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MiR-24 alleviates cardiomyocyte apoptosis after myocardial infarction via targeting BIM

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Abstract. – OBJECTIVE: Ischemia hypoxia induces cardiomyocyte (CM) apoptosis in the process of acute myocardial infarction (AMI). It was showed that pro-apoptosis factor BIM participates in regulating tumor cell apoptosis under ischemia or hypoxia condition, while its role in CM apoptosis after AMI is still unclear. It was revealed that miR-24 expression was significantly reduced in myocardial tissue after AMI. Bioinformatics analysis exhibits that miR-24 is targeted to the 3'-UTR of BIM. This study aims to investigate the role of miR-24 in mediating BIM expression and CM apoptosis.

PATIENTS AND METHODS: Dual-luciferase assay was used to confirm the targeted regulation between miR-24 and BIM. Cells were cultured under ischemia hypoxia for 12 h after transfection for 48 h. Cell apoptosis was tested by using flow cyto[illegible] The caspase activity was detected by[illegible] spectrophotometry. Wistar rats were divide[illegible] to four groups, including Sham, AMI, AMI+agomir trol, and AMI + agomir-24 groups. Cardiac func was evaluated by using echocardiography. CM apo tosis was determined by using [illegible] Infarctio area was measured by using [illegible]staining. MiR-24 targeted suppressed [illegible]expres[illegible].

RESULTS: MiR-24 mi[illegible]nd/or [illegible] sfection significantly d[illegible] B[illegible] inhibited caspase-9 a[illegible]casp[illegible]tivities, and reduced cell apopto[illegible]H9C2 ce[illegible]R-24 expression was decreas[illegible]le BIM level[illegible]up-regulated in myoca[illegible]AMI. Agomi[illegible]njection down-regulate[illegible]e BIM[illegible]ssion in myocardium, reduced C[illegible]poptosis, r[illegible]ed infarction area, and impr[illegible] cardiac functio[illegible]ts.

CO[illegible]SIONS: MiR-24 was[illegible]educed, whereas BIM[illegible] enha[illegible] in the CM after AMI. MiR-24 up[illegible]atio[illegible]ys a critical role in decreasing BIM e[illegible], redu[illegible]CM apoptosis, and im[illegible]ving [illegible]c fun[illegible] after AMI.

Key[illegible]ds
[illegible]24, BIM, [illegible]e myocardial infarction, Cardiomy[illegible]poptosis.

Introduction

Acute myocardial infarction (AMI) refers to the myocardial necrosis induced by coronary artery acute and persist[illegible]schem[illegible]ypoxia. [illegible]e AMI mainly exhibi[illegible]sev[illegible]and su[illegible]tained retrosternal pain[illegible]ch[illegible]mplica[illegible]with cardiac arrhyth[illegible], shock, [illegible]eart[illegible]re. The AMI is also [illegible]f the most [illegible] causes of cardiac de[illegible]diomyocyte[illegible]M) ischemia hypoxia [illegible]ne pr[illegible]of myocardial infarction can cause CM irrev[illegible] death or apoptosis, res[illegible] cardiac insu[illegible]ncy[3]. CM apoptosis a[illegible]ecrosis caused by myocardial ischemia hypoxia may induce [illegible]entricular remodeling, deteriorating cardiac f[illegible]tion, and forming continuous c[illegible] function[illegible]ect[4]. Particular factors in CM alw[illegible] disorders after ischemia and hypoxia[illegible]ation of related factors expression may reduce cell apoptosis, thus to provide new [illegible] for treating myocardial infarction and [illegible]g myocardial function. Bcl-2 interacting mediator of cell death (BIM), also known as the Bcl-2-like protein 11 (BCL2L11), belongs to the subfamily member of Bcl-2 family containing BH3-only structural domain[5,6]. As a kind of important apoptosis regulatory protein, BIM widely distributes in a variety of tissues and cells. It is closely related to promoting cell apoptosis[7]. It was showed that BIM participates in tumor cell apoptosis under ischemia[8] or hypoxia[9,10]. However, the role of BIM in regulating CM apoptosis after AMI still needs to be investigated. MiRNAs are a type of endogenous single strand non-coding RNA at the length of 22-25 nucleotides. The miRNAs can negatively regulate target gene expression via complete or incomplete complimentary binding with 3'-untranslated region (3'-UTR) to degrade mRNA or block mRNA translation at posttranscriptional level[11]. More and more evidence suggested that the abnormal expression and dysfunctions of miRNAs were involved in cardiac remodeling after myocardial infarction[12]. The results indicated that the miR-24 was significantly decreased in myocardium after infarction[13]. Bioinformatics analysis showed the complementary binding site between miR-24 and the 3'-UTR of BIM mRNA. This work explored

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the role of miR-24 in regulating BIM expression and affecting CM apoptosis.

Materials and Methods

Main reagents and materials

Healthy male Wistar rats at 8-10 weeks and weighted 220-250 g were purchased from Shanghai Fudan University, Shanghai, China. Rat myocardial cell line H9C2 was got from Shanghai Cell Bank of Chinese Academy of Sciences. Cell culture reagents were bought from Gibco (Grand Island, NY, USA). RNA extraction reagent Trizol was purchased from Invitrogen Life Technologies (Carlsbad, CA, USA); reverse transcription and Real-time PCR kits were from TaKaRa (Dalian, China); micrON™ agomir-24, micrON™ agomir-control, miR-24 mimic, miR-24 inhibitor, and negative control were designed and synthetized by RiboBio (Guangzhou, China). Rabbit anti-BIM and β-actin antibodies were obtained from Abcam (Cambridge, MA, USA). Caspase-3 and caspase-9 activities detection kits, and TUNEL apoptosis detection kit were b[illegible]ht from Beyotime (Shanghai, China). Hors[illegible] peroxidase (HRP) labeled goat anti rabbit se[illegible]d-ary antibody was purchased from Jackson Im[illegible] no Research (West Grove, PA, USA). Dual-lu[illegible] erase® reporter assay system an[illegible]-promot[illegible] plasmid was bought from P[illegible]g[illegible]Madison, WI, USA).

Rat AMI modeling and [illegible]

Wistar rats were k[illegible]pt in [illegible]environment with standard fe[illegible]g and free[illegible]king. The diurnal cycle w[illegible] humidity w[illegible]%, and room temper[illegible]e wa[illegible]ween 20°C and 22°C. The paddi[illegible] was chang[illegible]ice a week, while the bas[illegible]was cleaned tw[illegible]week. The rat was a[illegible]netize[illegible]by using 10% chloral hydrate intr[illegible]itonea[illegible]ection at 5 mg/g. Electrocardiog[illegible]w[illegible]onnect[illegible]for monitoring. The neck w[illegible]sected[illegible]separate trachea. Then, [illegible]ache[illegible]i[illegible]uted and connected to animal breathing machine with breathing ratio at 1:2, breathing frequency at 60 times/min, and ti[illegible]10-12 mL. The chest was opened at the fourth left intercostal and the pericardium was opened to expose the heart. Next, the anterior descending branch was identified and ligated by 6-0 no-traumatic sutures. ECG monitor exhibited ST segment arch lift for 0.1 mV or T wave high, pale myocardium, and abate pulse were applied to confirm the AMI model success. The Wistar rats were randomly equally divided into four groups with 15 in each group. The rats in sham group receiv[illegible] anterior descending branch ligatio[illegible]he ra[illegible] in AMI group were modeled as [illegible]. The rats in AMI + agomir-control receiv[illegible]10^3 mol/L agomir-control local injection at [illegible]before AMI modeling. Five poi[illegible]were se[illegible]on the surface of myocardi[illegible]with 10 μl inj[illegible] in each point. The r[illegible]in AM[illegible]agomi[illegible] received 2×10^3 mol/[illegible]mir[illegible]ocal i[illegible]ection at 24 h before A[illegible]mo[illegible]ive po[illegible]were selected on th[illegible]rface [illegible]ocar[illegible]n, with 10 μl injecti[illegible]each poin[illegible]age of all experimen[illegible]ls was str[illegible]abided by the Jinsha[illegible]Hospi[illegible]udan University Animal Management Commi[illegible]Regulation Ordinance and[illegible]ed by Jinsha[illegible]spital, Fudan University experimental Animal Ethics Committee.

Echocardiography

[illegible] rats in [illegible] group were anesthetized by 10%[illegible]ate (Sigma-Aldrich, St. Louis, MI, US[illegible]peritoneal injection at 1 week after modeling to perform echocardiography. Left ventricular end systolic diameter and left ventricular [illegible] diameter were recorded at the level of papillary muscle prior to mitral valve through the left ventricular stort axis view. Left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS) were automatically calculated.

Rat myocardium sample preparation

The tissue samples at MI region were extracted from rats at 1 week after modeling and used for RNA and protein extraction, and frozen section.

TUNEL assay

Myocardium sample was extracted from the rats at 1 week after modeling to prepare frozen section. TUNEL detection kit was used to measure cell apoptosis (Sigma-Aldrich, St. Louis, MI, USA). The section was fixed in 4% paraformaldehyde (Sigma-Aldrich) for 60 min and washed by phosphate-buffered saline (PBS) for twice. Next, the section was treated by using 0.15 Triton X-100 on ice for 2 min and was added with 50 μl TUNEL detection fluid prepared by TdT enzyme and fluorescence label liquid at 37°C for 60 min. At last, the section was tested under 488 nm to analyze cell apoptosis.

Myocardial infarction area measurement

The rats received 1 ml 2% evans blue intravenous injection at 1 week after modeling. Then the left ventricular wall tissue was extracted and cut into slice at 3 mm. Next, the slice was incubated in 2% TTC at 37°C for 15 min. After washed by normal saline and fixed by 4% paraformaldehyde, the slice was observed under the microscope. The infarcted myocardium exhibited as grey white, while the non-infarcted myocardium presented as brick red. Myocardium infarction area = infarction area/(infarction area + non-infarction area) × 100%.

Rat CM cell transfection and ischemia hypoxia treatment

Rat CM cells H9C2 were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Grand Island, NY, USA) containing 10% FBS (Gibco) and maintained in 37°C and 5% CO_2. Cells in logarithmic phase were divided into five groups, including mimic NC, miR-24 mimic, si-NC, si-BIM, and miR-24 mimic + si-BIM. After 48 h, the cells were incubated in low-glucose DMEM medium without FBS to simulate [illegible]emia environment, and then cultured in in[illegible] with 1% O_2, 5% CO_2, and 94% N_2 to si[illegible]te hypoxia for 12 h.

Luciferase reporter gene v[illegible] construction

The 3'-UTR of BIM ge[illegible]s amp[illegible]d based on HEK293 cell genom[illegible]R pro[illegible] cycled and connected [illegible]o p[illegible]M[illegible] tra[illegible] DH5α competent c[illegible] (Tak[illegible] Dalian, China). After colony [illegible]R, the pos[illegible]lone was screened and t[illegible]d with corr[illegible]equence was applied f[illegible]ell t[illegible]ction.

Lucifer[illegible] reporter gen[illegible]ay

Th[illegible]EK29[illegible] cells were transfected with 500 [illegible]GL3-[illegible]-3'UTR, 30 nmol miR-24 nucleo[illegible]fra[illegible]nt, and [illegible] ng pRL-TK mixture mediate[illegible] Lipof[illegible]mine 2000 (Invitrogen [illegible] Techn[illegible]ies). After incubated for 6 h, Op[illegible]EM [illegible] was changed to DMEM me[illegible]m supplemented with 10% FBS and 1% p[illegible]tomycin for another 48 h. Lu[illegible]rase activity was detected according to the [illegible]nual provided by the kit (Invitrogen Life [illegible]ologies). After washed by PBS for twice, the[illegible]ls were added with 100 µl PLB for 30 min at room temperature and centrifuged at 1,000 ×g for 10 min. The supernatant was added with 100 µl LAR II and tested immediately in chemiluminescence apparatus for fluorescence I. Then the solution was added with 100 µl Stop&Glo solution to test fluorescence II. The [illegible] fluorescence I and II was treated a[illegible]ative e[illegible]pression level of luciferase activi[illegible]

qRT-PCR

The reverse transcripti[illegible]stem co[illegible]d 2 µg total RNA, 0.75 µL d[illegible] at 10 mmol/[illegible] RT Buffer (5×), 1.2 µL [illegible] prim[illegible] µmol/L, [illegible] µL reverse transcrip[illegible] 0.5 µL [illegible]ase inhibitor, and ddH_2O. The [illegible]ctio[illegible]rform[illegible] 16°C for 30 min, 42°[illegible] 15 mi[illegible] 85°[illegible] 5 min. The PCR pri[illegible]sed were a[illegible] miR-24P_F: 5′-TCACA[illegible]ACATTGC[illegible]G-3′, miR-24P_R: 5′-[illegible]GGT[illegible]CTGCTCTCTGTCTC-3′; U6P_F: 5′-ATTGGAAC[illegible]ACAGAGAAGATT-3′, U6[illegible] 5′-GGAACC[illegible]CACGAATTTG-3′; B[illegible]P_F: 5′-TAAGTTCTGAGTGTGACCGAGA-3′, [illegible]IP_R: 5′-GCTC[illegible]TCTGTAGGGAGGTAGG-3′; [illegible]tinP_F: 5′-GA[illegible]CCTAAGGCCAAC-3′, β-acti[illegible] 5′-TGTC[illegible]CACGATTTCC-3′. The PCR reac[illegible] Invitrogen Life Technologies) was co[illegible] of 4.5 µl 2×SYBR Green Mixture, 0.5 µl forward and reverse primer at 5 µM, 1 µl [illegible] and 3.5 µl ddH_2O. The reaction was per[illegible]n ABI ViiA7 at 95°C for 5 min, followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. Comparative Ct method ($2^{-\Delta\Delta CT}$) was applied for data analysis. U6 and β-actin were selected as housekeeping control. Each test was repeated for 3 times.

Western blot

Total protein was extracted from the cells and quantified by bicinchoninic acid assay (BCA) method. A total of 50 µg protein was separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membrane (Bio-Rad Laboratories, Hercules, CA, USA). After blocked by 5% skim milk at room temperature for 60 min, the membrane was incubated in primary antibody at 4°C overnight (Santa Cruz Biotechnology, Santa Cruz, CA, USA). After washed by PBST, the membrane was further incubated in HRP labeled secondary antibody (Santa Cruz Biotechnology) at room temperature for 60 min. At last, the membrane was treated by ECL chemiluminescence reagent (Amersham, Piscataway, NJ, USA) and was developed for analysis.

Caspase-3 and caspase-9 activities detection

The standard substrate pNA (10 mM) was diluted to 0, 10, 20, 50, 100, and 200 μM, respectively. Their absorbance at 405 nm was tested to draw the standard curve. The cells were digested and centrifuged at 600 ×g and 4°C for 5 min. After cells were washed by PBS, a total of 2×10^6 cells were added to 100 μl lysis at 4°C for 15 min. After the supernatant was centrifuged at 18,000 ×g for 10 min, it was moved to a precooled Ep tube. For caspase-3, a total of 10 μl Ac-DEVD-pNA (2 mM) was added to the solution and incubated at 37°C for 120 min to test absorbance at 405 nm. For caspase-9, a total of 10 μl Ac-LEHD-pNA (2 mM) was added to the solution and incubated at 37°C for 2 h to test absorbance at 405 nm.

Flow cytometry

The cells were digested by enzyme and were collected. After, the cells were resuspended in 100 μl binding buffer, and 5 μl Annexin V-FITC and 5 μl propidium iodide (PI) were add[illegible] at room temperature avoiding of light for [illegible] Then, the cells were tested on flow cyto[illegible]y (Beckman Coulter, Miami, FL, USA).

Statistical Analysis

SPSS 18.0 software (SPSS Inc., Chicago, IL, USA) was applied for data analysis. Measurement data was presented as mean ± standard[illegible] and compared by *t*-test. $p<0.05$ was [illegible]cted [illegible] significant difference.

Result[illegible]

MiR-24 targeted re[illegible]ted B[illegible]xpressi[illegible]

Dual luciferase r[illegible]r as[illegible] exhibit[illegible]d that miR-24 mimic or i[illegible]bit[illegible]tion si[illegible]cantly reduced or enha[illegible]d the re[illegible]lucife[illegible] activity in HEK293 c[illegible]spectively, [illegible]g that BIM was the ta[illegible]f miR-218 ([illegible]re 1A-C). It suggested [illegible]t miR[illegible]n target bind with 3'-UTR of BIM mRNA to reg[illegible]s expression.

I[illegible]emia hypoxia intervention induced [illegible]C2 cell ape[illegible]osis and down-regulated [illegible]-24 expres[illegible]n

[illegible]hemia hy[illegible]a treatment *in vitro* signifi-can[illegible]9C2 cell apoptosis compared with co[illegible]nal cultured H9C2 cells (Figure [illegible]A). MiR-24 expression was reduced, while BIM [illegible]arkedly was enhanced after intervention [illegible] B-C).

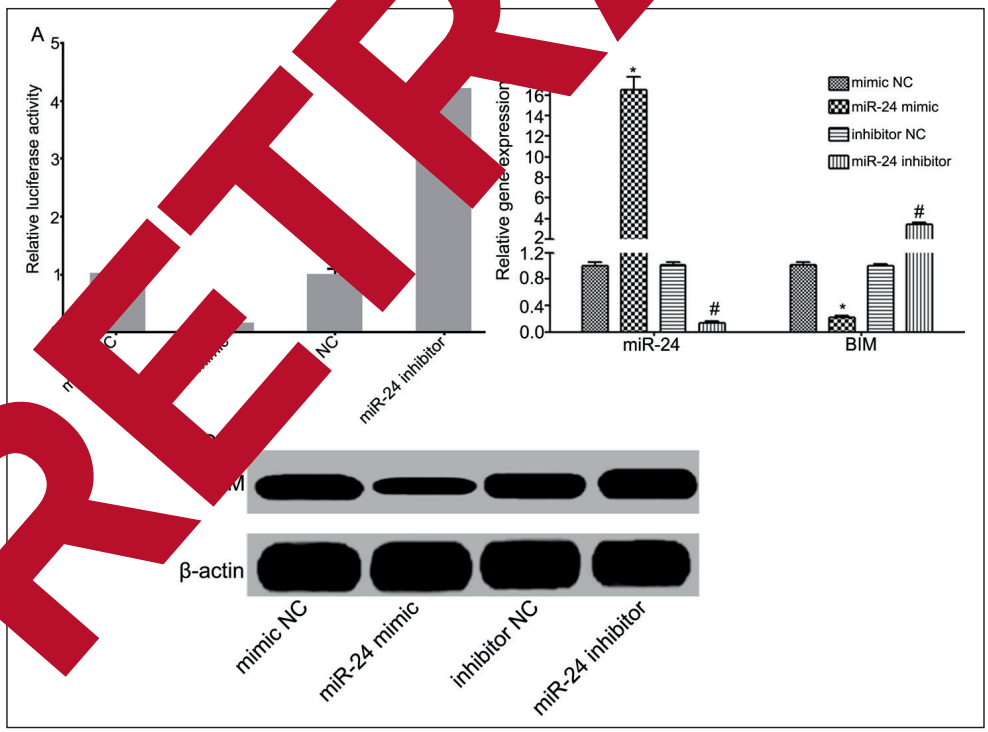

Figure 1. MiR-24 targets and regulates BIM expression. **A,** Dual luciferase reporter assay. **B,** qRT-PCR detection of miR-24 and BIM gene expression. **C,** Western blot detection of BIM protein expression. *$p<0.05$, compared with mimic NC. #$p < 0.05$, compared with inhibitor NC.

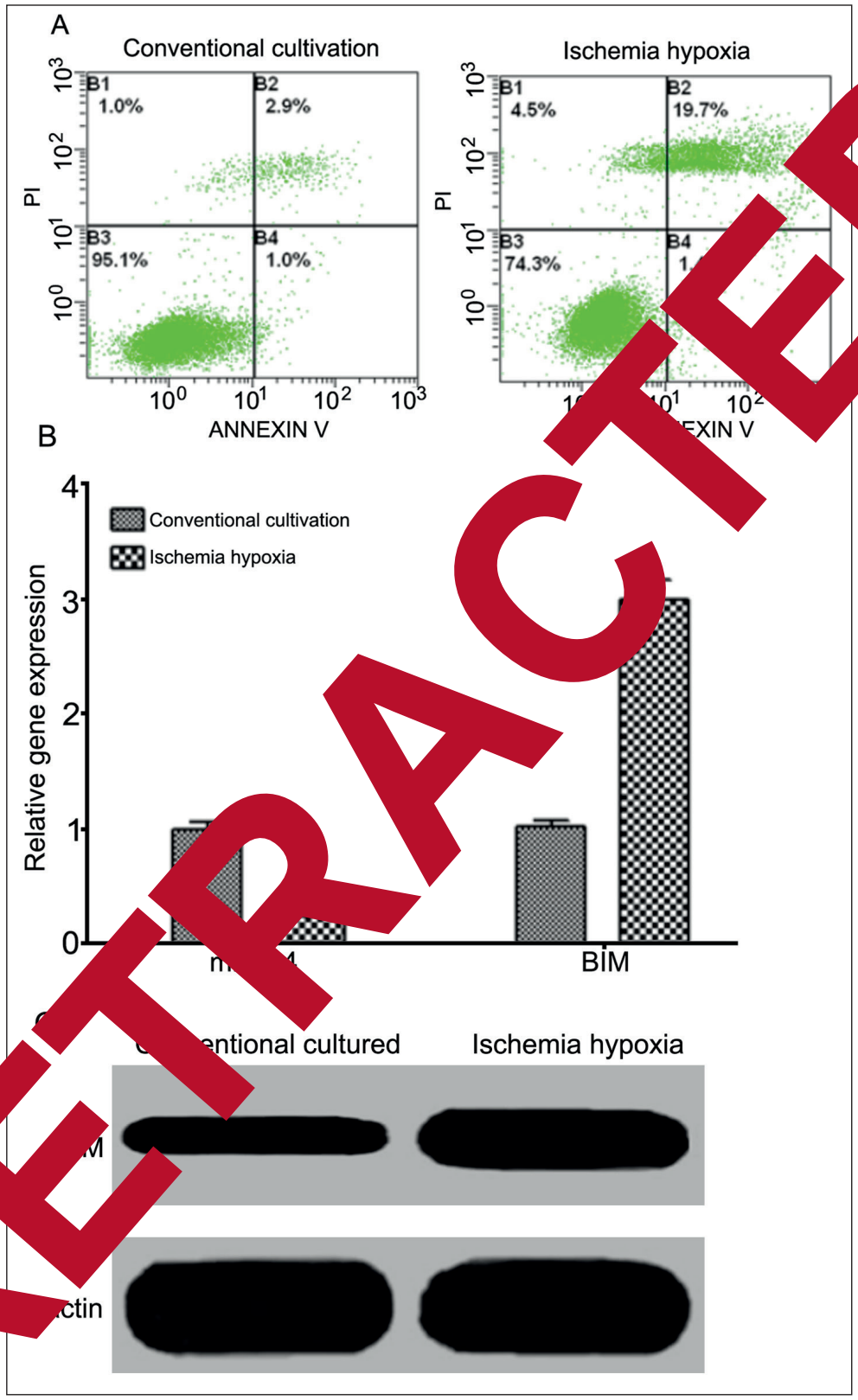

Figure 2. Ischemia hypoxia intervention induces H9C2 cell apoptosis and down-regulates miR-24 expression. **A,** Flow cytometry detection of cell apoptosis. **B,** qRT-PCR detection of miR-24 and BIM gene expression. **C,** Western blot detection of BIM protein expression. *$p<0.05$, compared with normal cells.

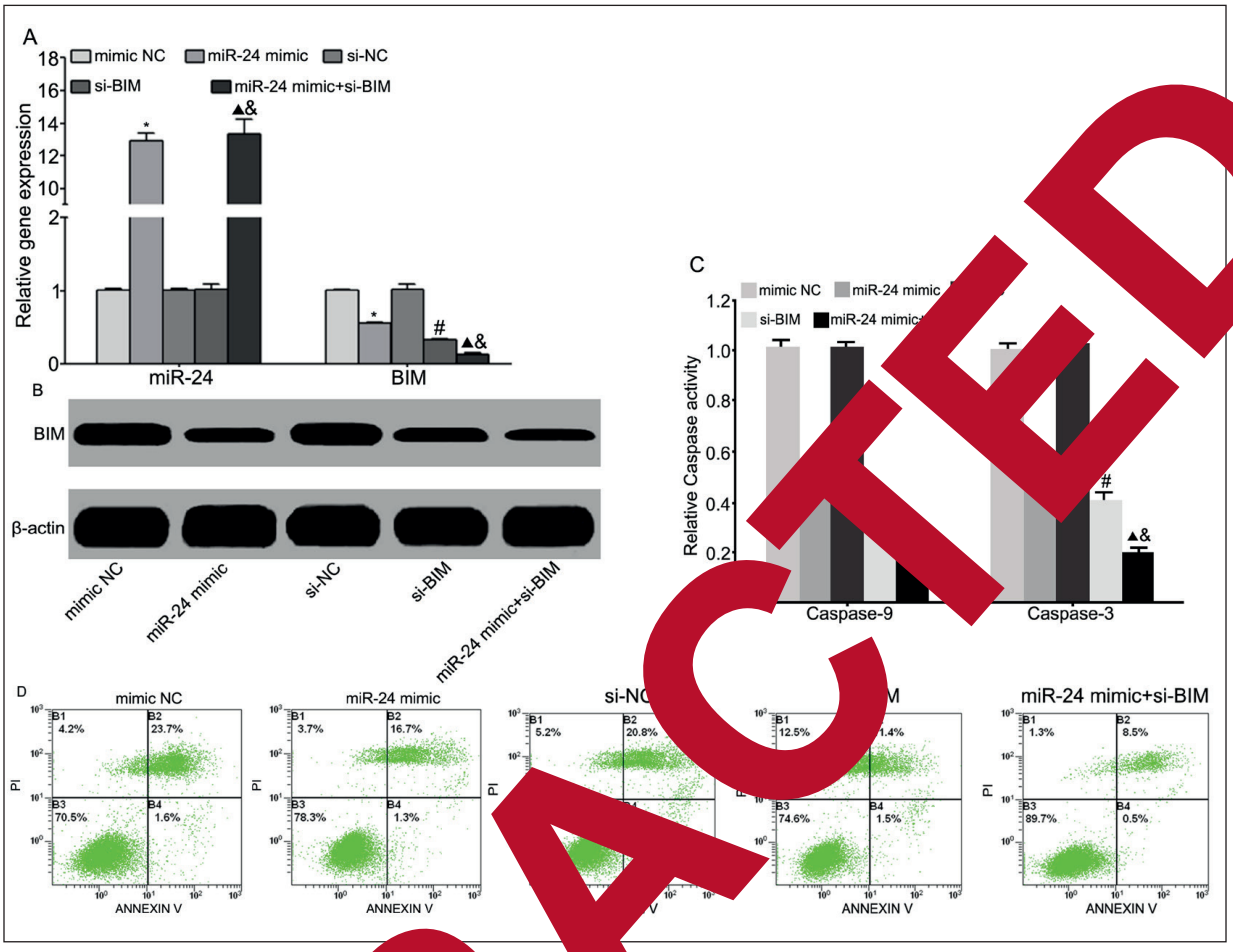

Figure 3. MiR-24 elevation reduc… apoptosis … ed by ischemia hypoxia. ***A,*** qRT-PCR detection of gene expression. ***B,*** Western blot detecti… BIM p… expressio…, Spectrophotometry detection of caspase activity. ***D,*** Flow cytometry detection of cell apo… *p<0.0… R-24 mimic compared with mimic NC. #p<0.05, si-BIM compared with si-NC. Δp<0.05, miR-24 mimic + … M com… NC. & p<0.05, miR-24 mimic + si-BIM compared with si-NC.

MiR-24 elevati… educed H… cell apoptosis in… y ischemia… oxia

Compared … NC, miR-24 mimic transfectio… ignificantly … essed BIM expression in … 2 cells (Figure … B) and reduced cell a… tosis i…duced by i…chemia hypoxia. Co…ed wi…-NC, BIM silence obviously enha… a… sis res…nce of H9C2 cell after it was … by is…mia hypoxia. Caspase-3 … acti… w…educed, whereas apoptosis sen…ity to …mia hypoxia declined to the low… level in H…C2 cells after miR-24 over-ex-p… …M silence (Figure 3 C-D).

…regulation of miR-24 protected CM …tosis after AMI

…was showed that compared with sham group, LVEF and LVFS markedly decreased (Table I), CM apoptosis significantly elevated (Table II), infarction area enlarged (Table III), miR-24 apparently reduced, and BIM level enhanced (Figure 4A-B) in AMI group. Compared with agomir-control, agomir-24 intra-myocardial injection improved LVEF and LVFS (Table I), decreased CM apoptosis (Table II), reduced infarction area

Table I. Echocardiographic detection (%, n=10, Mean ± SD).

Item	Grouping	7 days after modeling
LVEF	Sham	82.5±4.3
	AMI	65.3±2.2
	AMI+agomir-control	64.7±3.1
	AMI+agomir-24	73.5±2.4
LVFS	Sham	47.7±3.3
	AMI	35.1±2.8
	AMI+agomir-control	36.4±3.0
	AMI+agomir-24	42.6±2.9

Table II. CM apoptosis comparison (%, n=10, Mean ± SD).

Group	7 days after modeling
Sham	5.5±1.1
AMI	29.5±5.1
AMI+agomir-control	30.7±6.2
AMI+agomir-24	19.6±3.7

Table III. Myocardial infarction area comparison (%, n=10, Mean ± SD).

Group	7 days after modeling
Sham	0.0±0.0
AMI	42.7±
AMI+agomir-control	44.1±
AMI+agomir-24	27.2±2.8

(Table III), and declined BIM expression (Figure 4 A-B). It indicated that miR-24 elevation can downregulate BIM expression, reduce CM apoptosis after infarction, narrow infarction area, and improve cardiac function.

Discussion

Myocardial infarction caused by myocardial ischemia anoxic is a type of cardiovascular disease seriously harm to human health and one of the most common causes of heart failure[14]. AMI is featured as complicated pathogenesis, critical condition and a rapid progress. It is easy to endanger patient's life or c serious sequ is not treated in time t prese here are million people deat cardi scular isease worldwide every r. n a hal them died of AMI[16]. s repo hat th cidence of AMI in C was 45-55/1 ollowing the aggra geing socie speeding up of model life rh improvement of living standards, changes in nd increase of mental pre e incidence MI exhibits rising t year by year in our country. Ischemia oxia may c e CM irreversible apoptosis ecrosis, ind ventricular remodeling, de- te te cardia nction, and form sustainable card efect in the process of MI[4]. CM

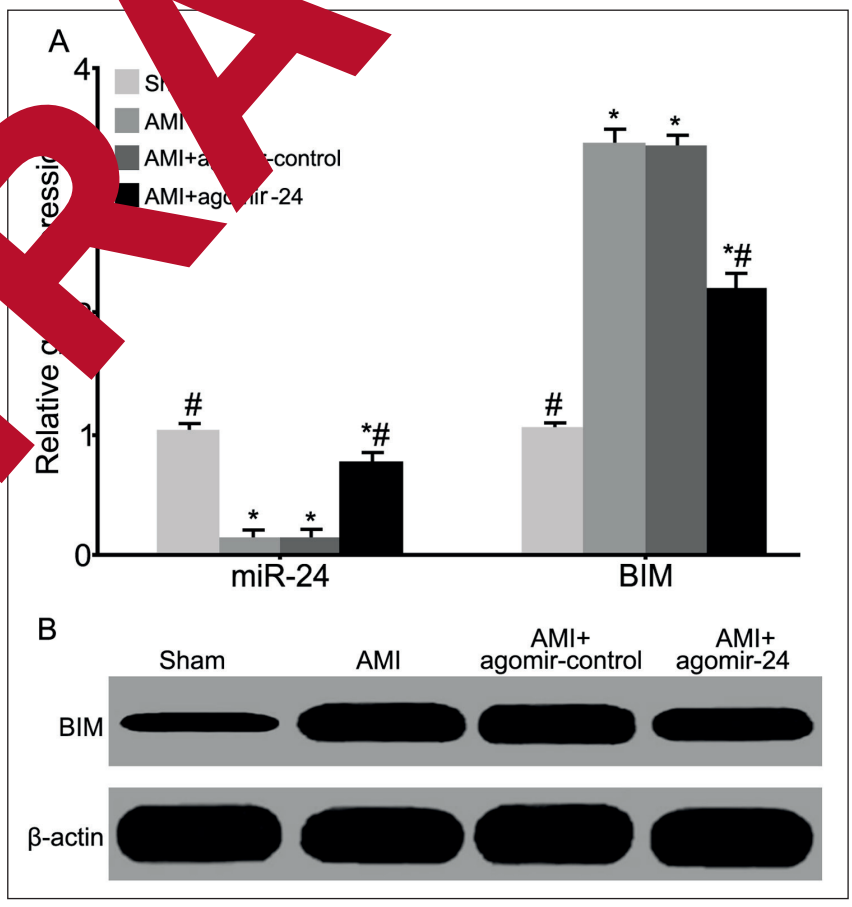

Figure 4. Up-regulation of miR-24 protected CM apoptosis after AMI. **A,** RT-PCR detection of gene expression. **B,** Western blot detection of BIM protein expression. *p<0.05, compared with Sham. #p<0.05, compared with AMI.

apoptosis plays an important role in the pathophysiology process of cardiac dysfunction after infarction, heart failure, and cardiac remodeling. Therefore, reducing CM apoptosis is of great significance in improving myocardial function after infarction and delaying the cardiac remodeling process[18]. CM apoptosis and necrosis are one of the major pathological mechanisms of cardiac insufficiency after AMI. Necrosis often occurs in the late phase of ischemia, whereas apoptosis occurs earlier and throughout the entire process. Thus, how to effectively reduce or avoid CM apoptosis after infarction became a hot issue in clinic[19]. Particular factors in CM appear to be disorder after ischemia and hypoxia. Regulation of related factors expression may reduce cell apoptosis, thus providing a new thought for treating myocardial infarction and improving myocardial function. BIM, a member of the subfamily of Bcl-2 protein family containing BH3-only domain structure, is the core regulatory protein in mitochondrial apoptosis pathway. It is a key factor in mediating apoptosis in various cell types[7]. The BH3-only domain structure of BIM is the main domain to promote apoptosis, which transfers Bax from cytoplasm to the mitochondria and increases the permeability of mitochondrial membrane. It further leads to cytochrome C-release to cytoplasm, activating caspase-9 and downstreaming caspase-3, resulting mitochondrial dependent endogenous pathway activation[20]. It was demonstrated that BIM participated in tumor cell apoptosis induction[8] or hypoxia[9,10]. However, the role of BIM in regulating CM apoptosis after AMI needs to be investigated. It was found that miR-24 obviously decreased in myocardium after infarction[13]. Bioinformatics analysis showed the complementary binding sites between miR-24 and the 3'-UTR of BIM mRNA. This paper explored the role of miR-24 in regulating BIM expression and affecting CM apoptosis. Dual luciferase reporter assay exhibited that miR-24 mimic or inhibitor transfection significantly reduced or enhanced BIM expression in H9C2 cells, suggesting that miR-24 can directly bind with 3'-UTR of BIM mRNA to regulate its expression. Ischemia hypoxia *in vitro* significantly induced H9C2 cell apoptosis, down-regulated miR-24 expression, and elevated BIM level compared with conventional cultured H9C2 cells, indicating that miR-24 reduction may play a role in increasing BIM expression and promoting CM apoptosis after ischemia hypoxia. Gao et al[21] reported that hypoxia obviously up-regulated BIM expression in CM cultured *in vitro* and induced CM apoptosis. Huang et al[10] revealed the role of BIM upregulation in low glucose hypoxia induced apoptosis. Our results suggested that ischemia hypoxia up-regulated BIM expression in H9C2 cells and facilitated cell apoptosis, which was in accordance to Gao et al[21] and Huang et al[10] findings. MiR-24 mimic and/or BIM siRNA transfection significantly suppressed BIM expression in H9C2 cells and reduced cell apoptosis induced by ischemia hypoxia. Compared with si-NC, BIM silence obviously enhanced apoptosis resistance of H9C2 cell, while it was reversed by ischemia hypoxia. Animal experiment revealed that miR-24 apparently elevated and BIM level enhanced in AMI group, which was in accordance with *in vitro* results. Liu et al[22] demonstrated BIM overexpressed in myocardium from hypoxia infarction in animal experiment, which was similar to our results. Kholamin et al[23] indicated that compared with control, miR-24 expression in peripheral blood from AMI patients was apparently reduced. Moreover, miR-24 elevation down-regulated BIM expression, reduced CM apoptosis after infarction, narrowed infarction area, and improved cardiac function. Izarra et al[24] showed that over-expression of miR-133a reduced BIM expression in cardiac progenitor cells (CPCs) and promoted transplanted CPCs proliferation and survival in the heart tissue from rat MI model to improve cardiac function and reduce the myocardial fibrosis after myocardial infarction. It suggested the protecting and treatment effect of reducing BIM in infarcted myocardium. Bhuiyan et al[25] presented that as a kind of myocardial protective drug, vanadyl sulfate can reduce BIM level and increase FLIP expression in CM, and inhibit caspase-3 and caspase-9 activities through activating AKT signaling pathway. Our results revealed the role of down-regulating BIM in protecting CM from apoptosis after infarction and improving heart function, which was similar to Izarra et al[24] study. Hu et al[26] reported that over-expression of miR-21, miR-24, and miR-221 enhanced CPCs resistance to serum starvation, prolonged transplanted CPCs survival in rat AMI model, and improved cardiac function. Guo et al[27] discovered that compared with wild type mice, myh6-miR-24 transgenic mice appeared sensitivity reduction to AMI, presenting as declined CM apoptosis after AMI, narrowed infarction area, and improved cardiac function. In addition to CM protection, Wang et al[13] showed over-expres-

sion of miR-24 can significantly reduce secretion of TGF-β and phosphorylation of Smad 2/3 in cardiac fibroblasts to improve cardiac function after AMI.

Conclusions

MiR-24 was reduced, whereas BIM was enhanced in CM after AMI. MiR-24 up-regulation plays a critical role in decreasing BIM expression, reducing CM apoptosis, and improving cardiac function after AMI.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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