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Overexpression of microRNA-135b-5p attenuates acute myocardial infarction injury through its anti-oxidant and anti-apptotic properties

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Abstract

Objective: Myocardial infarction (MI) remains the leading cause of morbidity and mortality due partly to the limited regenerative capacity of cardiomyocytes to replace cardiomyocyte lost due to apoptosis. Inhibiting cardiomyocyte apoptosis is recognized as an effective therapeutic approach for MI. MicroRNAs (miRNAs, miRs), which regulate target genes at the post-transcriptional level, play a significant role in the regulation of cardiovascular diseases such as MI. MicroRNA-135b (miR-135b) has a protective effect on cardiomyocytes. However, the role of miR-135b in cardiomyocyte apoptosis in infarct myocardium needs further clarification. Methods: We generated α-MHC-miR-135b transgenic mice to investigate the role of miR-135b in myocardial injury after MI. MiR-135b mimic and negative control (NC) were transfected into H_2O_2-induced cardiomyocytes to evaluate the effect of overexpression of miR-135b on the levels of reactive oxygen species (ROS) and apoptosis. Results: Our results showed that overexpression of miR-135b had protective effect on cardiomyocyte injury both in vivo and in vitro. MiR-135b inhibited cardiomyocyte apoptosis and ROS generation, downregulated pro-apoptosis proteins (cleaved-caspase-3 and Bax), and increased anti-apoptosis protein (Bcl-2). Moreover, miR-135b showed an inhibitory effect on apoptosis-related protein target transient receptor potential vanilloid-type 4 (TRPV4) cation channel. Conclusion: MiR-135b might be considered a new molecular target for potential replacement therapy as antiapoptotic cardioprotection in the setting of MI.

Keywords

myocardial infarction; apoptosis; microRNA-135b; TRPV4

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1 Introduction

Myocardial infarction (MI) is a metabolic catastrophe characterized by contractile dysfunction, arrhythmia, and cell death due to reduced supply of oxygen and nutrients to the myocardium[1-2]. Coronary occlusion trigger a large burst of cell death within the area rendered ischemic over the first 6 to 24 hours[3]. Apoptosis is one of the main mechanisms regulating cell death, which is initiated by intracellular stimuli, such as reactive oxygen species (ROS) or Ca^{2+}. Since cardiomyocytes are terminally differentiated and have no or little regenerative potential, preventing cardiomyocyte apoptosis after MI injury is a vital therapeutic strategy[4].

MicroRNAs (miRNAs, miRs) are a class of small single-stranded and highly conserved non-coding RNAs, which are widely studied for their modulation of several pathological aspects of MI, including cardiomyocyte apoptosis, inflammation, angiogenesis, and fibrosis[5]. Compelling evidence has demonstrated that the miR-135 family is closely associated with cardiovascular diseases. MiR-135b, a member of the miR-135 subfamily, protects cardiomyocytes from ischemic injury through restraining the NLRP3/caspase-1/IL-1β pathway[6]. Past studies have pointed out a regulatory relationship between miR-135b and cardiomyocytic apoptosis in chronic heart failure[7]. However, the anti-apoptotic mechanisms of miR-135b in MI remained incompletely delineated.

Transient receptor potential vanilloid 4 (TRPV4), a cation channel permeable mainly to Ca^{2+} (Ca^{2+}:Na^{+} permeability ratio=6:1), plays a crucial role in a multitude of physiological processes. Interventions targeting TRPV4 have demonstrated a therapeutic potential in a variety of diseases, including edema, pain, gastrointestinal diseases, and lung diseases[8]. TRPV4

is wildly expressed in the cardiovascular system, particularly in myocardium and isolated cardiomyocytes[9-11]. The roles of TRPV4 in regulating myocardial contractility, action potential, and fibrosis have been extensively studied. Recent studies found that TRPV4 is activated in myocardial ischemia/reperfusion (I/R), which causes calcium overload, oxidative stress, and RISK pathway inhibition, ultimately leading to cardiomyocyte apoptosis. Blocking TRPV4 has been considered a new approach for reducing cardiomyocyte apoptosis[12-13].

In the current study, we aimed to further clarify the role of miR-135b in cardiomyocyte apoptosis and the underlying mechanisms by gain of function approaches in a mouse model of MI injury and a cellular model of H_2O_2-induced cardiomyocytes apoptosis. The results demonstrated that overexpression of miR-135b prevented MI-induced damage by suppressing apoptosis and ROS generation through inhibiting TRPV4. The findings provide a theoretical basis for developing novel therapeutic strategies for protecting cardiomyocytes from MI-induced apoptosis.

2 Methods

2.1 Ethics statement

Male C57BL/6 mice weighing 20-24 g and neonatal mice were purchased from Liaoning Changsheng Biotechnology corporation. The study was approved by the ethics committee of Harbin Medical University. All institutions and guidelines on the care and use of laboratory animals were followed.

2.2 Myocardial infarction model

MI model was established by left coronary artery (LCA) ligation as previously described[6]. In sham operated mice, a thread was placed beneath the LCA without ligating. One day after MI, the hearts were harvested and infarct size was measured by TTC (triphenyl tetrazolium chloride, Solarbio, China) staining[14]. Myocardial specimens were dissected and the infarct border zones were retained for subsequent real-time PCR and western blot analyses.

2.3 Echocardiographic measurements

Cardiac function prior to surgery as a baseline control and after 24 h of MI was measured using two-dimensional M-mode echocardiography with Vevo1100 high-resolution imaging system (VisualSonics, Toronto, ON, Canada) equipped with a 30-MHz phased-array transducer. Ejection fraction (EF%) and fractional shortening (FS%) were calculated for the evaluation of cardiac function.

2.4 Transmission electron microscopy

Selected samples were fixed in 2.5% glutaraldehyde in 0.1 mol/L phosphate-buffered saline (PBS) (*pH* = 7.35), rinsed in buffer, and postfixed in PBS with 1% OsO4 for 2 h. Next, the samples were stained en bloc with 1% uranyl acetate, dehydrated using a graded ethanol series, and embedded in epoxy resin using standard methods. The stained sections were examined under a JEM-1200 electron microscope (JEOL Ltd., Tokyo, Japan) [15].

2.5 TUNEL staining

Cell apoptosis was assessed using In Situ Cell Death Detection Kit (11684817910, Roche, Basel, Switzerland). According to the manufacturer's instructions, normal cells showed blue nuclear Hoechst staining, and the TUNEL-positive cells displayed green nuclear staining. The stained slices were analyzed under an Upright Fluorescence Microscope (BX53, OLYMPUS, Tokyo, Japan).

2.6 Primary culture of neonatal mouse ventricular cardiomyocytes (NMVCs) and cardiomyocyte hypoxia injury model

NMVCs were cultured following the same methods as previously described in detail[6]. In brief, NMVCs were isolated from 1 to 3 days C57BL/6 mouse hearts. Cells were suspended in DMEM (Hyclone, USA) with 10% FBS, and cultured in a humidified incubator (95% air, 5% CO2) for 48 h. H_2O_2 was added to the culture medium for 24 h to mimic the ischemic condition in vitro.

2.7 Gene transfection

The miR-135b mimic and negative control (NC), commercially synthesized by Ribobio (Guangzhou, China), were transfected into NMVCs for miR-135b overexpression according to the manufacturer's protocol. H_2O_2 was added into the culture medium at 24 h after transfection of miR-135b mimic, followed by incubation for another 24 h.

2.8 In vitro cell viability

Cell viability was measured by a commercial Cell Counting Kit (CCK-8, Dojindo Molecular Technologies, Inc., Japan) according to the manufacturer's protocols. Varying concentrations of 25, 50, 100 and 200 μmol/L H_2O_2 were added separately after NMVCs had been seeded in a 96-well plate. After 24 h incubation, cell proliferation was measured and 10 μl CCK-8 reagent was added to each well and incubated at 37°C for 1 h. Absorbance was measured at 450 nm using a spectrophotometer.

2.9 Flow cytometry

Flow cytometry (BD LSRFortessa™, Franklin Lakes, NJ, USA) was performed to assess apoptosis of NMVCs using the Annexin V-FITC Apoptosis Detection Kit (Meilune, Dalian, China) according to the manufacturer's protocols. Culture medium was discarded, and the cells were washed with sterile PBS buffer twice and digested in trypsin digestion solution (Solarbio, USA). The cells in EP tubes were centrifuged and collected. Binding Buffer working solution was added to the cell precipitates. After mixing, 100 μL cell suspension was transferred into another EP. Annexin V-FITC 5 μL and PI 10 μL were added, respectively, and the cells were mixed and incubated in dark for 15 min. After staining and incubation, 400 μL Binding Buffer was added to each tube. After mixing, the apoptosis rate of cells was detected by flow cytometry (BD LSRFortessa™, Franklin Lakes, NJ, USA).

2.10 ROS assay

The intracellular ROS level was detected by dihydroethidium, a fluorescent probe for ROS (Beyotime Biotechnology, Shanghai, China), according to the manufacturer's protocols. Briefly, cells were incubated with dihydroethidium (10 μM) in the dark at 37°C for 20 min. Then, the cells were washed twice and examined under an inverted fluorescence microscope (Nikon, Tokyo, Japan) with an excitation wavelength of 370 nm and an emission wavelength of 594 nm. ROS emitted green fluorescence in images.

2.11 Real-time PCR

qPCR was performed as previously described.[6] Total RNA samples were extracted from cardiac tissues and cardiomyocytes by Trizol (Invitrogen, USA) and reverse transcribed to cDNA using Reverse Transcription Master Kit (Toyobo, Japan) according to the manufacturer's instructions. Real-time PCR was performed using SYBR qPCR Mix with ABI 7500 fast system (Applied Biosystems, USA). U6 and GAPDH served as internal controls. The relative quantification of gene expression was determined using the $2^{-\Delta\Delta CT}$ method. The sequences of qPCR primer pairs used in the present study are: miR-135b-5p (mouse) forward 5′-ATGTAGGGCTAAAAGCCATGGG-3′ and reverse 5′-ATCCAGTGCAGGGTCCGAGG-3′; U6 (mouse) forward 5′-GCTTCGGCAGCACATATACTAAAAT-3′ and reverse 5′-CGCTTCACGAATTTGCGTGTCAT-3′; caspase-3 (mouse) forward 5′-CTCGCTCTGGTACGGATGTG-3′ and reverse 5′-TCCCATAAATGACCCCTTCATCA-3′; Bax (mouse) forward 5′-AGACAGGGGCCTTTTTGCTAC-3′ and reverse 5′-AATTCGCCGGAGACACTCG-3′; Bcl-2 (mouse) forward 5′-GCTACCGTCGTGACTTCGC-3′ and reverse 5′-CCCCACCGAACTCAAAGAAGG-3′; TRPV4 (mouse) forward 5′-CGTCCAAACCTGCGAATGAAGTTC-3′ and reverse 5′-CCTCCATCTCTTGTTGTCACTGC-3′; GAPDH (mouse) forward 5′-AAGAAGGTGGTGAAGCAGGC-3′ and reverse 5′-TCCACCACCCTGTTGCTGTA-3′.

2.12 Western blot

Protein samples of 60-80 μg each, isolated from NMVCs, were separated by SDS-PAGE (8-12% polyacrylamide gels). Partitioned proteins were transferred to Pure Nitrocellulose Blotting Membrane (PALL, New York, USA). The membranes were incubated at 4°C overnight with primary antibodies for cleaved caspase-3 (Cell Signaling Technology #9661), Bax (Proteintech #60267-1-Ig), Bcl-2 (Proteintech #60178-1-Ig), and Trpv4 (Alomone labs #ACC-124), respectively. Then, the membranes were incubated with secondary antibodies (Jackson Immuno Research, West Grove, PA, USA) for 1h. Western blot bands were quantified using Quantity One Analyzer 4.6.2 Software.

2.13 Statistical analysis

Data are displayed as mean ± standard error with the statistical analyses processed using SPSS 13.0 Software. Differences between multiple groups were compared by one-way analysis of variance (ANOVA), and differences between two groups were compared by student t test. A two-tailed $P < 0.05$ was considered statistically significant. Graphs were generated using GraphPad Prism 5.0.

3 Results

3.1 Downregulation of miR-135b expression in ischemic hearts and H_2O_2-treated cardiomyocytes

We demonstrated that miR-135b expression is markedly downregulated in the myocardium of mice with MI (24 h post-MI) and in 200 μM H_2O_2-treated NMVCs. First, we established a MI model and confirmed that the cardiac function of the MI mice was significantly decreased (Fig. 1A-C). The results of TTC staining showed that ischemic tissues were in white color, whereas non-ischemic tissues were stained red in the MI group (Fig. 1D). The transmission electron microscopy demonstrated that the dying cells developed typical apoptosis morphology with mitochondrial swelling and myofibril breakage in ischemic border zone of MI hearts (Fig. 1E). TUNEL staining revealed that MI was accompanied by substantial cardiomyocyte apoptosis (Fig. 1F). As shown in Fig. 1G, miR-135b expression was progressively downregulated in the border area 1 day after MI. Then, we evaluated the effects of miR-135b on viability of cultured NMVCs exposed to 50, 100, or 200 μM H_2O_2 to simulate the oxidative stress-induced cell injury as in MI. Fig. 1H exhibits a significant concentration-dependent decrease in cell viability in the presence of H_2O_2. In addition, the expression of

miR-135b was considerably downregulated in 200 μM H_2O_2-treated NMVCs compared to the non-treated control group (Fig. 1I). The results were consistent with those obtained in vivo.

3.2 Overexpression of miR-135b protects cardiomyocytes against MI by inhibiting apoptosis and reactive oxygen species

To verify the anti-apoptotic property of miR-135b, we created a MI model in α-MHC-miR-135b transgenic mice (Tg mice) and wild-type mice (WT mice). As expected, miR-135b was significantly upregulated in cardiac tissues of transgenic mice (Fig. 2A). The results of echocardiography showed that overexpression of miR-135b attenuated myocardial damage after MI (Fig. 2B-D). The size of infarcted cardiac tissues was significantly smaller in Tg mice than in WT littermates with MI (Fig. 2E). The mitochondrial swelling and myofibril breakage caused by MI in the cardiac tissue were nearly abolished by overexpression of miR-135b in Tg mice, as evidenced by the results from electron microscopic examinations (Fig. 2F). Moreover, TUNEL staining demonstrated that miR-135b

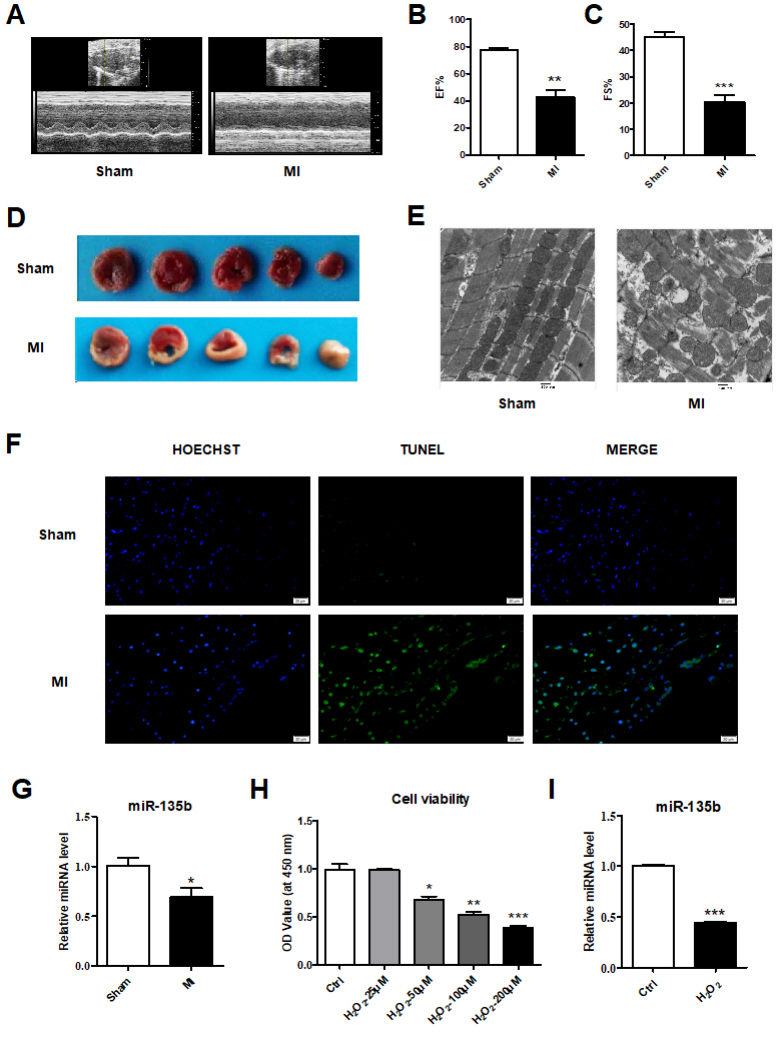

Fig. 1 MI induced functional and morphological changes in mouse hearts

(A) M-mode echocardiograms. (B) Ejection fraction and (C) Fractional shortening. (D) TTC staining results. (E) Election microscope images. Scale bars = 500 nm. (F) TUNEL staining results. The mRNA expression of miR-135b (G) in vivo and (I) in vitro. (H) CCK8 assay result. $n = 3$; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* Sham or Ctrl. The data are presented as mean ± SD.

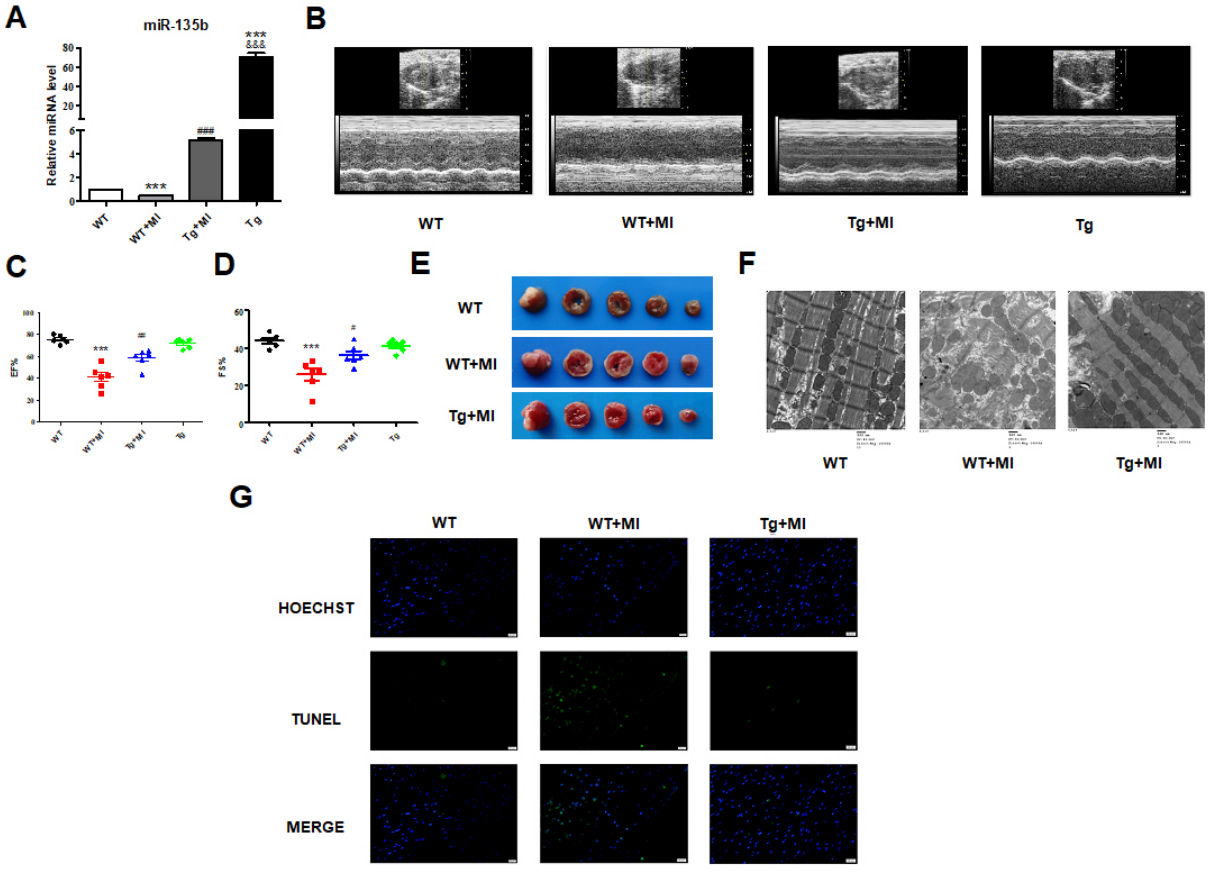

Fig. 2 Overexpression of miR-135b in transgenic (Tg) mice reverses MI-induced impairments of cardiac function

(A) The mRNA expression of miR-135b in vitro. (B) M-mode echocardiograms. (C) Ejection fraction and (D) Fractional shortening. (E) TTC staining results. (F) Election microscope images. Scale bars = 500 nm. (G) TUNEL staining results. $n = 3$; $^{***}P < 0.001$ *vs.* WT, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ *vs.* WT+MI. $^{\&\&\&}P < 0.001$ *vs.* Tg+MI. The data are presented as mean ± SD.

overexpression suppressed cardiomyocyte apoptosis (Fig. 2G).

To investigate whether miR-135b downregulation is related to the decrease in cell viability, we transfected miR-135b mimic and its negative control construct into NMVCs and confirmed the overexpression of this miRNA (Fig. 3A). We then observed that overexpression of miR-135b effectively rescued the cardiomyocyte death induced by H_2O_2 with a prominent increase in cell viability (Fig. 3B). The finding indicates that miR-135b has a protective effect on cardiomyocytes. To clarify whether the decrease in cell viability was due to apoptotic cell death, we performed Annexin V staining to detect apoptosis. Our results demonstrated that the percentage of cells stained with Annexin V was markedly increased after 24 h H_2O_2 treatment indicating the early stage of NMVCs apoptosis, which was significantly reduced after transfection with miR-135b mimic (Fig. 3C). Specifically, 79.77% of NMVCs underwent apoptosis after 24 h H_2O_2 incubation; yet miR-135b mimic reduced the apoptotic cell death down to 26.30%, compared to 80.68% Annexin V-positive staining in NMVCs transfected with miR-135b negative control construct (Fig. 3D). These data support that miR-135b is an antiapoptotic miRNA. Since ROS overproduction can induce apoptosis, we detected ROS levels in NMVCs by immunofluorescence. The results showed that the promoting effects of H_2O_2 on ROS generation were abrogated by miR-135b mimic, suggesting that miR-135b suppressed ROS generation (Fig. 3E). These data indicate that overexpression of miR-135b alleviates the damage caused by MI.

3.3 Effects of overexpression miR-135b on the expression of apoptosis related proteins

We went on to assess the possible alterations of expression of

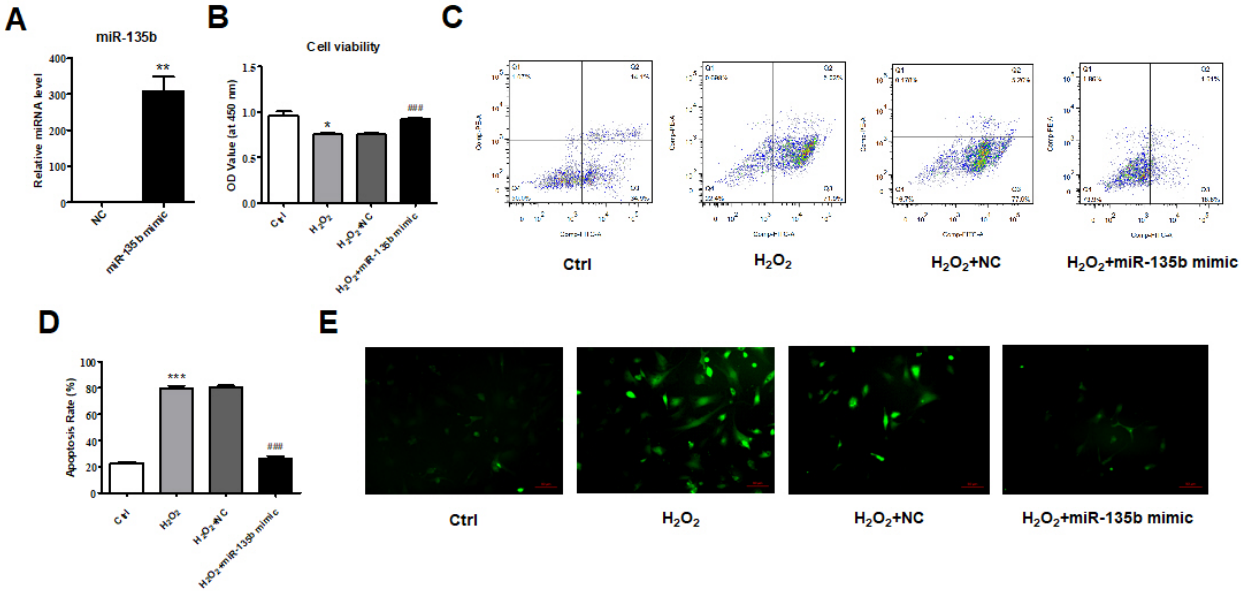

Fig. 3 Forced expression of miR-135b suppresses ROS generation and apoptosis in primary cultured neonatal mouse ventricular cells (NMVCs)

(A) The mRNA expression of miR-135b *in vitro*. (B) CCK8 assay result. (C) Flow cytometry results and (D) corresponding statistical result *in vitro*. (E) ROS generation *in vitro*. $n = 3$; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs*. Ctrl or NC, $^{\#\#\#}P < 0.001$ *vs*. H_2O_2. The data are presented as mean ± SD.

apoptosis-associated proteins. Caspase-3, the main apoptotic executioner protein, is activated as indicated by the increased level of cleaved caspase-3. Bax and Bcl-2 are opposing apoptosis regulators, and their ratio determines the likelihood of apoptosis induction. Reducing Bax and/or increasing Bcl-2 intends to suppress apoptotic cell death. The results showed that the mRNA and protein expression levels of cleaved caspase-3[8], Bax and Bax/Bcl-2 ratio were increased in wild-type control mice with MI, and these detrimental changes were mitigated by miR-135b overexpression in transgenic mice (Fig. 4A, B, D, E, F, H). In contrast to the changes of Bax, the expression of Bcl-2 mRNA and protein was significantly downregulated in wild-type mice subjected to MI, which was reversed by miR-135b (Fig. 4C, G). Similarly, the mRNA and protein levels of cleaved caspase-3, Bax, and Bax/Bcl-2 ratio were markedly elevated in H_2O_2-treated NMVCs but substantially reduced following transfection of miR-135b mimics (Fig. 5A, B, D, E, F, H). Moreover, the expression levels of Bcl-2 mRNA and protein were significantly downregulated by H_2O_2, which was reversed by miR-135b mimic (Fig. 5C, G).

3.4 The effect of miR-135b on TRPV4 expression in cardiomyocytes

To explore the mechanism by which miR-135b produces anti-apoptotic effect, we analyzed the potential target genes of miR-135b using RNAhybrid. The database predicts that miR-135b has the potential to bind the 3'-UTR of TRPV4 mRNA in both human and mouse sequences that are broadly conserved among species (Fig. 6A). TRPV4 is a proapoptotic protein and its inhibition can alleviate cardiomyocyte apoptosis[12, 16]. We therefore investigated the regulatory effects of miR-135b on TRPV4 expression in NMVCs transfected with a miR-135b mimic and in α-MHC-miR135b transgenic mice. We found that the expression levels of TRPV4 mRNA and protein were considerably increased in wild-type control mice with MI and in H_2O_2-treated NMVCs relative to miR-135b-overexpressing Tg mice and miR-135b mimic-treated NMVCs, respectively (Fig. 6B, C, D, E). These results indicate that miR-135b elicits anti-apoptotic effect in MI by inhibiting the expression of TRPV4.

4 Discussion

This study presents the following findings: (1) Overexpression of miR-135b protects cardiomyocytes against apoptotic cell death induced by MI in both wild-type and Tg mice and H_2O_2 treatment in cultured NMVCs; (2) miR-135b produces cardioprotective effects by suppressing ROS generation and apoptosis; (3) MiR-135b attenuates cardiomyocyte apoptosis by repressing TRPV4 as its target gene.

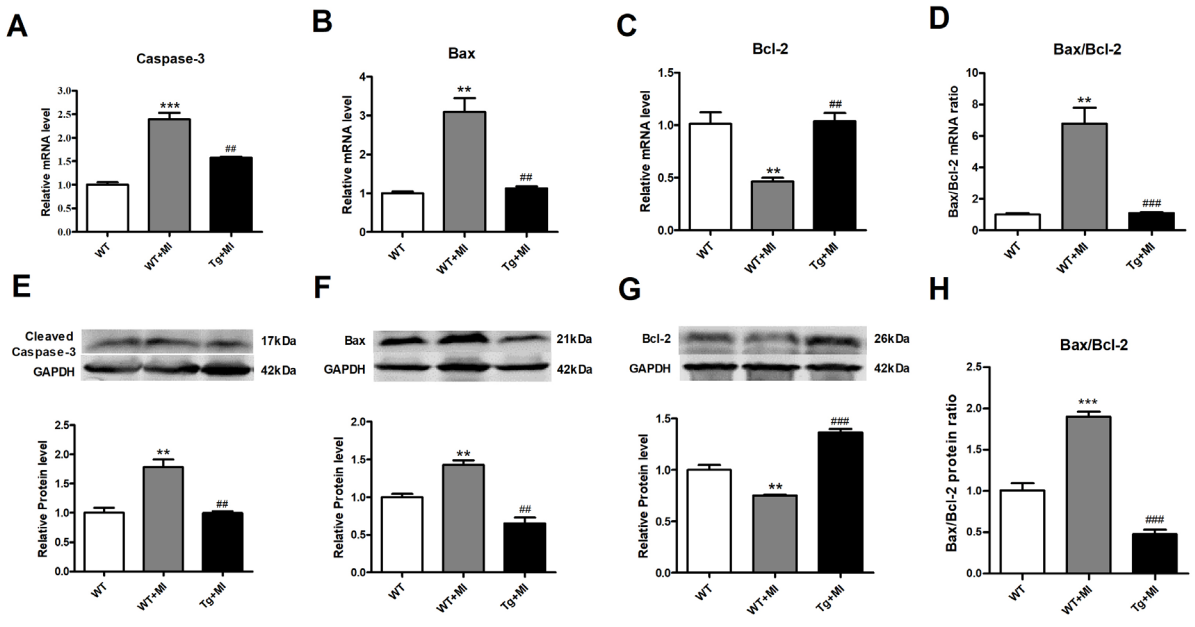

Fig. 4 Overexpression of miR-135b alters the expression of apoptosis-related protein regulators in Tg mice

The mRNA expression of (A) Caspase-3 (B) Bax (C) Bcl-2 and (D) Bax/Bcl-2 mRNA ratio *in vitro*. The protein expression of (E) Caspase-3 (F) Bax (G) Bcl-2 and (H) Bax/Bcl-2 protein ratio *in vitro*. $n = 3$; $^{**}P < 0.01$, $^{***}P < 0.001$ *vs*. WT, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ *vs*. WT+MI. The data are presented as mean ± SD.

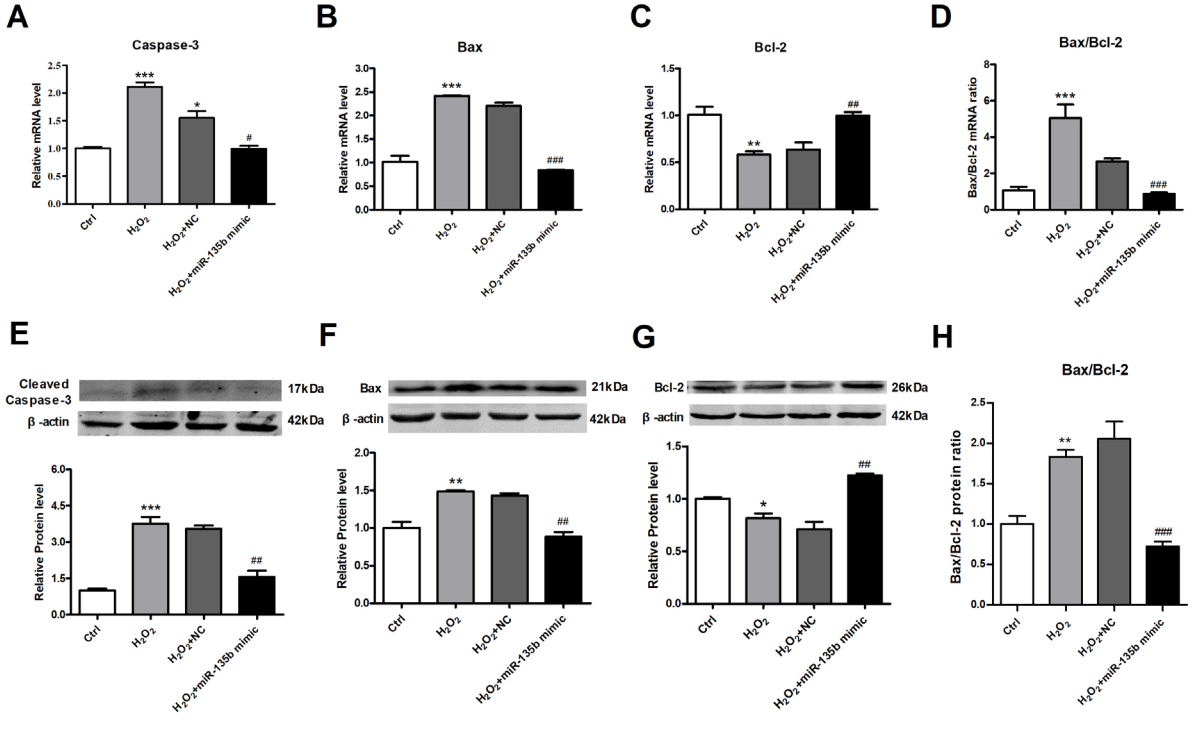

Fig. 5 Forced expression of miR-135b alters the expression of apoptosis-related protein regulators in cultured NMVCs

The mRNA expression of (A) Caspase-3 (B) Bax (C) Bcl-2 and (D) Bax/Bcl-2 mRNA ratio *in vitro*. The protein expression of (E) Caspase-3 (F) Bax (G) Bcl-2 and (H) Bax/Bcl-2 protein ratio *in vitro*. $n = 3$; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs*. WT, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ *vs*. WT+MI. The data are presented as mean ± SD.

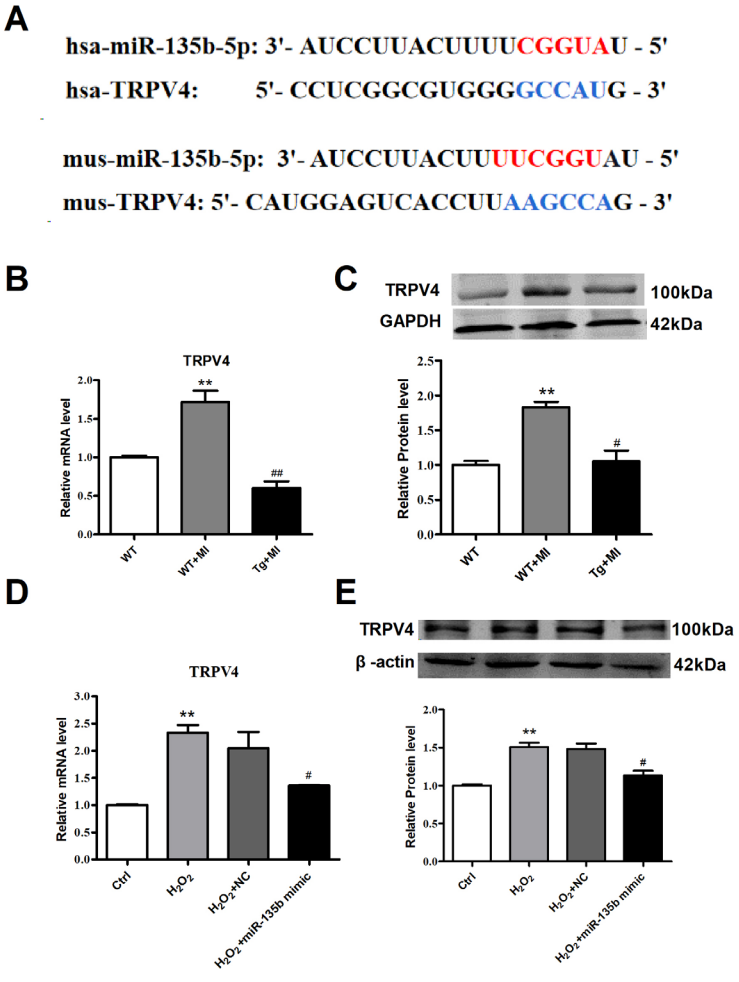

Fig. 6 miR-135b represses the expression of TRPV4 both in vivo and in vitro

(A) The predicted binding site for miR-135b in TRPV4 sequences of humans and mice. (B) The mRNA and (C) protein expression of TRPV4 *in vivo*. (D) The mRNA and (E) protein expression of TRPV4 *in vitro*. $n = 3$; $^{**}P < 0.01$ *vs*. WT or Ctrl, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs*. WT+MI or H_2O_2. The data are presented as mean ± SD.

Coronary occlusion triggers a large burst of cell death in the ischemic area within the first 6 to 24 hours[3]. Apoptosis is one of the main mechanisms of cell death, which is initiated by intracellular stimuli, such as reactive oxygen species (ROS) or Ca^{2+} overload. Multiple studies have demonstrated a causal connection between cardiomyocyte apoptosis and MI[17]. It is believed that inhibiting cardiomyocyte apoptosis during the early stage of MI is a effective maneuver for reducing infarct size and promoting cardiac repair, which is a key approach for treating ischemic heart diseases[6]. The present study unraveled that the expression of miR-135b was decreased 24 h after MI, which coincided with the timing of apoptosis as reported by a study[18]. Although it had been proved that miR-135b attenuates the protective effect of sevoflurane anesthesia on myocardial ischemia-reperfusion-induced apoptosis[19], but the direct evidence still lacks to be provided. The same possibility of regulation is also hinted in other studies. For example, overexpression of miR-135 can protect cardiomyocytes from ischemia/reperfusion injury in diabetic mice[20]. In the cardiomyocytes of patients with chronic heart failure, miR-135a/b regulates cell proliferation and apoptosis through HMOX1[7]. Our results were consistent with Li's conclusion that overexpression of miR-135b had a protective effect on MI[6]. To explore the mechanism underlying the role of miR-135b, our study used α-MHC-miR-135b transgenic mice and wild-type mice following 24 h post-MI to detect the effect on apoptosis. We observed here

that miR-135b is an anti-apoptotic miRNA with its overexpression inhibiting cardiomyocyte apoptosis induced by oxidative stress. Yet, more studies are needed to clarify the role of miR-135b in different cardiac diseases.

In this study, we also first demonstrated the upregulated expression of TRPV4 in the myocardium of mice with MI. TRPV4, is a Ca^{2+} permeable non-selective cation channel in heart. The excessive increase of TRPV4 protein and activity are associated with a variety of pathological mechanisms of cardiovascular diseases. As TRPV4 directly or indirectly regulates intracellular calcium and affects ion homeostasis, cell contractility and ECM formation, it has been thoroughly studied in diseases such as arrhythmia, myocardial fibrosis, and cardiac dysfunction[21-23]. Recent studies found that inhibition of TRPV4 is considered as an important target for reducing cardiomyocyte apoptosis. This is due to blocking TRPV4 reduced ROS generation and activated RISK signaling pathways, thus inhibiting cardiomyocyte apoptosis[12,16]. TRPV4 antagonism has therapeutic potential in dozens of diseases, but its safety profile in the human body remains yet to be determined, along with some other issues such as poor potency and selectivity[24-25]. More rigorous preclinical studies are required before it can reach the stage for clinical evaluations. Since miRNAs is one of human endogenous substances with high safety and simultaneously multi-target regulation, miRNA-target for therapeutic intervention and drug development is full of hope. Our recently study suggested that miR-135b have an inhibitory effect on TRPV4. Given the anti-apoptotic property of miR-135b, miR-135b mimic might be considered for its potential as a replacement therapy, and so might compounds that can upregulate expression of miR-135b, of the pathological processes associated with abnormally upregulated TRPV4.

Collectively, our results point to miR-135b as an anti-apoptotic miRNA in the heart and this property confers at least partially its cardioprotective effect against cardiomyocyte injury induced by oxidative stress as manifested in the setting of MI or other cardiac diseases as well. Thus, interfering miR-135b or miR-135b replacement might be an approach for relieving cardiac injury under pathological conditions.

Ethical approval

All procedures performed in the mouse studies were in accordance with the institutional guidelines for the care and use of animals.

Conflict of interest

The authors have declared that they have no competing interest exists.

Acknowledgments

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