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Visfatin relieves myocardial ischemia-reperfusion injury through activation of PI3K/Akt/HSP70 signaling axis

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Abstract. – OBJECTIVE: Myocardial ischemia-reperfusion injury (MIRI) is the most common complication of ischemic cardiomyopathy, which severely affects the prognosis of patients. The purpose of this study was to investigate the protective effects of visfatin on the myocardium after ischemia-reperfusion (I/R) and its mechanism.

MATERIALS AND METHODS: Sprague Dawley rats were used to construct the MIRI model and visfatin was administrated intraperitoneally in rats to determine the protective effect of visfatin on myocardium after I/R. In addition, visfatin was used to treat rat myocardial cell line H9c2 cells and detect its effect on H9c2 cells. The effect of visfatin on the PI3K/Akt/HSP70 signaling axis in H9c2 cells was also detected to determine the mechanism of the myocardial protection of visfatin.

RESULTS: The damage of cardiomyocytes in MIRI rats pretreated with visfatin was significantly improved compared with untreated MIRI rats. Visfatin also reduced the level of inflammation and apoptosis of cardiomyocytes in MIRI rats, reduced myocardial injury markers, and improved cardiac function. *In vitro*, visfatin also reduced inflammatory and apoptotic factors in H9c2 cells. In addition, visfatin also promoted the activity of the PI3K/Akt signaling pathway and increased HSP70 expression in H9c2 cells. The inhibition of the PI3K/Akt signaling pathway was found to attenuate the promotion of HSP70 by visfatin. SiRNA-HSP70 also attenuated the protective effect of visfatin on H9c2 cells.

CONCLUSIONS: Visfatin reduces the inflammation and apoptosis levels of myocardial cells through the PI3K/Akt/HSP70 signaling axis, thereby reducing I/R-induced myocardial injury.

Key Words:
Visfatin, PI3K/Akt signaling pathway, Myocardial ischemia-reperfusion injury.

Introduction

Myocardial ischemia-reperfusion injury (MIRI) refers to the exacerbation of ischemic myocardial injury when the myocardium is restored to blood flow after ischemia. It mainly includes arrhythmia, myocardial contractile dysfunction, and irreversible myocardial reperfusion injury[1]. In recent years, with the widespread use of thrombolytic therapy, percutaneous coronary intervention, cardiovascular system surgery and extracorporeal circulation, MIRI has become more common[2]. The mechanisms of MIRI include excessive production of reactive oxygen species, overload of calcium ions, excessive apoptosis of cardiomyocytes, and inflammatory responses[3]. Therefore, reducing myocardial cell damage caused by ischemia-reperfusion (I/R) is of great significance for improving the prognosis of patients with myocardial I/R.

Heat shock proteins (HSPs), also called stress proteins, are a class of endogenous protective proteins produced by organisms under stress[4]. HSP70 levels are lower in normal cells, but can be significantly increased under stress[5]. At present, HSP70 has become the most concerned family in HSPs. Cardiomyocytes can also up-regulate the expression of HSP70 under stress[4]. Okubo et al[6] overexpressed HSP70 in rabbit hearts through gene transfection technology and found that HSP70 can reduce MIRI in rabbits. The P13K/Akt signal transduction pathway is one of the important signal transduction pathways and it has been found to mediate a variety of cytoprotective effects. Hao et al[7] found that activation of the PI3K/Akt signaling pathway can reduce MIRI in mice, and the inhibitor LY294002 can block its protective effect. In addition, Banerjee et al[8] found that the protective effect of P13K/

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Akt may also be related to the up-regulation of HSP expression. They placed Chinese voles lung fibroblasts V79 cells under high temperature for 4 weeks. Their results showed that Akt was activated after long-term high temperature stimulation, and the expressions of HSP70 and antioxidant enzyme MnSO also increased significantly. However, the levels of HSP70 and MnSO decreased significantly after Akt was inhibited.

Visfatin is fatty cytokine isolated from abdominal visceral tissues of humans and mice[9]. In addition, the expression of visfatin is not limited to visceral adipose tissue. It is expressed in various tissues such as bone marrow stromal cells, macrophages, spleen, liver, pancreas, brain, muscle tissue, and fetal membranes[10]. Visfatin is closely related to cardiovascular disease. Visfatin can activate phosphatidylinositol-3-OH, protein kinase B, mitogen-activated protein kinase 1 and kinase 2, extracellular regulatory kinase 1 and kinase 2, and other kinases, inhibit mitochondrial permeability transition channels, and reduce myocardium infarct area[11]. We found in this study that visfatin protects the myocardium after I/R and is related to the activation of the PI3K/Akt/HSP70 signaling axis. Therefore, we used rats and H9c2 cells to construct MIRI models and treated them with visfatin to study the effects of visfatin on MIRI.

Materials and Methods

Animal

A total of 40 8-week-old male Sprague Dawley rats were used in this study. Rats were housed in People's Hospital of Rizhao experimental Animal Center. Animal experiments were approved by People's Hospital of Rizhao Animal Experiment Ethics Committee. Rats were placed in a Specific Pathogen Free (SPF) barrier facility (22-24°C, 12 hours of alternating light) and fed on clean diet. Rats were randomly divided into a sham group, a MIRI group, a MIRI + visfatin (2 ng/g) group and a MIRI + visfatin (4 ng/g) group. Rats treated with visfatin were injected intraperitoneally daily for one week before modeling.

Rat MIRI Model

After weighing the rats, we anesthetize the rats with 2% sodium pentobarbital (40 mg/kg). The rats were fixed on the operating table and we used scissors to gently remove the fur from the rat's chest. A small animal ventilator (ALC-V8S, Beijing, China) is used to maintain rat breathing. We used sterile scissors to cut the skin of the left chest at 2-3 cm above the xiphoid and isolate the subcutaneous tissue. After exposing the rat heart, we gently tore the capsule with forceps and ligated the left anterior descending coronary artery with suture. After successful ligation of the blood vessel, the heart at the distal end of the ligature became white and the ECG showed ST elevation. We then loosen the suture 30 minutes later. After 24 hours, we used ultrasonic cardiogram to measure the cardiac function of the rats and sacrifice the rats.

Ultrasonic Cardiogram

Twenty-four hours after rat myocardial reperfusion, we used ultrasonic cardiogram to measure rat cardiac function. The rats were placed in an ultrasound machine and we measured the left ventricular end-systolic volume (ESV), left ventricular end-diastolic volume (EDV), left ventricular end-systolic diameter (LVIDs) and left ventricular end-diastolic diameter (LVIDd) of rats using the Philips system (1E33, Philips, Eindhoven, The Netherlands). Left ventricular ejection fraction (EF) = (EDV-ESV)/EDV. Left ventricular fraction shortening (FS) = (LVIDd-LVIDs)/LVIDd.

Histology and Hematoxylin-Eosin (HE) Staining

Rat myocardial tissue was immersed in 4% paraformaldehyde fixative for 24 hours. Then, we dehydrate myocardial tissue in gradient alcohol and put it in xylene and paraffin to make paraffin blocks. A microtome (RM2235, Leica, Wetzlar, Germany) is used to make paraffin sections of myocardial tissue. Paraffin sections were baked in a 37°C oven for 24 hours. We then dewaxed and hydrated the paraffin sections in xylene. After washing the sections with running water, we stained the paraffin sections in hematoxylin staining solution (Beyotime, Shanghai, China) for 1 minute. We then differentiated the myocardial tissue using hydrochloric acid alcohol for 3 seconds and immediately washed the sections in running water for 3 minutes. The sections were then stained in eosin staining solution (Beyotime, Shanghai, China) for 1 minute, then dehydrated and mounted.

Immunohistochemistry (IHC) Staining

After dewaxing and hydrating the paraffin sections, we put the paraffin sections in citrate buffer and heated to 95°C for 10 minutes. After

the buffer cooled naturally, we took the sections and added 3% H_2O_2 to the sections. After half an hour, we blocked the sections with 10% goat serum for 1 hour. We then incubated the sections with primary antibody diluent (caspase3, ab13847; caspase9, ab32539, Abcam, Cambridge, MA, USA) at 4°C overnight. After washing the sections with phosphate-buffered saline (PBS), we incubated the sections with secondary antibody diluent (ab150077, Abcam, Cambridge, MA, USA) for 1 hour at room temperature. Then, we used diaminobenzidine (DAB) developer to color the myocardial tissue on the section. Finally, we stained the nuclei with hematoxylin and mounted the sections.

Cell Culture and Transfection

Rat myocardial cell line H9c2 cells were used in this study. We cultured H9c2 cells using Dulbecco's Modified Eagle's Medium (DMEM) medium (Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA). Cells were cultured in a sterile incubator with 5% CO_2 and 37°C. Hypoxia/reoxygenation (H/R) method was used to induce I/R damage in H9c2 cells. After the growth density of H9c2 cells reaches 50-60%, we discard the culture medium in the culture dish and replace it with sterile phosphate-buffered saline (PBS). After the H9c2 cells were cultured in PBS for 4 hours, we replaced the PBS in the culture dish with DEME complete medium.

After the cell growth density reached 30-50%, we used Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA, USA) to transfect small interfering RNA (siRNA)-HSP70 into H9c2 cells according to the manufacturer's instructions. SiRNA-HSP70 Oligo sense: GAUCCUCUGAUUCCUAAAUTT; anti-sense: AUUUAGGAAUCAGAGGAUCTT.

Western Blot

Radioimmunoprecipitation assay (RIPA) lysate (Invitrogen, Carlsbad, CA, USA) was used to extract total proteins from H9c2 cells. After washing H9c2 cells with PBS, we added an appropriate amount of RIPA lysate to the culture dish and mixed on ice. We then collected the RIPA lysate and removed the sediment by centrifugation. 5 × loading buffer was added to the RIPA lysate and we heated the RIPA lysate to 100°C for 5 minutes through water bath. The extracted protein was stored in a -80°C refrigerator. We configured 10% gel and added the same amount of protein. After electrophoresis and transfer, the proteins are transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). We then blocked the non-specific antigen on the PVDF membrane with 10% skim milk for 1 hour. The primary antibody diluent (Akt, ab179463; p-Akt, ab8805, Abcam, Cambridge, MA, USA) was used to incubate the PVDF membrane at 4°C overnight. After washing the PVDF membrane with phosphate-buffered saline and tween-20 (PBST), we incubated the PVDF membrane with secondary antibody diluent (ab150077, Abcam, Cambridge, MA, USA) for 1 hour at room temperature. Finally, we used enhanced chemiluminescence (ECL) solution to detect protein bands.

RNA Isolation and quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA from H9c2 cells. After measuring the RNA concentration using a spectrophotometer (Mettler Toledo, Shanghai, China), we reversed the mRNA into complementary deoxyribose nucleic acid (cDNA) using SuperScript IV (Invitrogen, Carlsbad, CA, USA). The total reverse transcription system was 20 μL (4 μL 5 × SSIV Buffer + 1 μL 10 mM dNTP mix + 1 μL 100 mM DTT + 1 μL Ribonuclease Inhibitor + 1 μL 50 μM Oligo d $(T)_{20}$ primer + DEPC-treated water). The extracted RNA was stored in a -80°C refrigerator. SYBR Green mix (Invitrogen, Carlsbad, CA, USA) was used to amplify the cDNA. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression was used as control. The relative expression of mRNA is expressed as $2^{-\Delta\Delta Ct}$. The sequences of qRT-PCR were shown in Table I.

Enzyme Linked Immunosorbent Assay (ELISA)

Rat serum was collected to detect expression of inflammatory factors and myocardial injury markers. At the end of rat modeling, we collected 2 mL of blood from the rat's abdominal aorta. Then, we obtained the serum by centrifugation and stored the serum in a -80°C refrigerator. Enzyme-linked immunosorbent assay (ELISA) kits (Invitrogen, Carlsbad, CA, USA) were used to detect the concentrations of IL-1β, TNF-α, cTnI and CK-MB in rat serum. The same ELISA kit was also used to detect the levels of IL-1β and TNF-α in H9c2 cells.

Table I. qRT-PCR primers sequences.

Name	Sense/Anti-sense	Sequences (5'-3')
IL-1β	Sense	CGCGTCTATCGATTACATGC
	Anti-Sense	TCTAGCAGCTATATGCGCAT
TNF-α	Sense	GCTCATCGAGCTACGACGTGT
	Anti-Sense	ATATCGCAGCTACGTACGTACGT
Caspase 3	Sense	AATGGGCTCATGCGACGAGC
	Anti-Sense	ACGACGTTGCGATGCGTACG
Caspase 9	Sense	GTCTACGATCGTACTGACTGAC
	Anti-Sense	CTATCGATCGTACATGCATG
HSP70	Sense	AGCTACTAGCAGCGTACGCT
	Anti-Sense	AATCGTCGAGCTACGATCGCG
GAPDH	Anti-Sense	GGTTTCGACACCGTGTGGCATC
	Anti-Sense	AGCGCCCTTTGGGCTATCGAC

Immunocytofluorescence (IF) Staining

H9c2 cells were transfected into 24-well plates. After treating H9c2 cells, we took the 24-well plate and discarded the medium. We fixed the H9c2 cells with fixative and broke the cell membrane with 0.2% Triton-100. We, then, incubated H9c2 cells with 10% goat serum for 1 hour. Primary antibody (caspase3, ab13847; caspase9, ab32539; HSP70, ab16892, Abcam, Cambridge, MA, USA) diluent was used to incubate H9c2 cells at 4°C overnight. After washing the cells with PBS, we incubated the cells with fluorescent secondary antibody diluent (ab150081, Abcam, Cambridge, MA, USA) for 1 hour at room temperature. Finally, we stained the nucleus with 4',6-diamidino-2-phenylindole (DAPI) and observed the staining results with a fluorescence microscope.

Cell Counting Kit-8 (CCK-8) Assay

H9c2 cells were transfected into 96-well plates. After processing H9c2 cells, we added 10 μL of CCK-8 reagent (Dojindo Molecular Technologies, Kumamoto, Japan) to each well. We, then, incubated the 96-well plate in a 37°C incubator for 2 hours. Finally, we use a microplate reader to detect the absorbance of each well at 450 nm.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 21.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 7.0 statistical software (La Jolla, CA, USA) were used to analyze the results of this study. The measurement data in this study were represented as mean ± SD (standard deviation). One-way analysis of variance followed by Post-Hoc Test (Least Significant Difference) was used for comparison among groups. All experiments were repeated more than three times. $p<0.05$ was considered statistically significant.

Results

Visfatin Relieves MIRI In Rats

To determine the effect of visfatin on MIRI in rats, we constructed MIRI model and pretreated rats with visfatin. The results of HE staining (Figure 1A) showed that the cardiomyocytes of the MIRI group were disorderly arranged. The structure of the cardiomyocytes of the rats in the visfatin (2 ng/g) and visfatin (4 ng/g) group was better than that of MIRI group. IHC staining (Figure 1A) detected the apoptotic protein caspase 3/9 in myocardial tissue. The expression of caspase 3/9 in the myocardial tissue of the MIRI group was higher than that of the sham group, while the expression of caspase 3/9 in the myocardial tissue of the rats treated with visfatin decreased. In addition, we used ELISA to detect inflammatory factors in the rat serum and found that the expression of IL-1β and TNF-α in the serum of the MIRI group was higher than that of the sham group, while visfatin could reduce their expression level (Figure 1B). In addition, myocardial injury markers were also detected to determine the differences in myocardial injury of rats. The expressions of cTnI and CK-MB in the MIRI group were higher than those in the sham group, and the application of visfatin could reduce their expression (Figure 1C, 1D). In addition, visfatin can also promote the recovery of cardiac function in rats, which was manifested by an increase in EF and FS (Figure 1E).

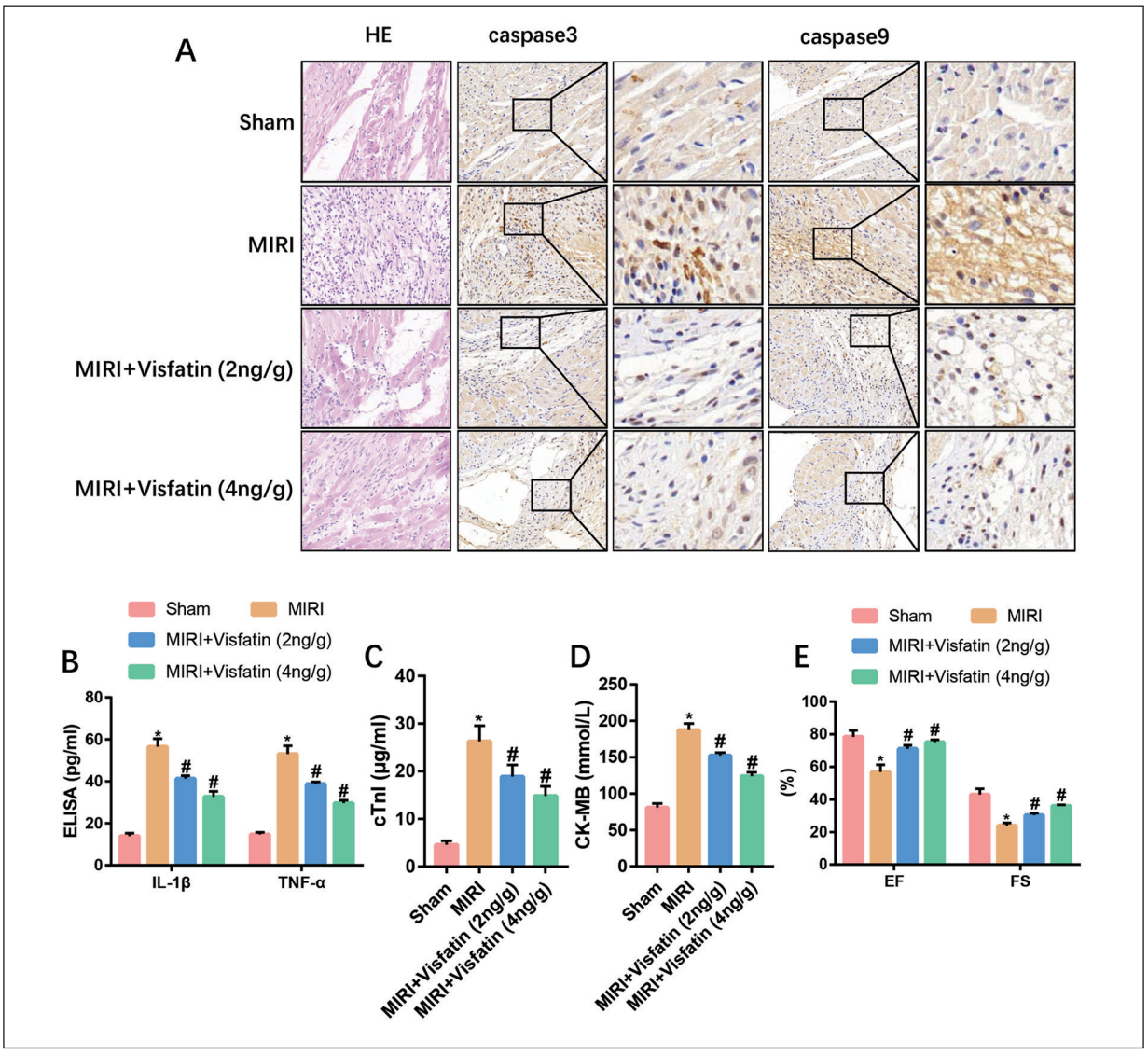

Figure 1. Visfatin relieves MIRI in rats. **A,** HE staining and IHC staining in rat myocardial tissue (magnification: 200×). **B,** ELISA detected the expression of IL-1β and TNFα in rat serum. **C, D,** Expression of cTnI and CK-MB in rat serum. **E,** Ultrasonic electrocardiogram results of rats. ("*" means $p<0.05$ *vs.* the Sham group and "#" means $p<0.05$ *vs.* the MIRI group).

Visfatin Reduces H/R-Induced H9c2 Cell Damage

To further determine the protective effects of visfatin on cardiomyocytes, we used visfatin to treat H9c2 cells for *in vitro* studies. CCK-8 assay examined the viability of H9c2 cells and found that 400 ng/mL of visfatin had the best effect on H9c2 cells (Figure 2A). We constructed I/R injury at the cellular level through H/R. ELISA detected the IL-1β and TNF-α in H9c2 cells. Visfatin was found to reduce inflammation level in H9c2 cells (Figure 2B). IF staining detected the expression of the apoptotic protein caspase 3/9 and found that the visfatin reduced the expression of caspase 3/9 in H9c2 cells (Figure 2C, 2D). qRT-PCR results were similar to IF staining and ELISA (Figure 2E).

Visfatin activates PI3K/Akt/HSP70 Signal Axis in H9c2 Cells

To determine the mechanism by which visfatin protects myocardial cells, we focused on the PI3K/Akt signaling pathway. Western blot (Figure 3A) detected the expression of AKT and p-AKT and it was found that the visfatin can increase the ratio of p-AKT and AKT (Figure 3B). qRT-

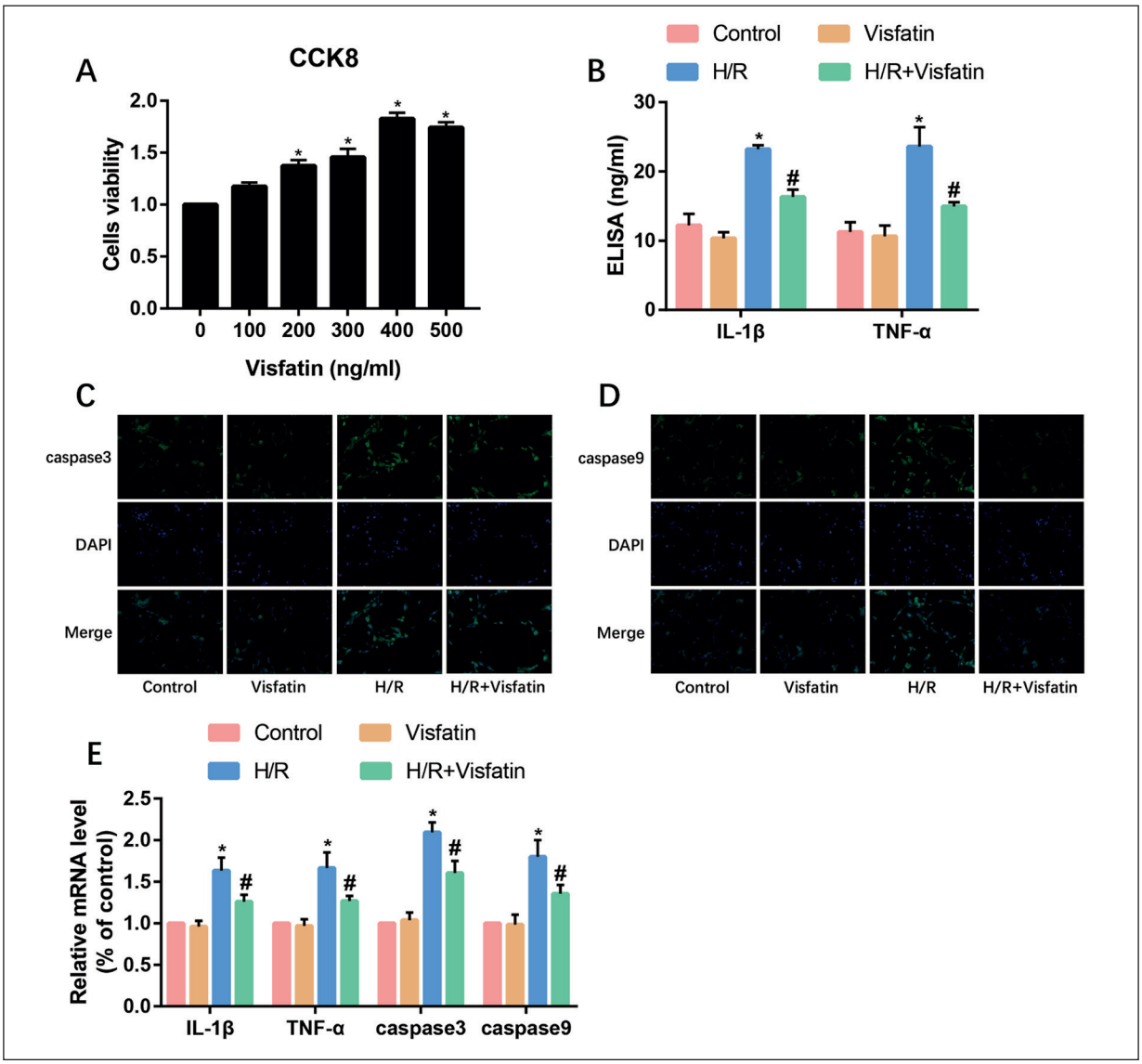

Figure 2. Visfatin reduces H/R-induced H9c2 cell damage. **A,** CCK-8 results in H9c2 cells. **B,** ELISA detected the expression of IL-1β and TNFα in H9c2 cells. **C, D,** IF staining results of caspase3 and caspase9 in H9c2 cells (magnification: 200×). **E,** Expression of IL-1β, TNFα, caspase3 and caspase9 mRNA in H9c2 cells. ("*" means $p<0.05$ *vs.* the Control group and "#" means $p<0.05$ *vs.* the H/R group).

PCR also found that the expression of HSP70 in H9c2 cells in the H/R group increased compared with the control group, and the expression of HSP70 was further increased after the treatment with visfatin in H9c2, indicating that visfatin can increase the expression of HSP70 (Figure 3C). LY294002 is an inhibitor of the PI3K/Akt signaling pathway. After LY294002 (1 μM) was used to treat H9c2 cells, IF staining (Figure 3D) and qRT-PCR (Figure 3E) detected HSP70 and found that the expression of HSP70 was reduced after stimulation of LY294002, indicating that the expression of HSP70 is regulated by the PI3K/Akt signaling pathway. qRT-PCR also detected IL-β, TNF-α, caspase3, and caspase 9 and found that LY294002 attenuated the protective effect of visfatin on H9c2 cells (Figure 3E).

SiRNA-HSP70 Attenuates the Protective Effect of Visfatin on H9c2 Cells

To demonstrate that the PI3K/Akt/HSP70 signaling axis mediates the myocardial protective effects of visfatin, we used siRNA-HSP70 to suppress HSP70 expression. qRT-PCR was

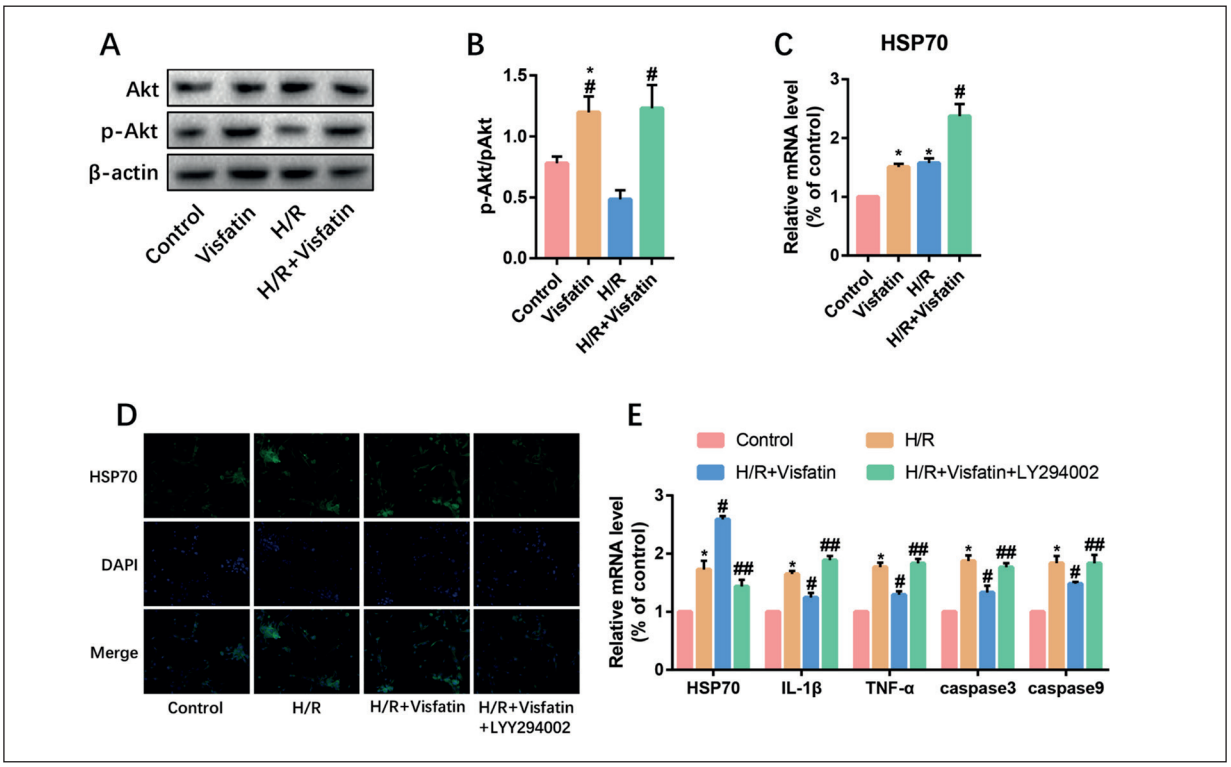

Figure 3. Visfatin activates PI3K/Akt/HSP70 signal axis in H9c2 cells. **A,** Western blot results of Akt and p-Akt in H9c2 cells. **B,** Ratio of expression of p-Akt and Akt. **C,** Expression of HSP70 mRNA in H9c2 cells. **D,** IF staining results of HSP70 in H9c2 cells (magnification: 200×). **E,** Expression of HSP70, IL-1β, TNFα, caspase3 and caspase9 mRNA in H9c2 cells. ("*" means $p<0.05$ *vs.* the Control group, "#" means $p<0.05$ *vs.* the H/R group and "##" means $p<0.05$ *vs.* the H/R+Visfatin group).

used to detect the transfection efficiency of siRNA-HSP70 (Figure 4A). The CCK-8 assay examined the viability of H9c2 cells and found that siRNA-HSP70 reduced cell viability (Figure 4B). The expressions of inflammatory factors were detected by ELISA. SiRNA-HSP70 was found to attenuate the anti-inflammatory effects of visfatin on H9c2 cells (Figure 4C). IF staining detected the expression of the apoptotic protein caspase 3/9 and found that siRNA-HSP70 also attenuated the anti-apoptotic effect of visfatin on H9c2 cells (Figure 4D, 4E). qRT-PCR results were similar to ELISA and IF staining (Figure 4F).

Discussion

Currently, MIRI is still the leading cause of mortality and disability worldwide. Timely reperfusion therapy is the most effective method in clinic, but it may cause further damage to cardiomyocytes after blood supply is restored[12]. Visfatin has been linked to a variety of cardiovascular diseases. Chiu et al[13] showed that at the cellular level, during angiotensin II-induced cardiomyocyte hypertrophy, cardiomyocytes entered the decompensated stage and the expression of endolipidins was further elevated. This suggested that endolipidins can be used as predictors to predict the development of cardiac hypertrophy. Chyl-Surdacka et al[14] showed that plasma levels of visfatin were positively correlated with high-density lipoprotein levels and negatively correlated with low-density lipoprotein levels. Abnormal lipid metabolism is an important risk factor for coronary heart disease. Therefore, visfatin is highly correlated with the course of coronary heart disease and MIRI. We found that visfatin has significant protective effect on myocardium after I/R. After intraperitoneal injection of visfatin into rats, the myocardial injury of rats was obviously improved and the level of inflammation and apoptosis of cardiomyocytes decreased. In addition, the PI3K/Akt signaling pathway was significantly activated and the expression of HSP70 was also significantly increased in visfatin-stimulated H9c2 cells. HSP70 is an endogenous protective substance that plays an important role in resisting MIRI. However, LY294002, an inhibitor of the PI3K/

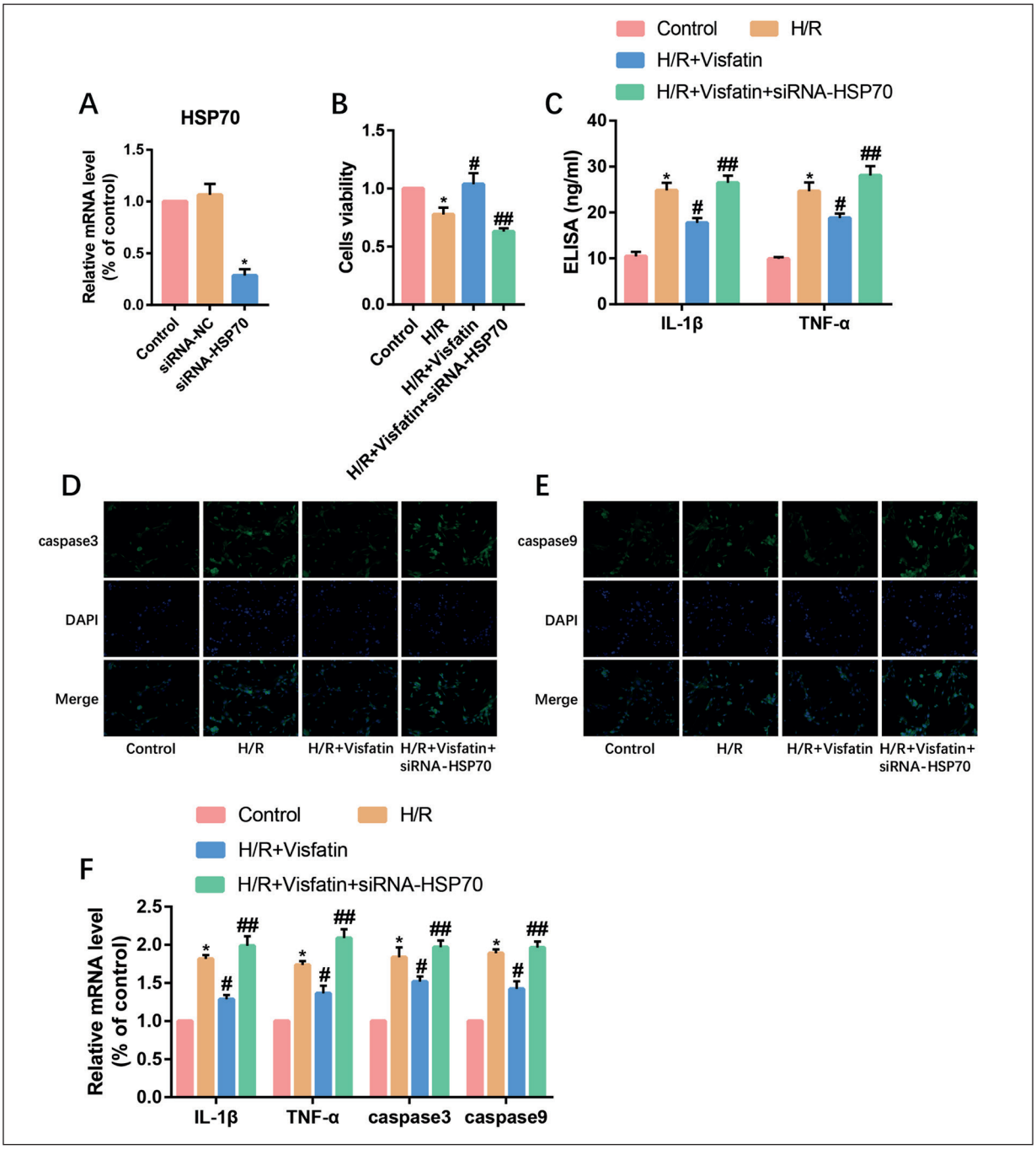

Figure 4. SiRNA-HSP70 attenuates the protective effect of visfatin on H9c2 cells. **A,** Expression of HSP70 mRNA in H9c2 cells. **B,** CCK-8 results in H9c2 cells; **C,** ELISA detected the expression of IL-1β and TNFα in H9c2 cells. **D, E,** IF staining results of caspase3 and caspase9 in H9c2 cells (magnification: 200×). **F,** Expression of IL-1β, TNFα, caspase3 and caspase9 mRNA in H9c2 cells. ("*" means $p<0.05$ *vs.* the Control group, "#" means $p<0.05$ *vs.* the H/R group and "##" means $p<0.05$ *vs.* the H/R+Visfatin group).

Akt signaling pathway, reduced the promotion of HSP70 by visfatin, indicating that the positive regulation of visfatin on HSP70 is related to the activation of the PI3K/Akt signaling pathway. After the use of siRNA-HSP70 to reduce the expression of HSP70 in H9c2 cells, the protective effect of visfatin on H9c2 cells was significantly reduced. It was reconfirmed that the anti-MIRI effect of visfatin was related to the activation of PI3K/Akt/HSP70 signaling axis.

The main manifestations of MIRI in the early stage are excessive inflammatory response and myocardial cell apoptosis[15]. The degree of inflammatory response and apoptosis determines the prognosis of MIRI. Therefore, effectively inhibiting the levels of inflammation and apoptosis of cardiomyocytes after I/R is the key to treating MIRI[16]. During the inflammatory response, polymorphonuclear (PMN) infiltration plays an important role. Coronary microvascular endothelial cells and vascular endothelial cells were damaged during I/R, and endothelial function and structure were also damaged, resulting in the significant weakening of the anti-neutrophil adhesion of endothelial tissue. The accumulation and adhesion of PMN block the blood capillary, and increase the release of inflammatory factors such as IL-1β, IL-6 and TNF-α[17]. Inflammatory factors can activate more inflammatory cells to release more inflammatory factors through the NF-κB signaling pathway[18]. Apoptosis is another major pathological change of I/R injury. BCl-2 gene family regulates apoptosis through the mitochondrial pathway, and the most representative genes controlling 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. Bax promotes apoptosis by promoting the release of cytochrome C, activating caspase9, and forming a dimer with Bcl-2 to inhibit Bcl-2 activity, while Bcl-2's anti-apoptotic effect is through inhibiting the release of mitochondrial cytochrome C and inhibiting the activation of the caspase family[19]. We have found *in vivo* and *in vitro* that visfatin can effectively inhibit the level of inflammation and apoptosis of cardiomyocytes. The expression of caspase3 and caspase9 in cardiomyocytes of MIRI rats increased and inflammatory factors in serum increased significantly. After intraperitoneal injection of visfatin into rats, the apoptosis level of rat cardiomyocytes and serum inflammatory factors were significantly reduced. These results indicate that visfatin has a significant therapeutic effect on I/R-induced myocardial injury.

PI3K/Akt signaling pathway is necessary for the regulation of cardiomyocyte survival and apoptosis in ischemic cardiomyopathy[20]. PI3K/Akt signaling pathway is a classic pro-proliferation and anti-apoptotic signaling pathway. Activation of this pathway has a variety of positive effects on cell survival[21]. Wu et al[22] reported that higenamine can activate β2-adrenergic receptors to reduce MIRI. This protective effect depends on activation of the PI3K/Akt signaling pathway through phosphorylation of β2-adrenergic. Arslan et al[23] also studied the protective effect of mesenchymal stem cell-derived exosomes on MIRI and found that exosomes exerted cardioprotective effect by activating the PI3K/Akt signaling pathway. To further study the protective mechanism of visfatin against MIRI, we focused on the PI3K/Akt signaling pathway. We found that H/R inhibited the activation of the PI3K/Akt signaling pathway, and p-Akt/Akt increased significantly after visfatin stimulated H9c2 cells, indicating that visfatin can significantly activate PI3K/Akt. In addition, after LY294002 inhibited the PI3K/Akt signaling pathway, the protective effect of visfatin on H9c2 cells decreased. These results indicated that visfatin protected the myocardium from I/R injury by activating the PI3K/Akt signaling pathway.

HSPs are family of stress-responsive proteins that are found in all species exposed to stress conditions. HSPs inhibit the phosphorylation of some proteins and improve the stability and function of these proteins[24]. HSP70 is an important stress response protein in the HSPs family and is called protective molecular chaperone. HSP70 can inhibit MIRI by regulating the nuclear translocation of other proteins. Zhou et al[25] found that the improvement of myocardial injury by teprenone was related to the up-regulation of HSP70 expression. Therefore, in this study, we observed that lipoproteins play a role in HSP70 expression in animal models of cardiomyocyte injury. We found that H/R increased the expression of HSP70 in H9c2 cells, and the stimulation of visfatin could further increase the level of HSP70 expression. But after LY294002 was used to inhibit PI3K/Akt signaling pathway, the level of HSP70 decreased and the damage of H9c2 cells also increased. These results indicate that visfatin promoted HSP70 expression by activating the PI3K/Akt signaling pathway, and thus relieved MIRI.

In previous studies, visfatin has been found to regulate glucose and lipid metabolism by promoting fat synthesis, accumulation and differentiation, and has been linked to obesity and type 2 diabetes[10]. However, this is the first study to investigate the effect of visfatin on MIRI. To sum up, visfatin plays an important role in myocardial protection through the PI3K/Akt/HSP70 signaling axis. We hope that this study will make an important contribution to the clinical treatment of MIRI.

Conclusions

Visfatin was first found to promote HSP70 expression through the PI3K/AKT/HSP70 signaling axis. HSP70 is an important cytokine that protects the heart muscle. By promoting the expression of HSP70, visfatin can reduce the level of inflammation and apoptosis of cardiomyocytes after I/R, thereby protecting the myocardium from MIRI.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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