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TRIM59 attenuates inflammation and apoptosis caused by myocardial ischemia reperfusion injury by activating the PI3K/Akt signaling pathway

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Abstract. – **OBJECTIVE:** Myocardial ischemia reperfusion injury (MIRI) is a common factor in heart-related diseases. The aim of this study was to explore the effect of TRIM59 gene on MIRI and its mechanism.

MATERIALS AND METHODS: Rats were used to construct MIRI models, and TRIM59 gene was overexpressed in myocardium by Entranster technique to detect the effects of TRIM59 on myocardial oxidative stress, myocardial injury and ATPase. In addition, rat myocardial H9c2 cells were cultured, and a hypoxia-reoxygenation model of H9c2 cells was constructed to detect the effect of TRIM59 overexpression on the inflammation and apoptosis of H9c2 cells. Finally, the PI3K/Akt signaling pathway inhibitor LY294002 was used to verify the effect of TRIM59 on the PI3K/Akt signaling pathway.

RESULTS: Overexpression of TRIM59 in vivo effectively reduced the expression of MDA, CK, and LDH, and increased the expression of SOD and the activity of Na^{+}-K^{+}-ATPase and Ca^{2+}-Mg^{2+}-ATPase. In addition, overexpression of TRIM59 in H9c2 cells significantly reduced the expression of inflammatory cytokines (IL-1β, IL-6, and TNF-α) and oxidative stress (ROS) levels. TRIM59 also significantly increased the activity of PI3K/Akt signaling pathway and promoted the phosphorylation of Akt.

CONCLUSIONS: TRIM59 reduces the level of inflammation and apoptosis of myocardial cells caused by MIRI by activating the PI3K/Akt signaling pathway, thereby reducing myocardial injury.

Key Words:
TRIM59, Inflammation, Apoptosis, Myocardial ischemia reperfusion injury, PI3K/Akt signaling pathway

Introduction

Heart-related diseases are one of the three major chronic diseases in humans and their incidence rates are increasing year by year[1]. At present, coronary interventional therapy, coronary artery bypass grafting, and coronary thrombolysis are common treatments for heart diseases, such as coronary heart disease[2]. However, with the widespread use of such treatments, the incidence of myocardial ischemia reperfusion injury (MIRI) has also increased year by year. Especially for myocardial infarction recanalization, coronary artery spasm, heart transplantation, and cardiopulmonary bypass, the incidence of MIRI is extremely high, which seriously affects the treatment effect[3]. MIRI refers to the phenomenon that the tissue damage is aggravated after the blood supply is restored on the basis of ischemia. The mechanism of MIRI is complex, and various factors of the body are involved in the development of such disease. Intracellular signal transduction pathways such as cell proliferation, oxidative stress, and apoptosis significantly affect the occurrence of this lesion[4].

The PI3K/Akt signaling pathway is a key pathway involved in the regulation of MIRI[5]. A variety of interventions, such as ischemic preconditioning (IPC) and statins, can actively activate the PI3K/Akt signaling pathway and reduce myocardial infarction[6]. Tong et al[7] found that IPC induced phosphorylation of Akt in an *in vitro* MIRI model, whereas the PI3K inhibitor LY294002 reduced myocardial protection induced by IPC and phosphorylation of Akt. In PI3K knockout mice, the cardioprotective effect of IPC is weakened and there is no activation of Akt, thus confirming that there is the activation of PI3K/Akt signaling pathway in IPC. Therefore, the PI3K/Akt signaling pathway plays an important protective role for MIRI.

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The TRIM family proteins are present in all multicellular animals and are a fairly large family that more than 80 have been found in humans[8]. TRIM family proteins are involved in a variety of physiological processes, including cell proliferation, DNA damage repair, intracellular signal transduction, and immune responses[9]. In recent years, most studies on the TRIM family have focused on tumors, but lack of relevant studies on the role of MIRI. TRIM59 can activate PI3K/Akt activation signaling pathway to promote cell proliferation and metabolism[10]. Whether TRIM59 can attenuate myocardial injury by activating PI3K/Akt signaling pathway in MIRI has not been studied. Therefore, rats were used to construct MIRI models to test the myocardial protective effect of TRIM59, and rat cardiomyocyte cell line H9c2 was cultured to construct a hypoxia-reoxygenation (HR) model. Then, the TRIM59 was overexpressed to detect the change of the inflammation and apoptosis of H9c2 cells.

Materials and Methods

Animals and Grouping

A total of 30 Sprague Dawley (SD) rats aged 6-8 weeks old and weighing 280-320 g were used in this study. They were raised in a standard environment (five in a cage) and divided into 4 groups, namely control group, MIRI group, NC group, and TRIM59 group. The rats in the control group were normally raised, while those in the IRI group, NC group, and the TRIM59 group were constructed as MIRI models. Rats in the TRIM59 group and NC group were transfected *in vivo* one week before constructing the MIRI model to overexpress TRIM59 or be set as negative control. This study was approved by the Animal Ethics Committee of Qingdao University Animal Center.

Operation Procedure of Rat MIRI

The rats were placed in the supine position on the surgical table after being anesthetized with 4% paraformaldehyde (10 μL/g). Next, the fur was removed from the chest and abdomen of the rat using scissors, and then, the chest was cut to expose the heart. After that, scissors were used to gently separate the pericardium and find the anterior descending coronary artery. Later, the anterior descending coronary artery was ligated with a suture for half an hour. The darkening of the anterior wall of the rat heart indicated that the ischemic model was successfully constructed. After half an hour of ligation, the suture was loosened to restore the heart to blood flow for 4 hours. Finally, the rats were sacrificed by aortic bleeding.

Entranster

Entranster™-*in vivo* kit (Engreen, Beijing, China) was used to transfect rat myocardial tissue *in vivo* to increase expression of TRIM59 in cardiomyocytes. After mixing the lentivirus overexpressing TRIM59 with Entranster, the mixture was injected into the pericardium of the rat. After 3 days, the transfection efficiency was verified by Western blot and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR).

Cell Culture and Treatment

The H9c2 cell line was purchased from Nanjing Cobioer Biotechnology Co., Ltd. (Nanjing, China), which were cultured in a 37°C and 5% CO_2 incubator using Dulbecco's Modified Eagle's Medium (DMEM) medium (Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS[illegible] Gibco, Rockville, MD, USA) and 1% penicillin plus streptomycin (Gibco, Rockville, MD, USA). LY294002 (Selleck, Houston, TX, USA) is an inhibitor of the PI3K/Akt signaling pathway and is used to inhibit the activity of the PI3K/Akt signaling pathway in H9c2 cells. MIRI models were established at the cellular level by HR. The medium in the logarithmic growth phase of H9c2 cells was changed to phosphate-buffered saline (PBS), and the cells were cultured in an incubator filled with 95% N_2 for 4 hours. After 4 hours, the PBS in the cell culture dish was replaced with the medium and placed back in the 37°C and 5% CO_2 incubator.

Cell Transfection

Lenti-TRIM59 and Lenti-NC were transfected in H9c2 cells using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Lenti-TRIM59 was used to increase the expression of TRIM59 in H9c2 cells. Lenti-NC transfected H9c2 cells were used as a negative control group.

Western Blot

A protein lysate (NCM Biotech, Newport, RI, USA) was used to lyse H9c2 cells and then, the cell debris was removed by centrifugation. The BCA method (Yifeixue, Nanjing, China) was used to detect protein concentration. 5% loading buffer

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(NCM Biotech, Newport, RI, USA) was used to dilute into the protein lysate. Next, an appropriate amount of protein dilution was added to each well of the electrophoresis gel and transferred the protein to the polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA) after sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). After that, 5% skim milk was used to block non-specific antigens, and primary antibodies (IL-1β, 1:3000, Rabbit, IL-6, 1:3000, Rabbit, TNF-α, 1:2000, Rabbit, caspase3, 1:1000, Rabbit, caspase9, 1:3000, Rabbit, Bcl-2, 1:2000, Rabbit, Bax, 1:1000, Rabbit, PI3K, 1:5000, Rabbit, Akt, 1:5000, Rabbit, p-Akt, 1:5000, Rabbit and β-actin, 1:3000, Rabbit, Abcam, Cambridge, MA, USA) were used to incubate the PVDF membrane at 4°C overnight. The next day, after washing the PVDF membrane, a secondary antibody (Goat anti-rabbit, 1:3000, Abcam, Cambridge, MA, USA) was applied to incubate PVDF membrane for 2 hours at room temperature. Finally, chemiluminescence was conducted to detect protein expression.

RNA Isolation and qRT-PCR

Total RNA was extracted from H9c2 cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). SuperScript IV reverse transcriptase (Invitrogen, Carlsbad, CA, USA) was used to reverse transcribe RNA into complementary deoxyribose nucleic acid (cDNA), and SYBR Green (Invitrogen, Carlsbad, CA, USA) was used to amplify cDNA. Then, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as endogenous controls. Finally, $2^{-\Delta\Delta Ct}$ method was used to calculate relative expression level. The primer sequences of mRNA are shown in Table I.

Malondialdehyde (MDA), Superoxide Dismutase (SOD), Creatine Kinase (CK), Lactate Dehydrogenase (LDH) Activity Assay

After the rats were sacrificed, appropriate size myocardial tissue was taken, and lysate was used to dissolve the tissue. MDA and SOD activity assay kits (Lianke, Hangzhou, China) were used to detect the expression of MDA and SOD in myocardial tissue. In addition, blood was taken from the heart and the serum of the rats was isolated. Next, CK and LDH activity assay kits (Lianke, Hangzhou, China) were used to detect the expression levels of CK and LDH in serum.

Na^{+}-K^{+}-ATPase and Ca^{2+}-Mg^{2+}-ATPase Activity Assay

After the rats were sacrificed, the apical tissue of the rat was taken and ground into powder to dissolve in PBS buffer. Thereafter, Na^{+}-K^{+}-ATPase and Ca^{2+}-Mg^{2+}-ATPase activity were detected by bioenzymatic colorimetry. In normal physiological processes, ATP released energy and decomposed into ADP and inorganic phosphorus,

Table I. RT-PCR primer sequences.

Name	Sense/Anti-sense	Sequence (5′-3′)
IL-1β	Sense	GCAACTGTTCCTGAACTCAACT
	Anti-sense	ATCTTTTGGGGTCCGTCAACT
IL-6	Sense	ACTCACCTCTTCAGAACGAATTG
	Anti-sense	CCATCTTTGGAAGGTTCAGGTTG
TNF-α	Sense	CTACCATCACCGCACTGAGAT
	Anti-sense	GGTCACTTCACCATAGTGGACA
Caspase3	Sense	CAGAATCATAAGCCCCTGGA
	Anti-sense	TCTGCGAGTCAGGCATTTG
Caspase9	Sense	TTCTTGAGCAACACCCTC
	Anti-sense	CGCATACACTGTCTACCT
Bax	Sense	CAGTTGAAGTTGCCATCAGC
	Anti-sense	CAGTTGAAGTTACCATCAGC
Bcl-2	Sense	GACTGAGTACCTGAACCGGCATC
	Anti-sense	CTGAGCAGCGTCTTCAGAGACA
PI3K	Sense	GGTGACTGTGTGGGACTTATTGA
	Anti-sense	CTGATGTAGTGTGTGGCTGTTGA
Akt	Sense	CAGGTTCACCCAGTGACAACTCA
	Anti-sense	CACGAGACAGGTGGAAGAAGAGC
GAPDH	Sense	ACAACTTTGGTATCGTGGAAGG
	Anti-sense	GCCATCACGCCACAGTTTC

and the activity of the ATPase could be determined by the amount of inorganic phosphorus.

Immunocytofluorescence (IF) Staining

Cell slides were placed in a 12-well plate and the cells were cultured in the 12-well plate to attach to the slides. After cell treatment, a 12-well plate was taken out, and the medium was discarded. 4% paraformaldehyde was used to fix cells, which were then immersed in 0.5% Triton-PBS for 20 minutes. Afterwards, 10% of goat serum as used to block non-specific antigens, and the cells were incubated with primary antibodies (IL-1β, 1:500, rabbit, Abcam, Cambridge, MA, USA, and Akt, 1:500, rabbit, Abcam, Cambridge, MA, USA) at 4°C overnight. The next day, after washing the cells, the cells were incubated for 1 hour at room temperature with a fluorescent secondary antibody (Goat anti-rabbit-FITC, 1:500, Abcam, Cambridge, MA, USA). After washing the cells, the slides were removed and fixed on glass slides using a 4',6-diamidino-2-phenylindole (DAPI) containing closure. Finally, the staining results were observed using a fluorescence microscope.

Enzyme Linked Immunosorbent Assay (ELISA)

After cell culture and treatment, the supernatant was taken. The ELISA kit ([illegible], Hangzhou, China) was used to detect the number of related molecules in the supernatant according to the manufacturer's instructions.

Flow Cytometry

Annexin V-FITC kit (KeyGen, Nanjing, China) was used to detect apoptosis levels. After discarding the medium, the cells were washed with PBS and collected in each group. Then, 5 μL of Annexin V-FITC and 5 μL of PI were added to each group. The cells were incubated in the dark for 15 minutes. After adding 400 μL of loading buffer to each group, the apoptotic rate was measured by flow cytometry.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 software (IBM Corp., Armonk, NY, USA) was used to perform statistical analysis on the data of this study. The mean ± standard deviation was used to represent the measurement data. Comparison between multiple groups was done using One-way ANOVA test followed by post-hoc test (Least Significant Difference). All experiments were repeated 3 times. $p<0.05$ was considered statistically significant.

Results

TRIM59 Attenuated Myocardial Damage Caused by MIRI in Rats

TRIM59 lentivirus was transfected into rat myocardium to determine the effect of TRIM59 on myocardial injury in rats. The results showed that the expressions of MDA, CK, and LDH in the MIRI group were significantly higher than those in the control group, while the expression of SOD was decreased. After transfected with TRIM59 in rats, the levels of MDA and serum CK and LDH in rats were significantly decreased compared with the NC group, and the expression of SOD was increased (Figure 1A-1D). In addition, the overexpression of TRIM59 also effectively increased the Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase activity, significantly higher than the NC group (Figure 1E-1F). This indicated that the transfection of TRIM59 *in vivo* in rat myocardial tissue significantly reduced myocardial damage caused by MIRI.

Overexpression of TRIM59 Reduced Inflammation Levels in H9c2 Cells

To examine the effect of TRIM59 on inflammation of cardiomyocytes, TRIM59 lentivirus was used to increase the expression of TRIM59 in H9c2 cells. Western blot (Figure 2A) and qRT-PCR (Figure 2B-2D) results showed that the expressions of the inflammatory factors IL-1β, IL-6, and TNF-α in H9c2 cells in HR group and HR+Lenti-NC group were significantly increased, and the overexpression of TRIM59 could reduce the expressions of inflammatory factors. The results of the ELISA (Figure 2E, 2F) were similar to those of the Western blot. The results of IF (Figure 2G) also showed that the overexpression of TRIM59 significantly reduced IL-1β expression.

Overexpression of TRIM59 Reduced Apoptosis Levels in H9c2 Cells

The results of Western blot (Figure 3A) and qRT-PCR (Figure 3B-3E) showed that H9c2 cells in HR group and HR+Lenti-NC group expressed more caspase3, caspase9, and Bax and less Bcl-2, while the overexpression of TRIM59 attenuated the effect of HR on apoptosis of H9c2. The results of flow cytometry (Figure 3F) also showed that the percentage of apoptosis in the HR+Lenti-TRIM59

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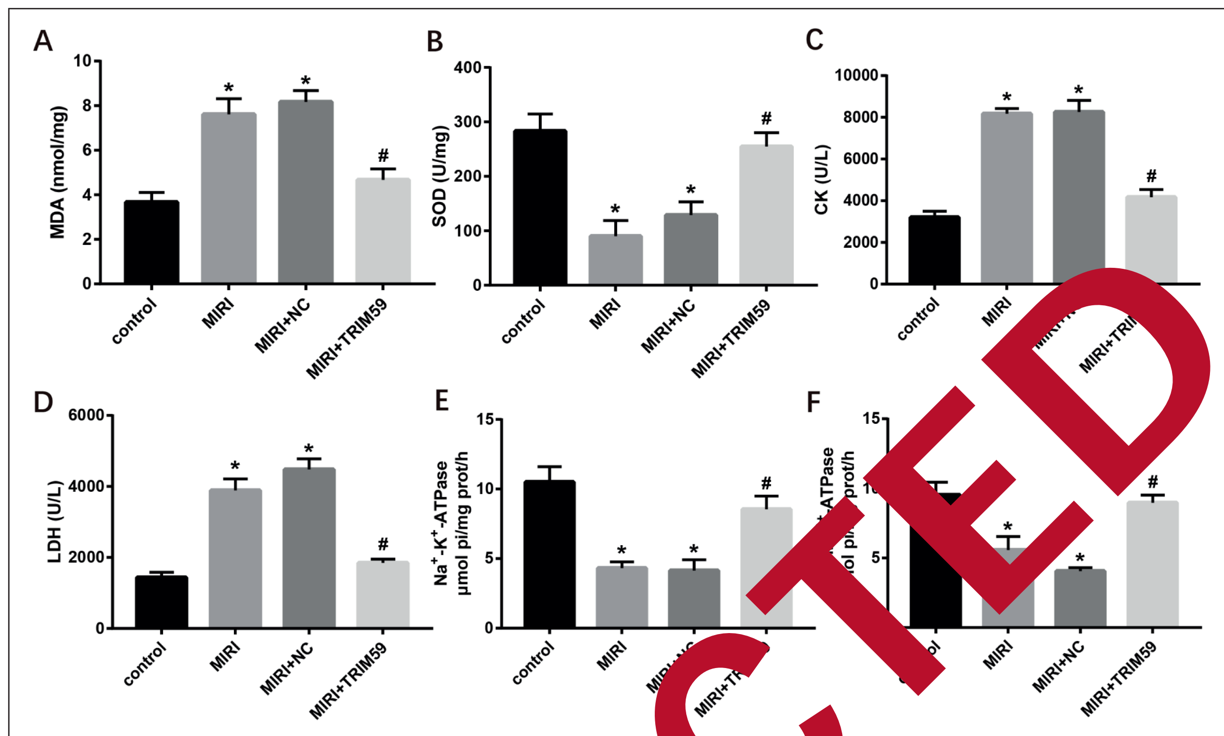

Figure 1. TRIM59 attenuates myocardial damage caused by MIRI in [illegible]. **A,** MDA activity assay. **B,** SOD activity assay. **C,** CK activity assay. **D,** LDH activity assay. **E,** Na^+-K^+-ATPase activity assay. **F,** Ca^{2+}-Mg^{2+}-ATPase activity. ("*" means there is a statistical difference with the control group and "#" means there is a statistical difference with the MIRI+NC group).

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group was significantly lower than that in the HR group and the HR+Lenti-NC group.

TRIM59 Increased the Activity of the PI3K/Akt Signaling Pathway

To examine the effect of TRIM59 on the PI3K/Akt signaling pathway, the expression levels and phosphorylation levels of PI3K/Akt signaling pathway-associated molecules were detected. Western blot (Figure 4A) results showed that the expressions of PI3K, Akt, and p-Akt in HR and HR+Lenti-NC group were significantly lower than those in the control group, and the overexpression of TRIM59 significantly increased their expression. The results of qRT-PCR (Figure 4B, 4C) were similar to those of Western blot. The results of IF (Figure 4D) also indicated that TRIM59 increased the expression level of Akt.

LY294002 Attenuated the Anti-Inflammatory and Anti-Apoptotic Effects of TRIM59

LY294002 was used to inhibit the activity of the PI3K/Akt signaling pathway. The results of Western blot (Figure 5A) and qRT-PCR (Figure 5B-5E) showed that the anti-inflammatory and anti-apoptotic effects of TRIM59 were significantly inhibited after inhibiting the activity of PI3K/Akt signaling pathway, showing the increase of IL-1β, TNF-α, and Bax and the decrease of Bcl-2.

Discussion

Multiple pathways can promote myocardial ischemia, causing hypoxia and hypoperfusion leading to atherosclerosis and acute myocardial infarction. Obstruction of arterial blood flow leads to hypoxia and causes dysfunction of electron transport chain in mitochondria. The reduction of ATP production in mitochondria induces anaerobic metabolism, dysfunction of sodium-potassium pump, and increased production of lactic acid leading to metabolic acidosis. After reperfusion, the recovered blood flows to the ischemic tissue, and reactive oxygen species (ROS) are increased. ROS causes oxidative stress to cause endothelial cell dys-

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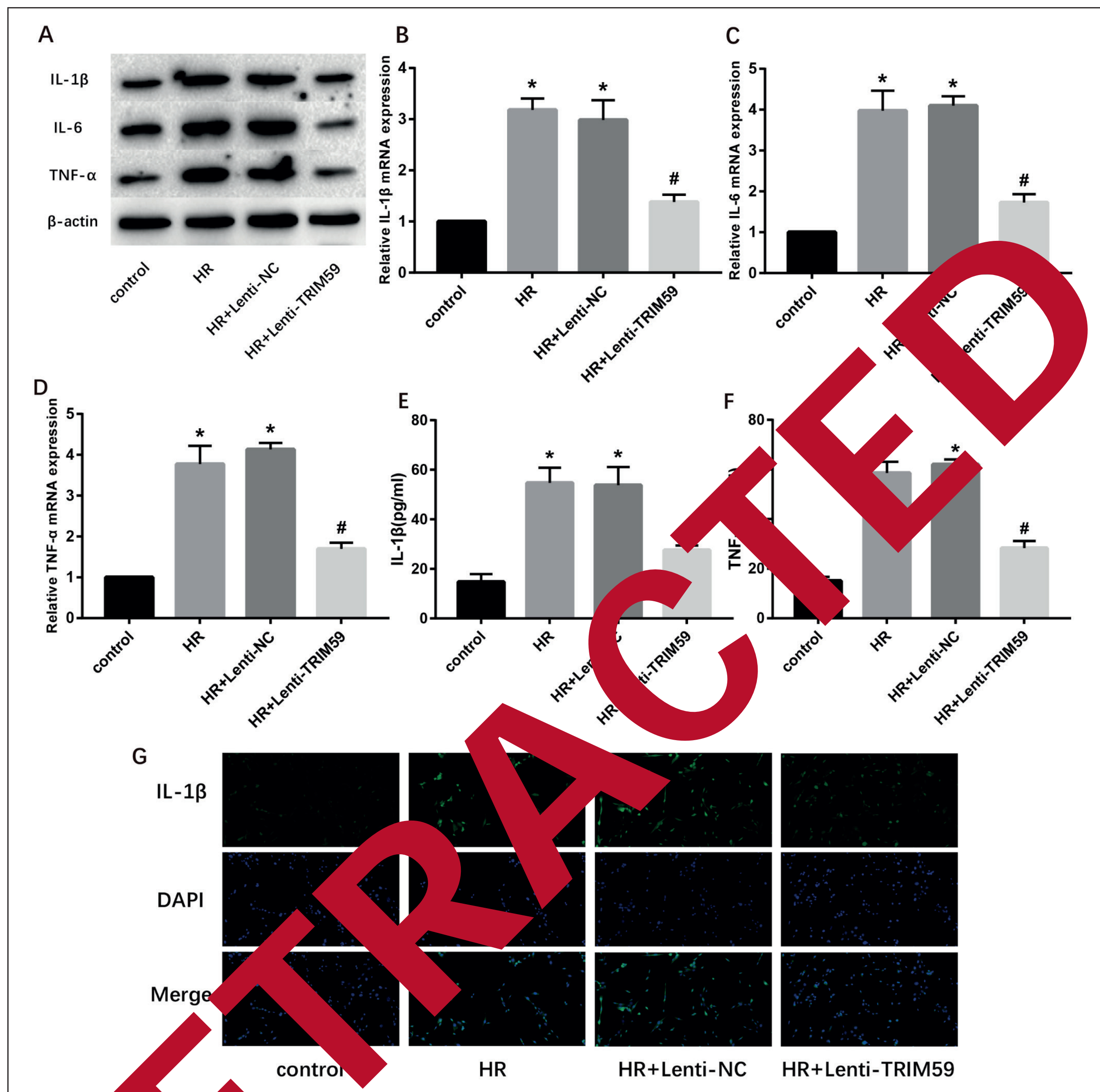

Figure 2. Overexpression of TRIM59 reduces inflammation levels in H9c2 cells. Expressions of IL-1β, IL-6 and TNF-α in four groups were detected by western blot (**A**) and qRT-PCR (**B-D**). ELISA detects the expressions of IL-1β (**E**) and TNF-α (**F**). IF (**G**) detects the expression of IL-1β (magnification: 100×). ("*" means there is a statistical difference with the control group and "#" means there is a statistical difference with the HR+Lenti-NC group).

functions, DNA damage, and inflammation[11]. Inflammation cascades and oxidative stress lead to endothelial cell structural damage. IRI is a dynamic change that may last for several days. The mechanism of IRI has not been fully elucidated, but it is currently considered that free radicals and ROS, intracellular calcium overload, leukocyte activation, and apoptosis are important pathogenesis of IRI[12]. Understanding the mechanism of MIRI may provide new strategies for future treatment and prevention.

In experiments *in vivo*, TRIM59 was overexpressed in myocardium by Entranster and a MIRI model was constructed in rats. It was found that the expression of MDA was decreased, and the SOD was increased in the rat myocardium of TRIM59 overexpression group, suggesting that TRIM59 reduces the level of oxidative stress

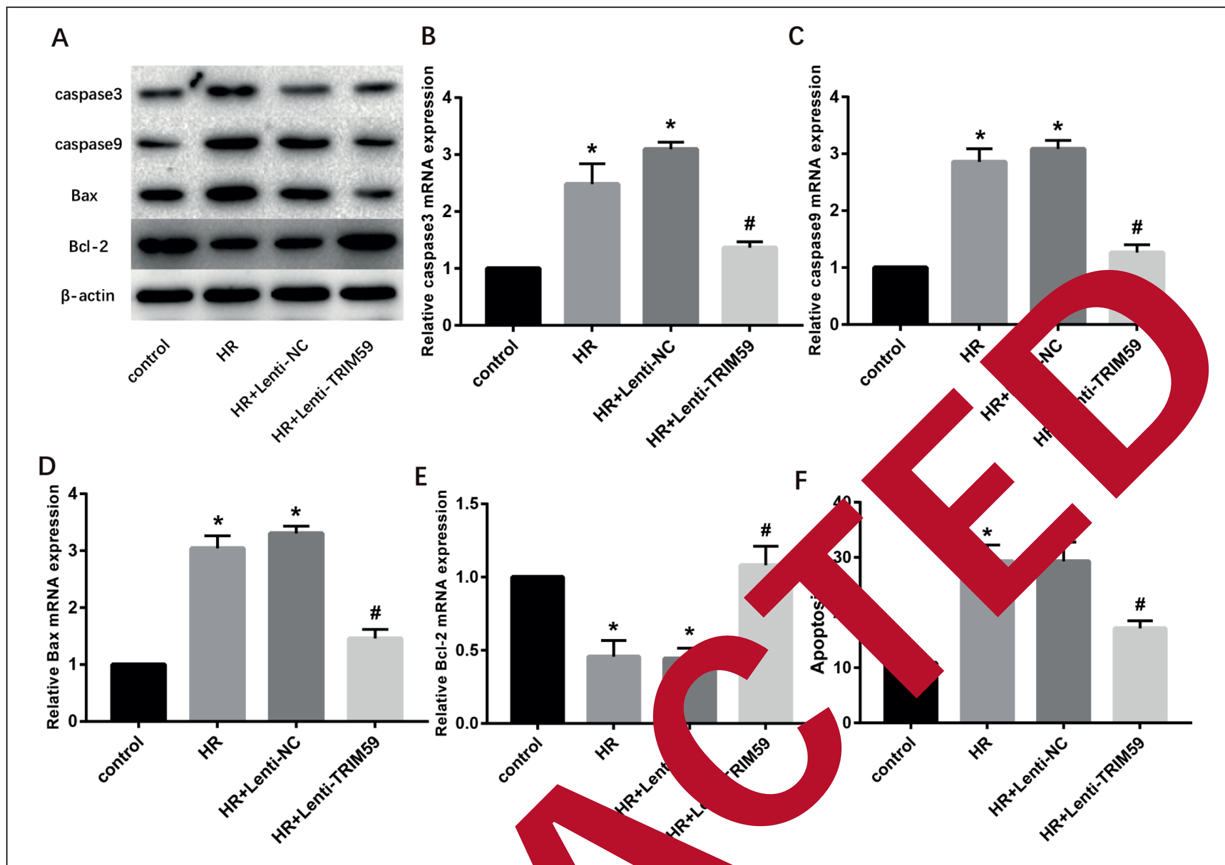

Figure 3. Overexpression of TRIM59 reduces apoptosis levels in H9c2 cells. Expressions of caspase3, caspase9, Bax and Bcl-2 in four groups are determined by Western blot (**A**) and RT-PCR (**B-E**). Cell apoptosis percentage was detected by flow cytometry (**F**). ("*" means there is a statistical difference with the control group and "#" means there is a statistical difference with the HR+Lenti-NC group).

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in cardiomyocytes and enhances the ability to scavenge oxygen free radicals. Besides, the reduction of myocardial injury markers CK and LDH in the TRIM59 overexpression group and Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase activity also confirmed the protective effect of TRIM59 on myocardium. The results of *in vivo* experiments demonstrated the protective effect of TRIM59 on the myocardium, so the mechanism of TRIM59 protecting cardiomyocytes was also explored by *in vitro* experiments. Lentiviral transfection was used to increase the expression of TRIM59 in H9c2 cells, and it was found that TRIM59 could inhibit inflammation and apoptosis in H9c2 cells. In addition, TRIM59 was found to increase the activity of the PI3K/Akt signaling pathway in H9c2 cells, whereas inhibition of the PI3K/Akt signaling pathway attenuated the protective effect of TRIM59 on H9c2 cells. The results of *in vivo* and *in vitro* experiments suggested that TRIM59 could reduce myocardial cell damage by inhibiting PI3K/Akt signaling pathway and reducing inflammation and apoptosis of cardiomyocytes.

A major pathological form of MIRI is apoptosis[13]. The Bcl-2 gene family regulates apoptosis by the mitochondrial pathway. The most representative control genes for apoptosis are Bcl-2 and Bax. Bcl-2 and Bax have high homology, Bcl-2 is an anti-apoptotic gene, and Bax is a pro-apoptotic gene. The role of Bax in promoting apoptosis is to promote the release of cytochrome C, activate caspase9, and form a dimer with Bcl-2 to inhibit Bcl-2 activity. The anti-apoptotic effect of Bcl-2 is to inhibit the activation of sarcosine aspartic protease by inhibiting the release of mitochondrial cytochrome C, and the ratio of Bax/Bcl-2 can further reflect the anti-apoptosis role of Bcl-2 gene family. Studies have detected that after MIRI, the body regulates the balance of Bax/Bcl-2 expression by regulating the content of Bcl-2 and Bax and reduces the degree of IRI[14]. A variety of

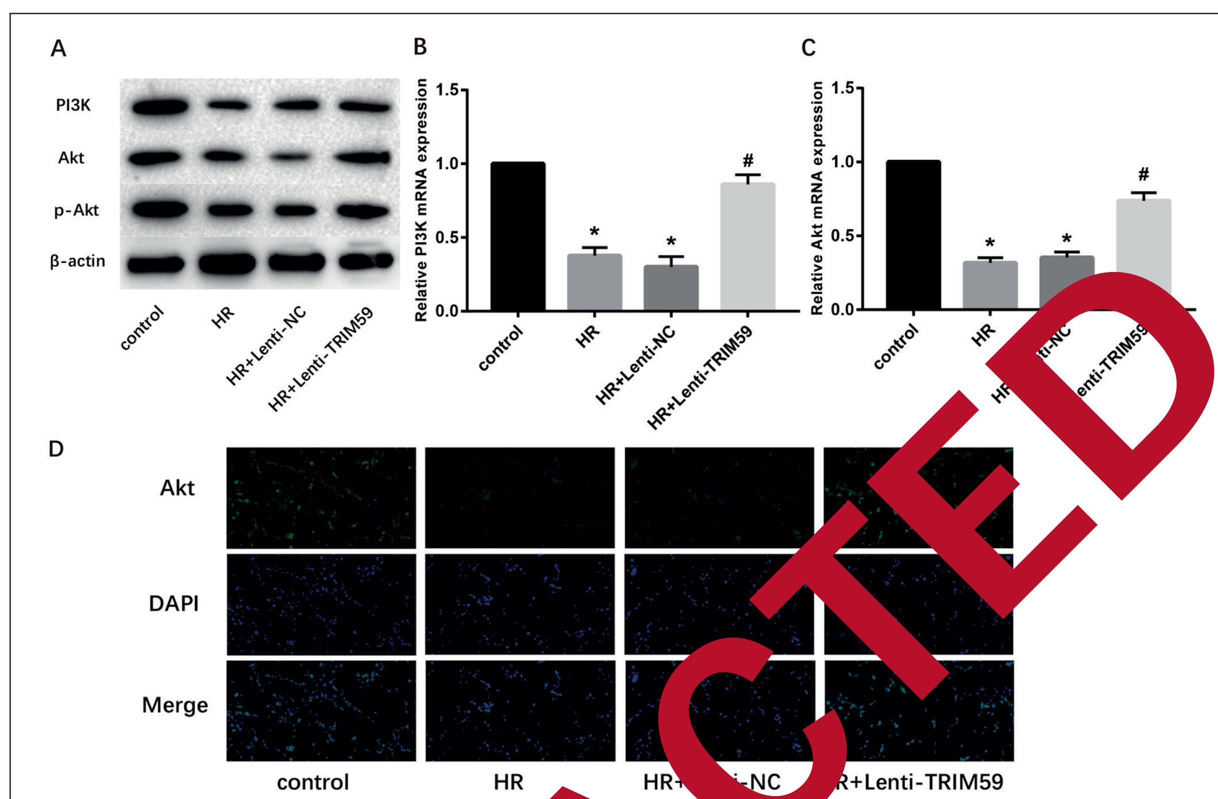

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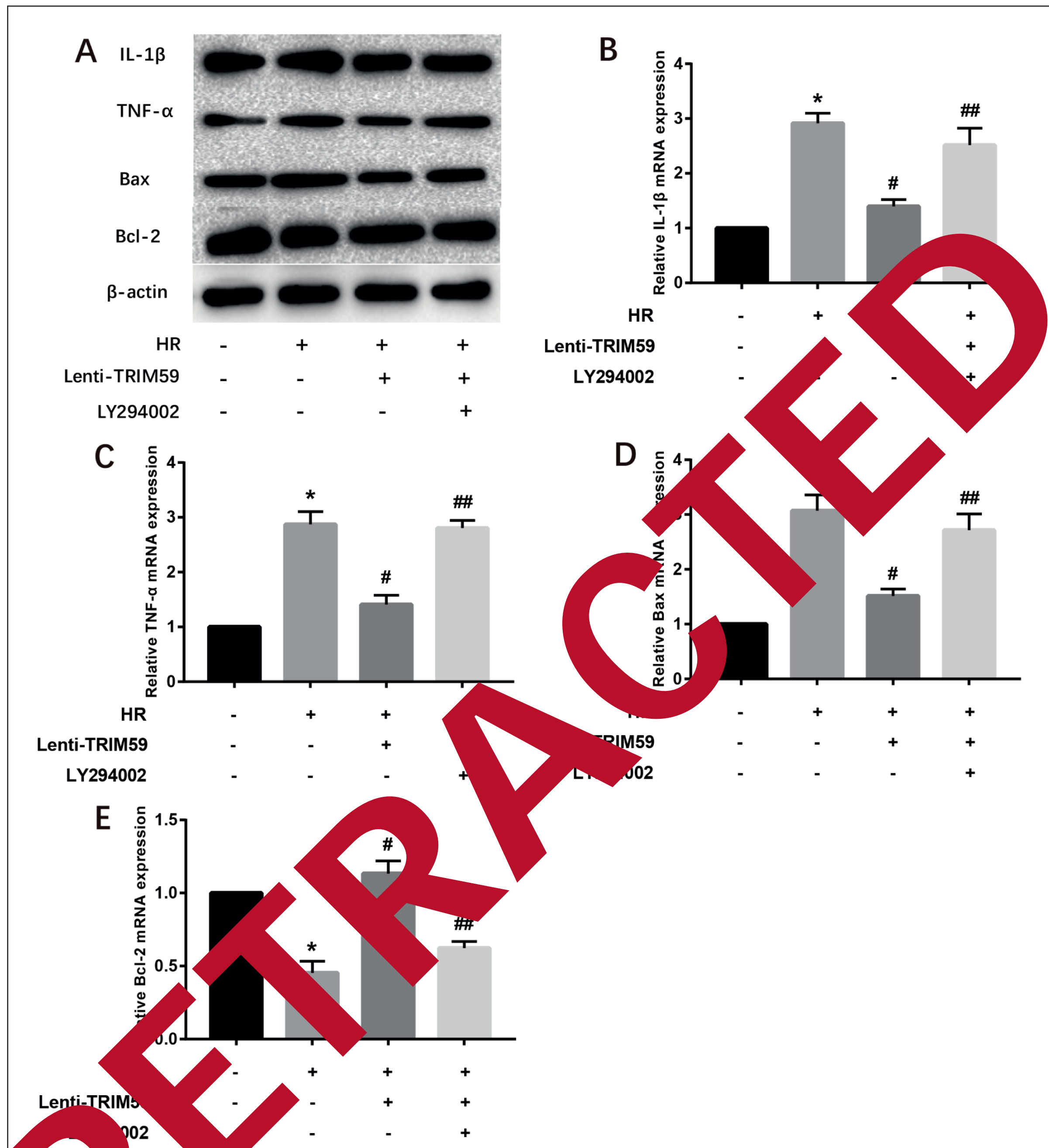
Figure 4. TRIM59 increases the activity of the PI3[illegible]kt [illegible] pathway. Expressions of PI3K, Akt and p-Akt in four groups are determined by Western blot (**A**) and qRT-P[illegible] (**B, C**)[illegible]ected the expression of Akt (magnification: 100×). (“*” means there is a statistical difference with the co[illegible]l [illegible]p an[illegible] “#” means there is a statistical difference with the HR+Lenti-NC group).

pretreatments have been found [illegible] redu[illegible] apopto-sis after IRI by increasi[illegible] Bcl-2 [illegible]ession [illegible]d decreasing Bax expre[illegible]n. The ca[illegible]e family also plays an impo[illegible]nt [illegible] in media[illegible]g apoptosis[15]. Caspase3 [illegible] one of t[illegible] important caspase family enzym[illegible] that induce ap[illegible]sis, and its role in apoptosi[illegible] irreplaceable[16]. Multiple members of the TRI[illegible] [illegible]il[illegible] have been found to be closely related to mit[illegible]ndrial [illegible]ction and affect cell ap[illegible]d o[illegible] bi[illegible]gical activities. Tan et [illegible] found [illegible]t TRI[illegible] promotes the formation of [illegible]chon[illegible]l complexes and maintains the nor-ma[illegible]ogica[illegible]nctions of mitochondria. Tomar et al[1[illegible]] [illegible]d that TRIM4 is a novel mitochondrial interacti[illegible]RING E3 ligase and could sensitizes the cells to hydrogen peroxide induced cell death. Xu et al[19] found that TRIM3, as a ubiquitin E3 ligase, could be recruited into the mitochondria of cells and affect the body’s immunity to the virus. In addition, TRIM10, TRIM17, and TRIM28 have been found to regulate the level of apoptosis in cells by affecting the mitochondria of cells[20,21]. Shen et al[22] explored the role of TRIM59 in the study of cholangiocarcinoma and found that silencing of the TRIM59 gene promotes apoptosis in cholangiocarcinoma cells *via* the mitochondrial pathway. The results of these studies suggested that TRIM59 may affect cell apoptosis through the mitochondrial pathway, which was consistent with our findings. Our study found that H9c2 cells overexpressing TRIM59 expressed more Bcl-2 and less Bax. In addition, the expression level of caspase 3/9 was also decreased after overexpression of TRIM59. This indicates that TRIM59 can effectively reduce apoptosis of H9c2 cells. The results of flow cytometry also showed this. The percentage of apoptotic cells in H9c2 cells overexpressing TRIM59 was significantly lower than that in hypoxia-reoxygenated cells. In addition, TRIM59 also effectively inhibited the expression of inflammatory factors IL-1β, IL-6, and TNF-α, and significantly reduced the inflammatory response of cardiomyocytes.

The PI3K/Akt signaling pathway is a conserved family of signal transduction enzymes[23]. The PI3K/Akt signaling pathway may be an en-



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dogenous negative feedback regulator and compensatory mechanism that regulates inflammatory and apoptotic events in cells undergoing noxious stimulation, apoptosis, survival, inflammatory responses, chemotactic, and proliferative activities[24]. PI3K is an important molecule in the signal transduction process of the growth factor superfamily. It is a dimer composed of catalytic subunit p110 and regulatory subunit p85. It can be catalyzed by various cytokines and physicochemical factors to cause the activation of subunit tyrosine kinase or G protein coupled receptor.

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Activated PI3K specifically catalyzes phosphorylation of phosphatidylinositol (PI)-3-hydroxyl to produce 3-phosphophosphatidylinositol (PIP), 4,5-diphosphophosphatidylinositol (PIP2), and 3,4,5-triphosphate phosphatidylinositol (PIP3). PIP2 and PIP3 act as second messengers to deliver extracellular signals to effector molecules downstream of PI3K for biological effects. There are many effector molecules downstream of PI3K. Protein kinase B (also known as Akt) is at the center of this pathway and is the most important target enzyme downstream of PI3K. It is mainly responsible for the transmission of biological information initiated by PI3K. Under the action of phosphatidylinositol-dependent kinase I, phosphorylation of the threonine and serine residues of Akt is fully activated, released from the membrane, enters the cytoplasm and nucleus, and further activates the intracellular enzyme cascade[25]. Studies have confirmed that the activation of Akt is a mechanism of partial myocardial protection. The protective signaling pathway of PI3K/Akt in reducing MIRI and its downstream targets is a new hot spot of clinical concern, providing a new idea for reducing MIRI[26]. In this research, overexpression of TRIM59 significantly increased expression and phosphorylation of PI3K/Akt signaling pathway-associated molecules. LY294002, an inhibitor of PI3K/Akt signaling pathway, significantly reduced the anti-inflammatory and anti-apoptotic effects of TRIM59 on cardiomyocytes. This suggests that the protective effect of TRIM59 on cardiomyocytes may be obtained by activating the PI3K/Akt signaling pathway.

To our knowledge, this is the first study to report the protective effects and mechanisms of TRIM59 on MIRI. It is believed that this will provide new targets and ideas for clinical treatment of MIRI.

Conclusions

The findings of this investigation showed that TRIM59 can reduce the inflammatory and apoptosis of cardiomyocytes in both *in vivo* and *in vitro* experiments, thereby protecting against myocardial damage caused by MIRI. Moreover, TRIM59 activates the myocardial protective signaling pathway (PI3K/Akt signaling pathway), and inhibition of the PI3K/Akt signaling pathway attenuates the protective effect of TRIM59 on the myocardium, suggesting that TRIM59 attenuates MIRI by activating the PI3K/Akt signaling pathway.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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RETRACTED