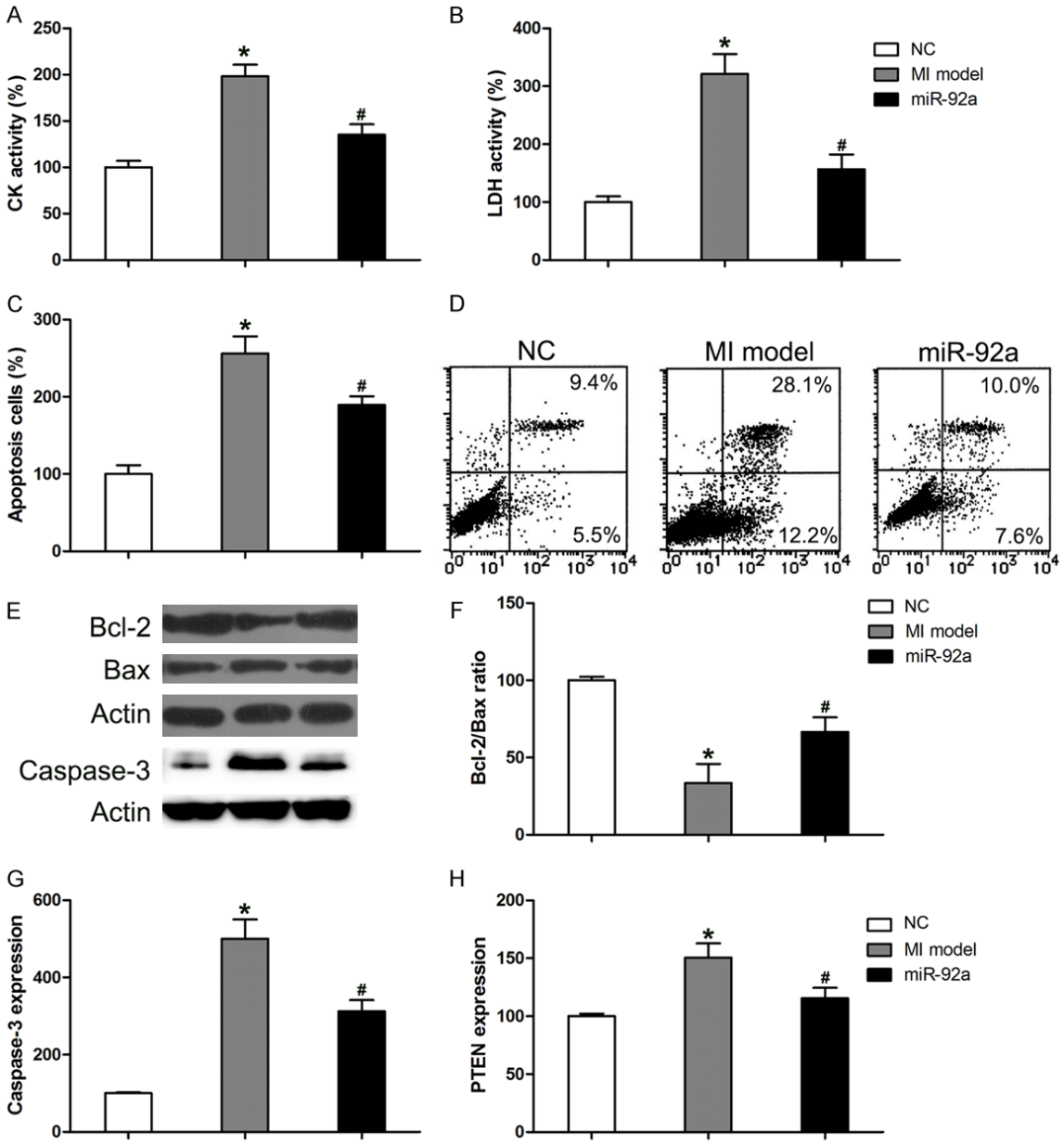

Figure 2. miR-92a protects cardiomyocytes against cell death in MI mice. (A) miR-92a agomir treatment increased CK and (B) LDH activity and promoted recovery of MI mice. n = 3. (C, D) Effect of miR-92a agomir on cardiac cell death assessed via Annexin V/PI dual staining. Apoptosis cell amount in negative control were normalized to 100%. The proportion of TUNEL-positive cells in different treatment groups. n = 3. (E-G) Effect of miR-92a agomir on expression of Bcl-2, Bax, and caspase-3 in MI mice. n = 5. (H) miR-92a inhibited up-regulation of PTEN expression in MI mice. n = 5. **P* < 0.05 vs. NC group; #*P* < 0.05 vs. MI group.

miR-92a agomir injection modulated serum LDH and CK concentrations, cell death, myocardial caspase-3 function, and PTEN expression in heart samples of MI mice

Following 1 h of miR-92a agomir treatment before construction of MI models, miR-92a expression in murine hearts was dramatically promoted by 406.21%. The activities of LDH and CK were remarkably promoted in MI mice compared to the controls. However, the increases in LDH and CK concentrations were inhibited by transfection with the miR-92a agomir (**Figure 2A**, **2B**).

Moreover, we evaluated *in vivo* myocardial cell death via Annexin V/PI dual staining (**Figure 2C**, **2D**). MI mice showed significant cell death

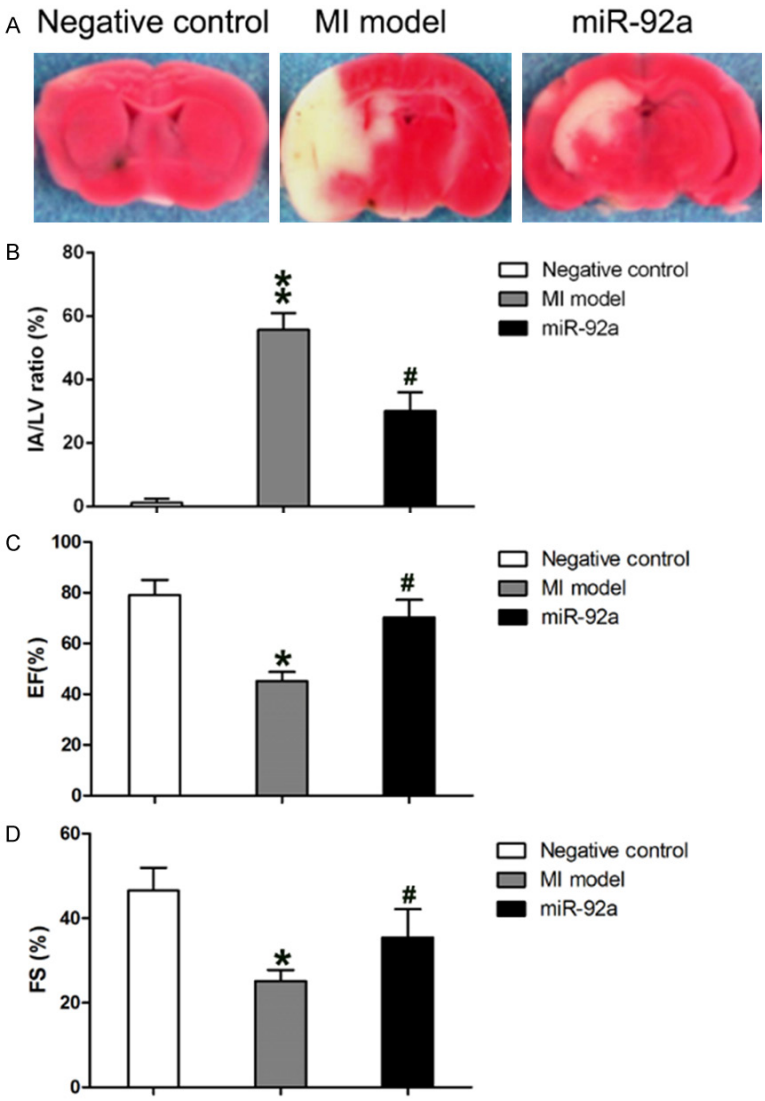

Figure 3. miR-92a protected against MI damage *in vivo*. (A) The original figure of infracted tissue. (B) IA/LV ratio analysis. LV, left ventricles; IA, infarct area. n = 3. $^{**}P < 0.01$ vs. MI group. (C) Ejection fractions (EF) and (D) fractional shortening (FS). n = 3. $^{*}P < 0.05$ vs. NC group; $^{\#}P < 0.05$ vs. MI group.

compared to the control. However, treatment with the miR-92a agomir suppressed myocardial cell death. miR-92a treatment counteracted the reduction in the Bcl-2/Bax ratio in MI mice, as shown in **Figure 2E**, **2F**. Furthermore, up-regulation of caspase-3 expression was observed in MI mice, which was counteracted by treatment with miR 92a-agomir (**Figure 2G**). The above findings indicate that miR-92a alleviates MI-related cell death in myocardial cells. Further, miR-92a agomir treatment abrogated PTEN over-expression in MI mice compared to the controls (**Figure 5H**).

miR-92a agomir reduced infarct size and promoted cardiac activity

To further explore the influence of miR-92a agomir on MI murine heart activity, we evaluated the changes in infarct size. The results revealed that miR-92a agomir treatment remarkably reduced the infarct size in MI mice (**Figure 3A** and **3B**). Moreover, ECG was performed to evaluate whether miR-92a facilitates recovery of heart function. miR-92a treatment increased EF and FS in MI heart samples, which indicated that heart activity was improved (**Figure 3C** and **3D**).

miR-92a overexpression promoted NMVC survival and inhibited cell death in H_2O_2-treated cells

Given that cardiomyocyte survival can be suppressed in a time- and concentration-dependent manner by H_2O_2 supplementation, we next asked whether miR-92a counteracted H_2O_2-induced cell death. Results of MTT assay revealed that cell survival was remarkably reduced in cells treated with 100 mM of H_2O_2. Transfection with the miR-92a mimic significantly enhanced survival of H_2O_2-treated NMVCs compared to that in the H_2O_2 and NC group (**Figure 4A**).

Given that miR-92a expression was inhibited in H_2O_2-treated NMCVs and MI models, we investigated whether miR-92a exerted a protective effect against H_2O_2-induced cell death in cardiomyocytes. Tunnel staining and FC results indicated significant cell death in H_2O_2-treated samples compared to the normal samples. Nevertheless, miR-92a transfection remarkably reduced H_2O_2-induced cell death, suggesting that miR-92a modulated NMCV stabilization by inhibiting cell death (**Figure 4B-E**).

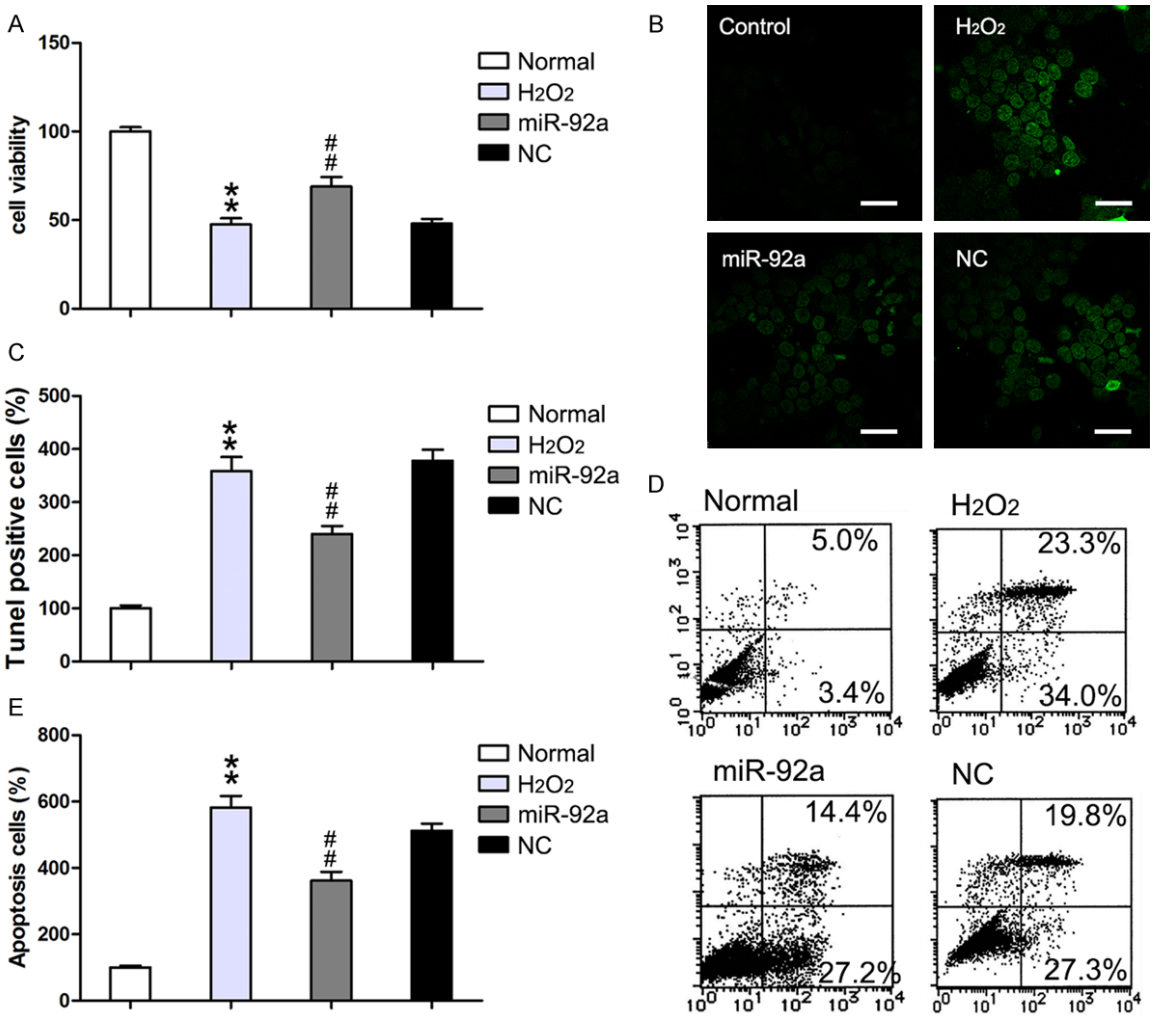

Figure 4. miR-92a overexpression promoted NMVCs survival and inhibited cell death H_2O_2-treated cells. A. Results of MTT assay showed that transfection with miR-92a mimic restored cell survival. B, C. miR-92a overexpression inhibited H_2O_2-induced cell death in NMCVs via TUNEL staining. D, E. NMCV death in each treatment group was evaluated via FC. $^{**}P < 0.01$ vs. Normal group; $^{\#\#}P < 0.01$ vs. H_2O_2 group.

miR-92a overexpression counteracted H_2O_2-induced down-regulation of Bcl-2 expression and upregulation of the expression of caspase-3 and Bax

Given that miR-92a enhanced cell survival and inhibited cell death in cardiomyocytes, we examined its potential role in regulating the expression of proteins involved in cell death. Bcl-2 functions as suppressor and caspase-3 promotes cell death. As shown in **Figure 5A-D**, H_2O_2 treatment down-regulated Bcl-2 expression but up-regulated both caspase-3 and Bax expression. In contrast, transfection with miR-92a mimic resulted in considerable reduction in caspase-3 and Bax expression compared to the NC-mimic group. Moreover, miR-92a overexpression promoted Bcl-2 expression. The above findings demonstrated that miR-92a suppressed H_2O_2-induced cell death.

miR-92a expression counteracted up-regulation of PTEN expression in H_2O_2-treated cardiomyocytes via direct binding to 3'-UTR

Bioinformatics analysis revealed that PTEN is a potential miR-92a target (**Figure 6A**). Dual double luciferase assay presented that miR-92a mimic suppressed luciferase activity, which was fused with the 3'-UTR wild type of *PTEN*, when compared to the negative controls. However, the luciferase activity of 3'-UTR

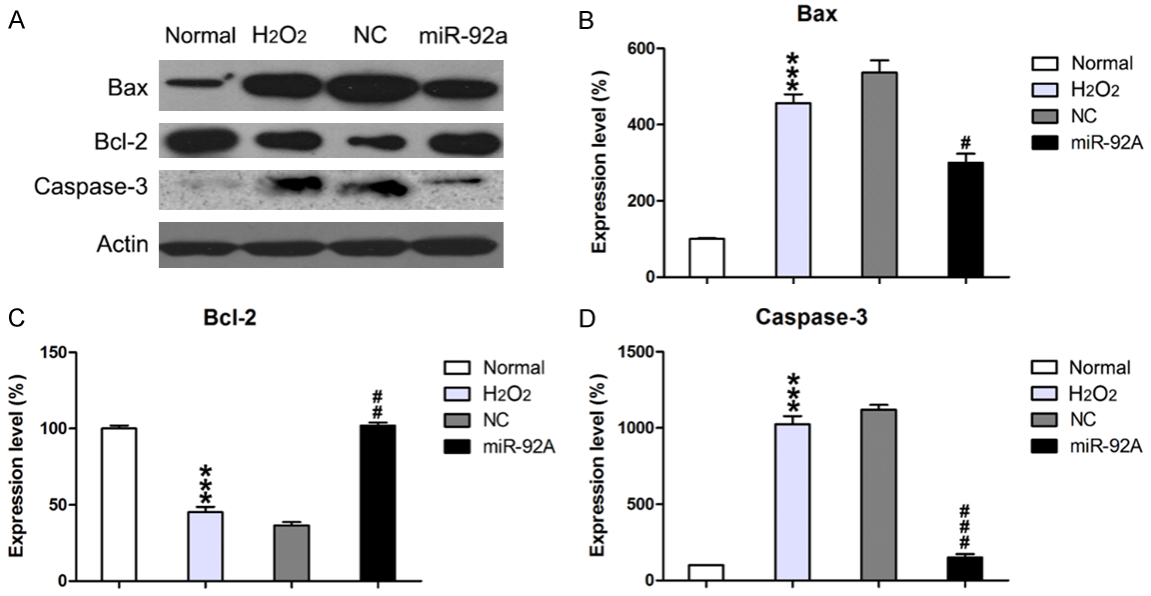

Figure 5. Effect of miR-92a on expression levels of Bax and Bcl-2. A. miR-92a expression suppressed H_2O_2-induced expression of Bax, Bcl-2, and caspase-3. B. Image pixel analysis of Bax expression bands. C. Image pixel analysis for Bcl-2 expression bands. D. Image pixel analysis of caspase-3 expression bands. ***P < 0.001 vs. Normal group; #P < 0.05, ##P < 0.01, ###p < 0.001 vs. H_2O_2 group.

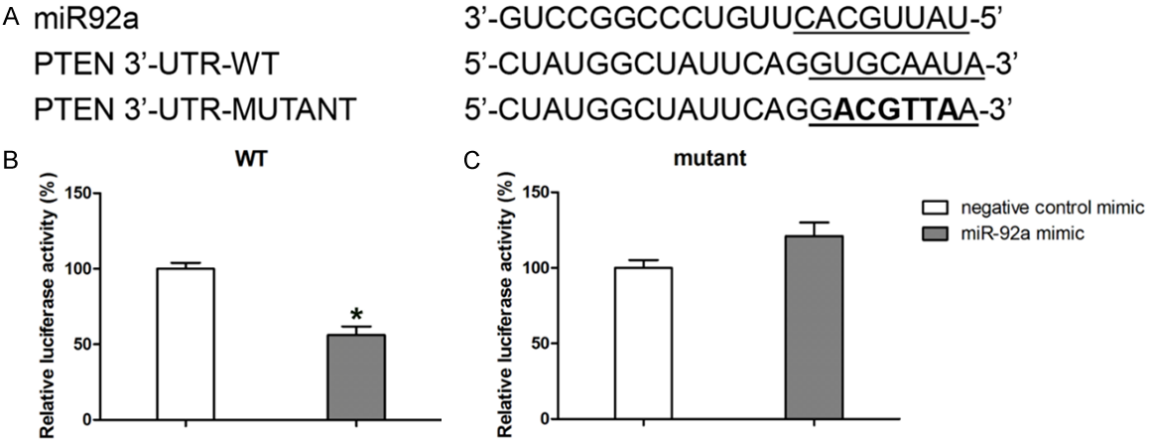

Figure 6. miR-92a targets PTEN. Dual LRA was carried out via co-transfection of luciferase reporter harboring the WT or mutant 3'-UTR of murine Fas with the miR-92a mimic into HEK293T cells. A. Bioinformatics analysis revealed that miR-92a binds to the 3'-UTR of Fas mRNA. Mutation in the putative binding site was identified. B, C. Effect of miR-92a overexpression on luciferase function in WT and mutant PTEN reporter constructs. n = 3. *P < 0.05. miR-92a overexpression dramatically reduced luciferase function in the WT 3'-UTR but not in the mutant 3'-UTR of PTEN mRNA.

mutant type did not show significant change (**Figure 6B** and **6C**), indicating that miR-92a directly targets the 3-UTR of PTEN.

To confirm the effect of miR-92a mimic on PTEN expression, we transfected miR-92a mimic in H_2O_2-treated cells, as **Figure 7A** showed that the expression level of PTEN was decreased due to miR-92a transfection. The data suggest that PTEN can be down-regulated by miR-92a over-expression.

As PTEN was up-regulated in H_2O_2 treated cell model, to prove that the H_2O_2-induced apoptosis was by targeting PTEN, PTEN was silenced to observe the cell apoptosis and caspase-3 activity. As **Figure 7B** and **7C** show that PTEN silencing decreased caspase-3 activity and

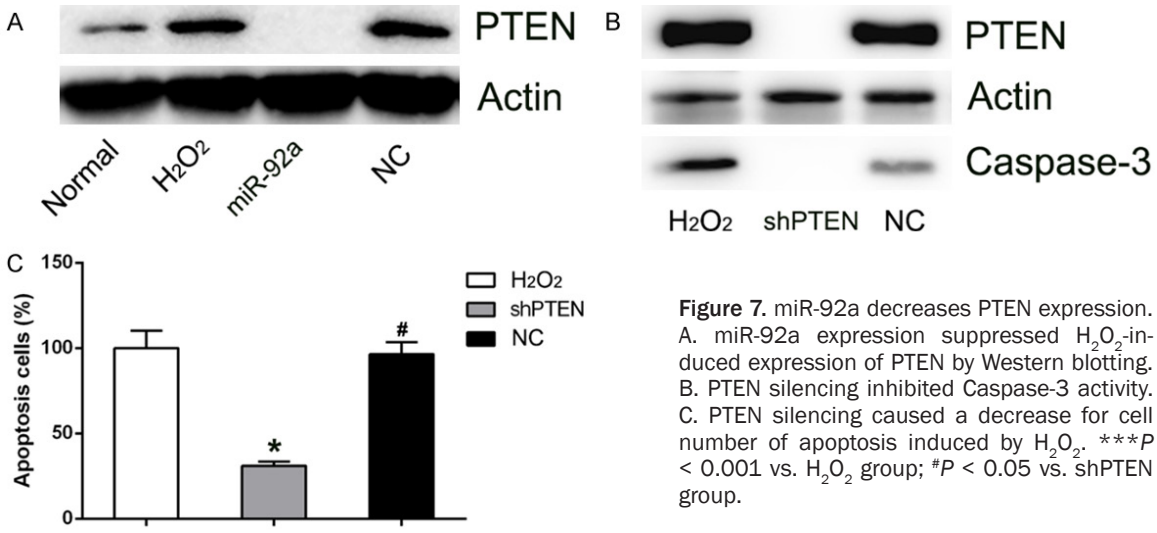

Figure 7. miR-92a decreases PTEN expression. A. miR-92a expression suppressed H_2O_2-induced expression of PTEN by Western blotting. B. PTEN silencing inhibited Caspase-3 activity. C. PTEN silencing caused a decrease for cell number of apoptosis induced by H_2O_2. ***P < 0.001 vs. H_2O_2 group; #P < 0.05 vs. shPTEN group.

apoptosis. Combined with the previous result, we speculate that miR-92a inhibited infarction-induced cell apoptosis via PTEN/caspase-3 pathway.

Discussion

Cell death is primarily responsible for cardiomyocyte malfunction and participates in MI damage [21, 22]. To elucidate the mechanisms underlying the etiology of cardiomyocyte death and associated cardiac illnesses, there is an urgent need to identify the factors that stimulate cell death and to identify promising treatment targets. In the present study, we examined the role of miR-92a in MI damage. The results revealed that miR-92a expression was down-regulated in MI mice and in H_2O_2-treated NMCVs. miR-92a overexpression prevented cardiomyocyte death by inhibiting the cell death-promoting pathways and promoting cell death-counteracting signals. Moreover, inhibition of cell death by miR-92a was found to be mediated by the PTEN pathway, which protects heart tissues against MI and promotes heart function.

Previous studies have demonstrated that the intrinsic mitochondrial pathway modulates cell death [16, 23]. In the present study, we evaluated the role of miR-92a in modulation of cell death via the PTEN pathway. Bax and Bcl-2 expression levels were evaluated to examine the effects of miR-92a on mitochondrial preservation. Bcl-2 can prevent the cytochrome C release from the mitochondria to the cytoplasm, leading to suppression of cardiomyocyte death [24]. By contrast, Bax counteracts the antagonistic activity of Bcl-2 against cell death, thereby promoting cell death [24]. As a result, the intrinsic mitochondrial pathway relies on the balance between the effects of Bcl-2 and Bax to regulate stress and cell death [25, 26]. Our findings revealed that H_2O_2 exposure stably up-regulated Bax expression and down-regulated Bcl-2 expression in NMVCs compared to the control. Results also showed that miR-92a over-expression counteracted down-regulation of Bcl-2 expression caused by MI and H_2O_2 stimulation, thereby, demonstrating that Bcl-2 suppresses miR-92a-associated cardiomyocyte death. In contrast, Bax expression was suppressed following miR-92a overexpression. Briefly, our findings suggest that miR-92a protects against H_2O_2-induced cell death in NMVCs.

Our current findings on miR-92a expression and NMVC death under oxidative stress (OS) are consistent with previously reported results using other cell lines. Ohyashiki and colleagues revealed that anti-miR-92a treatment promoted cell death in human lymphoid and myeloid cells [27]. Furthermore, miR-92a suppression promoted cell death in human glioma cells via direct binding to Bim [28]. Nevertheless, understanding the mechanisms by which miR-92a modulates H_2O_2-induced cell death is insufficient. Our findings suggest that miR-92a participates in the modulation of the PTEN/caspase axis, which acts as a crucial modulator of stress-induced cell death [29].

Dual LRA results showed that miR-92a modulated PTEN expression via direct binding to the 3'-UTR [29]. Nevertheless, our study revealed that miR-92a binds to PTEN via the 3'-UTR. Therefore, it is likely that the sequence of site 2 (503-510) of the miR-92a 3'-UTR harbors a 10-bp match with that of miR-92a. Therefore, PTEN is likely to associate with miR-92a in addition to miR-92a (105-112).

In summary, our findings demonstrate that MI damage and H_2O_2 treatment suppress miR-92a expression in NMCVs. Moreover, miR-92a protects against H_2O_2-induced cardiomyocyte death in NMCVs at least partly via the PTEN/caspase-3-associated apoptotic pathway. miR-92a overexpression in patients with MI damage is thus a promising strategy for restoring heart function.

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Disclosure of conflict of interest

None.

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