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MicroRNA-668-3p Protects Against Oxygen-Glucose Deprivation in a Rat H9c2 Cardiomyocyte Model of Ischemia-Reperfusion Injury by Targeting the Stromal Cell-Derived Factor-1 (SDF-1)/CXCR4 Signaling Pathway

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Ischemia-reperfusion injury (IRI) results from the restoration of blood supply to ischemic organs, including the heart. Expression of microRNA-668-3p (miR-668-3p) is known to protect the kidney from IRI. This study aimed to investigate the role of miR-668-3p in oxygen-glucose deprivation (OGD) in a rat H9c2 cardiomyocyte model of IRI.

Material/Methods: Rat H9c2 cardiomyocytes were cultured in glucose-free Dulbecco's modified Eagle's medium (DMEM) under anaerobic conditions, followed by oxygenation, to create the OGD model of IRI. The luciferase reporter assay evaluated the interaction between stromal cell-derived factor-1 (SDF-1), or CXC motif chemokine 12 (CXCL12), and miR-668-3p. Protein and mRNA levels of SDF-1, CXCR4, Bcl2, Bax, cleaved caspase-3, endothelial nitric oxide synthase (eNOS), and phosphorylated endothelial nitric oxide synthase (p-eNOS) were analyzed by Western blot and quantitative reverse transcription-polymerase chain reaction (RT-qPCR), and apoptosis were assessed by flow cytometry. Enzyme-linked immunosorbent assay (ELISA) measured reactive oxygen species (ROS), including malondialdehyde (MDA), nitric oxide (NO), p-eNOS, and the inflammatory cytokines, tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, and monocyte chemoattractant protein-1 (MCP-1) in H9c2 cell supernatants.

Results: In the OGD rat H9c2 cardiomyocyte model of IRI, miR-668-3p levels were reduced. Overexpression of miR-668-3p inhibited SDF-1, CXCR4, the expression of inflammatory cytokines, markers of oxidative stress, and p-eNOS. The overexpression of SDF-1 reversed these findings. Overexpression of SDF-1 promoted cell apoptosis, which was reduced by miR-668-3p.

Conclusions: In the OGD rat H9c2 cardiomyocyte model of IRI, miR-668-3p suppressed mediators of inflammation and oxidative stress and enhanced cell viability through the SDF-1/CXCR4 signaling pathway.

MeSH Keywords: Chemokine CXCL12 • MicroRNAs • Myocardial Ischemia • Myocytes, Cardiac • Receptors, CXCR4

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Background

Clinically, myocardial ischemia and infarction are associated with high morbidity and mortality worldwide [1]. The main cause of myocardial ischemia is coronary artery disease (CAD) due to atherosclerosis. However, microvascular disease associated with conditions such as diabetes mellitus can result in myocardial fibrosis and lead to heart failure [2]. Patients with CAD may undergo cardiac ischemic events that result in sudden death [3–5]. Thrombolytic therapy and percutaneous coronary intervention (PCI) are current treatment approaches for patients with myocardial ischemia and infarction, which have significantly reduced the mortality rate [6]. However, there continue to be significant population risk factors for CAD, and patient mortality rates from CAD remain high [7].

Following myocardial ischemia and infarction, if the patient survives and coronary artery perfusion is restored, further myocardium injury can be induced by myocardial ischemia-reperfusion injury (IRI). Myocardial IRI can increase the size of myocardial infarction and accelerate cardiac death, particularly in the older patient population [8]. Previous studies have shown that myocardial IRI associated with myocardial systolic and diastolic dysfunction was reduced by inhibiting the inflammatory response and oxidative stress [9]. Because of the prevalence of CAD and ischemic heart disease (IHD), there remains a need to investigate novel methods for the prevention, diagnosis, and treatment of myocardial ischemia and IRI.

Reduced bioavailability of nitric oxide (NO) is associated with endothelial dysfunction and results in oxidative stress and the generation of reactive oxygen species (ROS) and inflammation in pathological conditions [10]. NO is a key modulator of endothelial dysfunction in cardiovascular disease, including CAD [11]. Inflammation has important roles in the onset and progression of cardiovascular disease. The increased secretion levels of inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), IL-10 and IL-12, are correlated with the development of CAD [12]. IL-6 is a mediator of the inflammatory and immune response, and the association between the IL-6 gene polymorphism and cardiovascular disease has been demonstrated [13]. Previous studies have shown that increased production of ROS is a risk factor for the progression of cardiovascular disease, including myocardial infarction and heart failure [14]. Stocker et al. investigated the role of oxidative processes in atherosclerosis and cardiovascular events [15]. They demonstrated that inflammation was a primary process in atherogenesis, and oxidative stress was a secondary event [15]. There remains the possibility that anti-inflammatory agents and antioxidants are potential therapeutic approaches to reduce morbidity and mortality from CAD.

MicroRNAs (miRNAs) are small, endogenous, noncoding RNAs composed of 21–25 nucleotides, which regulate gene expression by targeting mRNA at the 3'-untranslated region (3'UTR) that leads to translational repression or target gene mRNA degradation. Previous studies have shown that microRNA-668-3p (miR-668-3p) has a protective role in acute kidney injury [16]. Also, Wei et al. showed that inhibition of miR-668-3p promoted renal tubular cell apoptosis and induced ischemic acute kidney injury in an animal model [17]. However, the role of miR-668-3p in myocardial ischemia remains unclear.

Recent studies have shown that stromal cell-derived factor 1 (SDF-1), also known as CXC motif chemokine 12 (CXCL12), and also CXCR4 signaling pathways may play important roles in hematopoiesis and immunity [18]. In women, estrogen exerts a cardiovascular protective effect on endothelial progenitor cells through estrogen receptor-α (ER-α) and the SDF-1/CXCR4 signaling pathway [19]. CXCL12 and CXCR4 gene polymorphisms are correlated with an increased genetic risk of CAD and the severity of coronary stenosis [19].

We found that SDF-1 was a potential target gene of miR-668-3p by searching the TargetScan database (*www.targetscan.org/*). Therefore, this study aimed to investigate the role of miR-668-3p in oxygen-glucose deprivation (OGD) in a rat H9c2 cardiomyocyte model of IRI.

Material and Methods

Cell culture and the oxygen-glucose deprivation (OGD) rat H9c2 cardiomyocyte model of ischemia-reperfusion injury (IRI)

H9c2 rat cardiomyocytes were purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA). H9c2 cells were cultured in a 5% CO_2 and 95% air humidified atmosphere at 37˚C in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Gibco, Thermo Fisher Scientific, Waltham, MA, USA), 100 µg/mL streptomycin, and 100 units/mL penicillin. The OGD rat H9c2 cardiomyocyte model of IRI was developed, as previously described [21]. Briefly, the cells were maintained in glucose-free DMEM and placed in an anaerobic chamber with 95% N_2 and 5% CO_2 at 37˚C. After 2 h, glucose was added, and the cells were cultured under normal growth conditions in 95% air and 5% CO_2 for a further 12 h.

Transfection of miRNAs

Bioinformatics analysis identified SDF-1 as a potential target gene of microRNA-668-3p (miR-668-3p) by searching the TargetScan database (*www.targetscan.org/*). To modulate miRNA

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expression levels, the H9c2 cells were transfected with either miR-668-3p mimic (4 μg) or miR-NC (negative control, 100 nM) in six-well plates with the transfection reagent Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. The miR-668-3p mimic and miR-NC were purchased from Biomics Biotechnologies Co., Ltd. (Nantong, Jiangsu, China). The culture medium was replaced with fresh DMEM containing 10% FBS following a 6–8 h incubation period. Quantitative reverse transcription-polymerase chain reaction (RT-qPCR) was performed to measure the transfection efficiency at 48 h post-transfection.

Quantitative real-time polymerase chain reaction (RT-qPCR)

Total RNA was extracted from the H9c2 cells, and the quality of the RNA was determined. The RNA concentration of each sample was adjusted to 500 ng/μL. The TaqMan MicroRNA Reverse Transcription kit was used to synthesize complementary DNA (cDNA), according to the manufacturer's instructions. A SYBR green qPCR assay kit (Takara, Minato-ku, Tokyo, Japan) was used to amplify and detect the expression levels of miR-668-3p, SDF-1, and CXCR4. U6 and GAPDH were used as internal controls for miRNA and mRNA, respectively. The expression levels of target genes and house-keeping genes were calculated with the $2^{\Delta\Delta Ct}$ method.

The primer sequences of miR-668-3p, SDF-1 and CXCR4 for qPCR amplification were as follows:
miR-668-3p, forward: 5'-TGTCACTCGGCTCGG- 3';
miR-668-3p, reverse: 5'-TGCGTGTCGTGGAGTC- 3';
U6, forward: 5'-CTCGCTTCGGCAGCACA- 3'
U6, reverse: 5'-ACGCTTCACGAATTTGCGT- 3';
SDF-1, forward: 5'-CCCGAAGCTAAAGTGGATTC- 3';
SDF-1, reverse; 5'-TTCAGAGCTGGGCTCCTACT- 3';
CXCR4, forward: 5'-GGCCCTCAAGACCACAGTC- 3';
CXCR4, reverse: 5'-TTAGCTGGAGTGAAAACTTG- 3';
GAPDH, forward: 5'-CTCACCGGATGCACCAATGTT- 3';
GAPDH, reverse: 5'-CGCGTTGCTCACAATGTTCAT- 3'.

Luciferase reporter assay

The firefly luciferase reporter plasmids, psiCHECK-SDF-1-3'-UTR-wild-type (wt) and psiCHECK-SDF-1-3'-UTR-mutant (mut), containing a wild-type and mutant miR-668-3p binding site in the 3'-UTR of SDF-1, were constructed by Shanghai GenePharma Co., Ltd and were co-transfected into H9c2 cells in 24-well plates with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). After 24 h, the firefly luciferase activity was measured and normalized to Renilla activity using the Nano-Glo Promega Dual-Luciferase reporter assay system (Promega, Madison, WI, USA). All the procedures were according to the manufacturer's instructions.

Western blot

After transfection, homogenized H9c2 cells were washed with cold PBS and lysed with RIPA buffer supplemented with protease inhibitors. Protein concentrations were measured by the BCA protein assay kit (Bio-Rad, Hercules, CA, USA). Total protein (25 μg) were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 5% dried skimmed milk powder for 2 h before incubation with primary antibodies at 4°C overnight. The primary antibodies included a rabbit antibody to SDF-1 (1: 1,000), a rabbit antibody to CXCR4 (1: 1,000), a rabbit antibody to Bcl2 (1: 1,000), a rabbit antibody to Bax (1: 1,000), a rabbit antibody to phospho-cleaved caspase-3 (1: 500), a rabbit antibody to caspase-3 (1: 1,000), a rabbit antibody to p-eNOS (1: 1,000), and a rabbit antibody to eNOS (1: 1,000), which were purchased from Cell Signaling Technology (Danvers, MA, USA). The membranes were washed three times with PBS and incubated with appropriate secondary antibodies for 2 h. The expression levels of proteins were evaluated with a Tanon-5200 Chemiluminescence Imager with enhanced chemiluminescence (ECL) Western blot substrate (Bio-Rad, Hercules, CA, USA).

Enzyme-linked immunosorbent assay (ELISA) for the measurement of inflammatory cytokines and markers of oxidative stress

The enzyme-linked immunosorbent assay (ELISA) was used to measure reactive oxygen species (ROS), including malondialdehyde (MDA), nitric oxide (NO), p-eNOS, and the inflammatory cytokines, tumor necrosis factor-α (TNF- α), interleukin-1β (IL-1β), IL-6, and monocyte chemoattractant protein-1 (MCP-1) in H9c2 cell supernatants. The measurements were performed according to the manufacturer's instructions for the Duo-Set enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA). The optical density (OD) value at 450–650 nm was determined by using a microplate reader.

The levels of malondialdehyde (MDA) and nitric oxide (NO) produced by H9c2 cells were measured by the enzyme activity assay kits (Beyotime, Shanghai, China) according to the manufacturer instructions. Briefly, samples (0.1 mL) and mixed reagent (0.4 mL) were added and mixed evenly in a 6-well plate. The concentrations of reactive oxygen species (ROS) in the cell supernatants was analyzed by 2'-7'-dichlorofluorescein diacetate (Beyotime, Shanghai, China). The cells were stained with 20 μM of dichloro-dihydro-fluorescein diacetate (DCFH-DA) at 37°C for 40 min in the dark. The absorbance was measured by a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). For lactate dehydrogenase (LDH), glutathione peroxidase (GSH-Px), and the superoxide dismutase (SOD) activity assay, H9c2 cells were disrupted and centrifuged to obtain the cell

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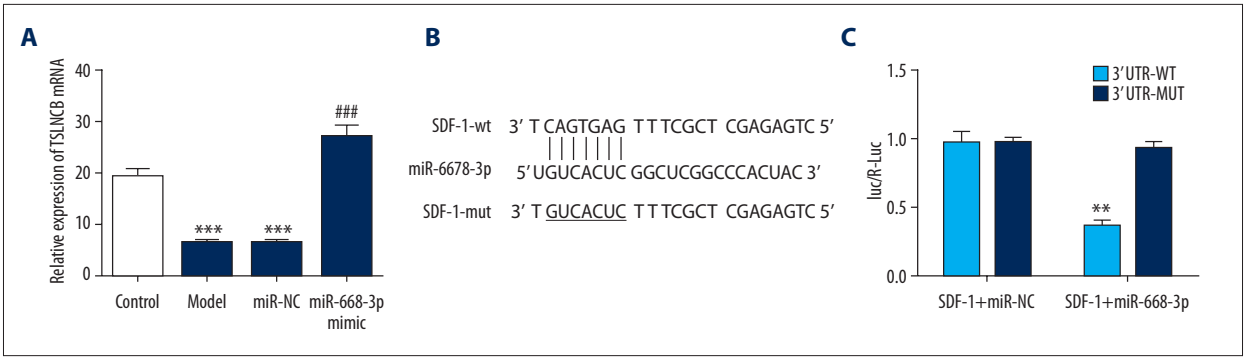

Figure 1. Levels of miR-668-3p were reduced in the oxygen-glucose deprivation (OGD) rat H9c2 cardiomyocyte model of ischemia-reperfusion injury (IRI) and directly targeted stromal cell-derived factor-1 (SDF-1). (**A**) The levels of miR-668-3p measured by quantitative reverse transcription-polymerase chain reaction (RT-qPCR). (**B**) The putative binding sites of miR-668-3p. (**C**) Luciferase reporter assay was employed to investigate whether miR-668-3p can directly target SDF-1. The data are expressed as the mean ± standard deviation (SD). *** P<0.001 *vs.* the control. ### P<0.001 *vs.* the model.

supernatant. The supernatant was mixed and incubated with the reaction buffer at 37°C for 30 min. The absorbance was measured using a microplate reader.

Cell apoptosis

H9c2 cells (1×10^6 cells/mL) were transfected with miR-668-3p mimic or miR-NC followed by washing and trypsinization. The cells were centrifuged and resuspended with binding buffer (100 µl), Annexin V-fluorescein isothiocyanate (FITC) (5 µL), and propidium iodide (PI) staining solution (10 µL), and mixed gently. The mixture of H9c2 cells was incubated for 30 min in the darkness. The Annexin V-FITC-positive and PI-negative cells were identified as apoptotic cells, and the percentage of apoptotic cells in each group was calculated by flow cytometry and analyzed with CellQuest software.

Statistical analysis

Data were expressed as the mean±standard deviation (SD). The differences between treated and control groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A P-value <0.05 was considered to be statistically significant.

Results

The levels of microRNA-668-3p (miR-668-3p) were reduced in the oxygen-glucose deprivation (OGD) rat H9c2 cardiomyocyte model of ischemia-reperfusion injury (IRI) and miR-668-3p directly targeted stromal cell-derived factor-1 (SDF-1)

Quantitative reverse transcription-polymerase chain reaction (RT-qPCR) showed that the levels of miR-668-3p were reduced in the OGD rat H9c2 cardiomyocyte model of IRI. After the H9c2 cells were transfected with the miR-668-3p mimic (100 nM), the level of miR-668-3p was significantly increased (Figure 1A). The luciferase reporter assay was used to investigate whether miR-668-3p directly targeted SDF-1. The putative miR-668-3p binding sites are shown in Figure 1B. Compared with the normal control (NC), the relative luciferase activity was significantly lower in H9c2 cells co-transfected with wild-type luciferase vector and miR-668-3p mimic. However, the luciferase activity of the plasmid harboring the mutated miR-668-3p binding site was unaltered (Figure 1C). These results showed that miR-668-3p specifically bound to the 3'UTR of the SDF-1 gene to inhibit its expression.

SDF-1 reversed the inhibitory effects of miR-668-3p on the inflammatory response and oxidative stress status

The expression levels of SDF-1 and chemokine receptor, CXCR4, were measured by Western blot and RT-qPCR. The SDF-1 expression level was upregulated in the OGD rat H9c2 cardiomyocyte model of IRI and CXCR4 expression levels showed no significant change. The expression levels of SDF-1 and CXCR4 were both significantly downregulated by miR-668-3p overexpression (Figure 2A, 2B). After the rat H9c2 cardiomyocytes in the model were co-transfected with miR-668-3p and SDF-1 plasmid, the expression levels of SDF-1 and CXCR4 were significantly increased compared with transfection with miR-668-3p (Figure 2C, 2D). The levels of inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, monocyte chemotactic protein 1(MCP-1), were significantly increased in the OGD rat H9c2 cardiomyocyte model of IRI when compared with the control group (Figure 3A). Also, treatment with the miR-668-3p mimetic inhibited the expression of the inflammatory cytokines, which was reversed by SDF-1 overexpression (Figure 3B).

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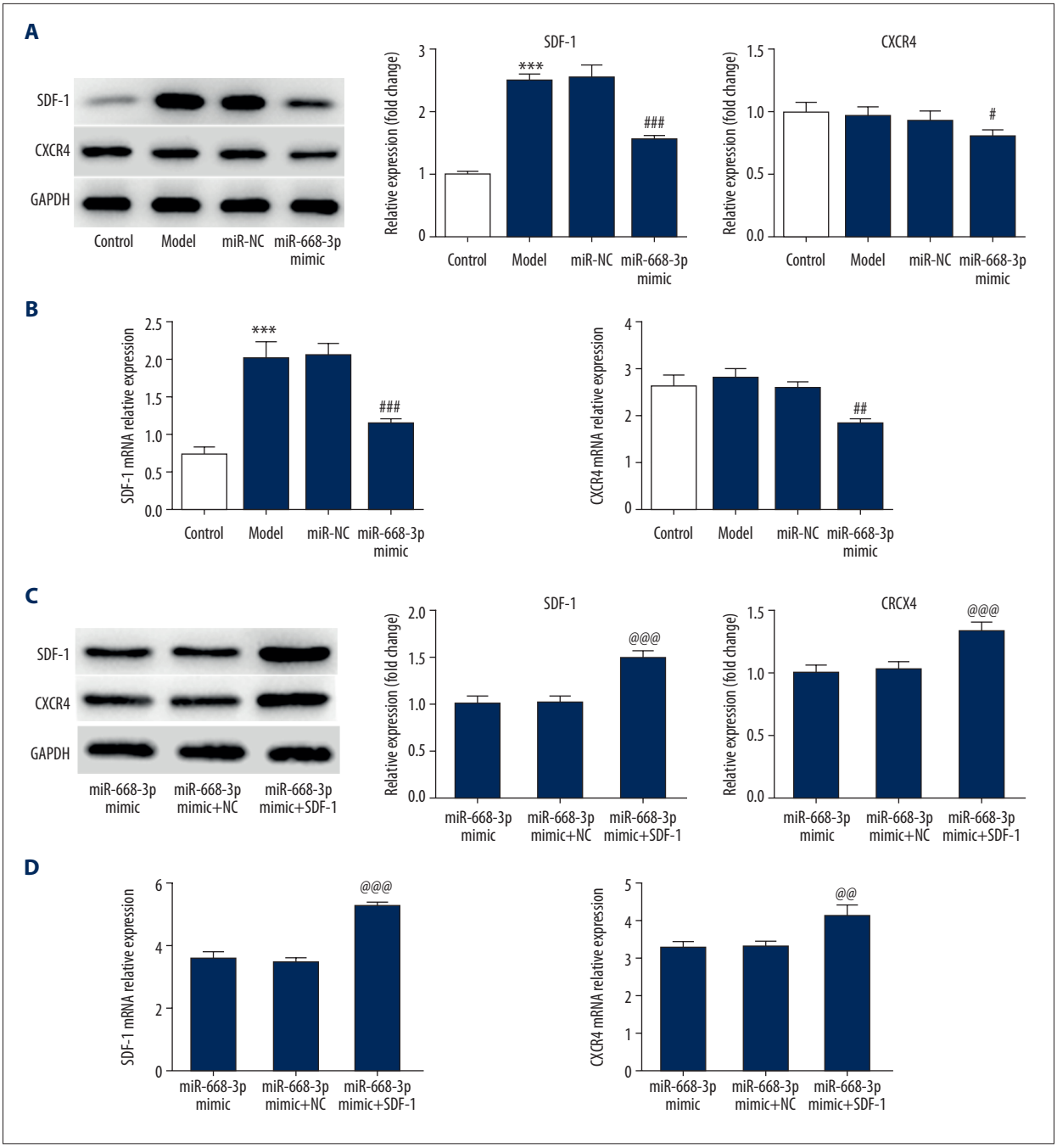

Figure 2. The expression levels of stromal cell-derived factor-1 (SDF-1) and CXCR4 measured by Western blot and quantitative reverse transcription-polymerase chain reaction (RT-qPCR). (**A, C**) The expression levels of SDF-1 and CXCR4 protein were measured by Western blot. (**B, D**) The expression levels of SDF-1 and CXCR4 mRNA were measured by RT-qPCR. The data are expressed as the mean±standard deviation (SD). *** P<0.001 *vs.* the control. # P<0.05, ## P<0.01, ### P<0.001 *vs.* the model. @@ P<0.01, @@@ P<0.001 *vs.* miR-668-3p+NC.

Levels of oxidative stress markers and antioxidant enzyme activities were measured with specific assay kits. Compared with normal H9c2 cells, increased levels of reactive oxygen species (ROS) and malondialdehyde (MDA) were found, with increased levels of lactate dehydrogenase (LDH), glutathione peroxidase (GSH-Px), and reduced the levels of superoxide dismutase (SOD) in the OGD rat H9c2 cardiomyocyte model of IRI. Overexpression of miR-668-3p reduced the levels of ROS and MDA levels, reduced the levels of LDH and GSH-Px, and increased the activity of SOD (Figure 3C). However, SDF-1

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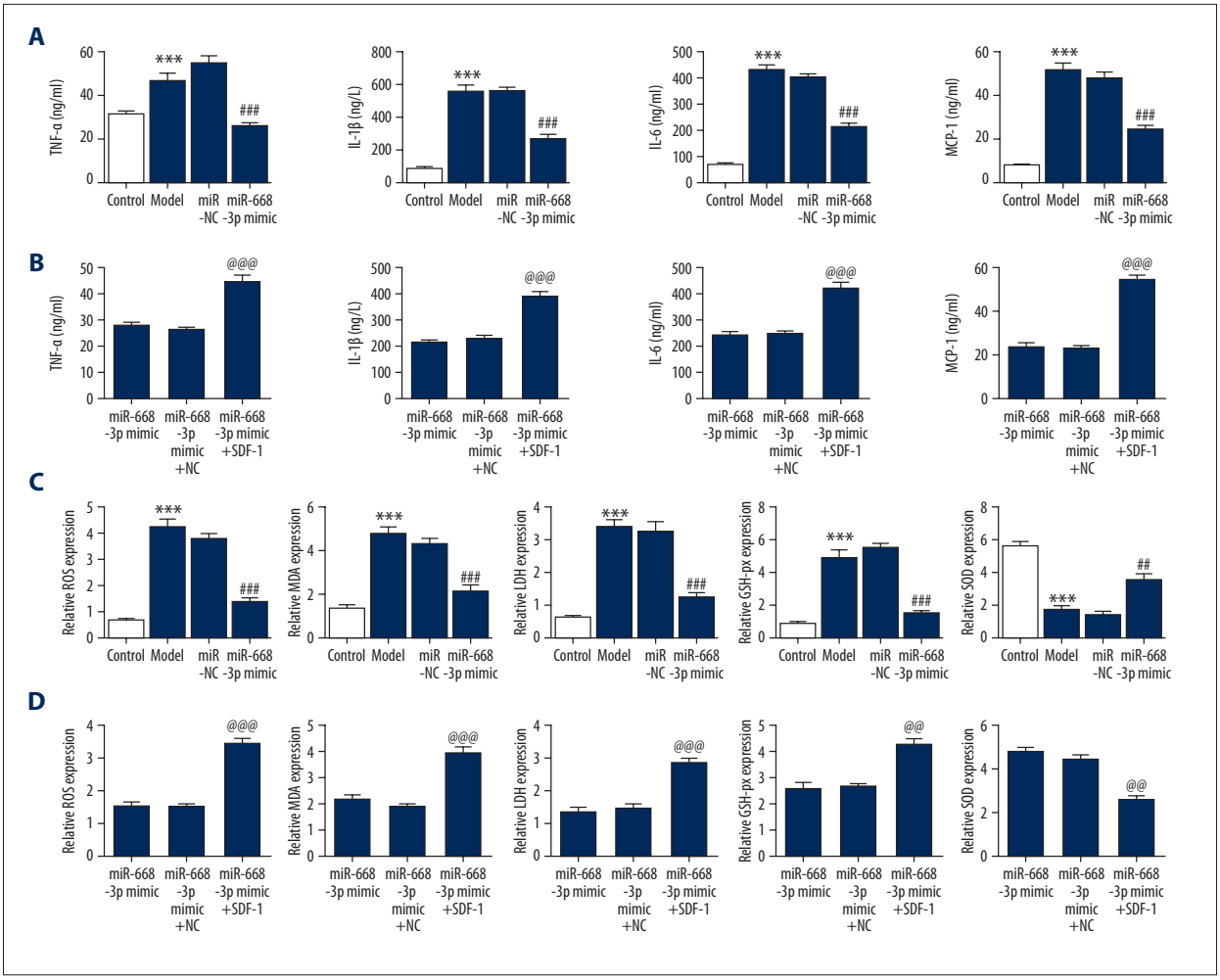

Figure 3. Stromal cell-derived factor-1 (SDF-1) reversed the effects of miR-668-3p on the inflammatory response and the oxidative stress status. (**A, B**) ELISA kits measured the levels of inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, monocyte chemotactic protein 1 (MCP-1). (**C, D**) Reactive oxygen species (ROS) and malondialdehyde (MDA), and the activities of lactate dehydrogenase (LDH), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) were measured. The data are expressed as the mean±standard deviation (SD). *** P<0.001 *vs.* the control. ## P<0.01, ### P<0.001 *vs.* the model. @@ P<0.01, @@@ P<0.001 *vs.* miR-668-3p+NC.

overexpression blocked these effects on oxidative stress products and antioxidant enzyme activities (Figure 3D). As shown in Figure 4A, 4B, the expression levels of NO and p-eNOS were significantly reduced in the OGD rat H9c2 cardiomyocyte model of IRI, and were reversed by treatment with a miR-668-3p mimic. The expression levels of NO and p-eNOS following co-transfection with miR-668-3p and in the SDF-1 group were lower than that following transfection with miR-669-3p, and total eNOS have levels showed no significant difference (Figure 4C, 4D).

SDF-1 reversed the inhibitory effects of miR-668-3p on H9c2 cardiomyocyte apoptosis

Flow cytometry showed that miR-668-3p overexpression suppressed the increased cell apoptosis rate in the OGD rat H9c2 cardiomyocyte model of IRI (Figure 5A, 5B). SDF-1 overexpression effectively induced cell apoptosis (Figure 5C, 5D). The expression levels of Bcl2, Bax, cleaved caspase-3, and caspase-3 proteins were detected by Western blot, which were significantly increased compared with the control group. However, Bcl2 expression was reduced in the OGD rat H9c2 cardiomyocyte model of IRI. Overexpression of miR-668-3p reversed the increased expression levels of apoptosis markers, which was abolished by SDF-1 overexpression, as shown in Figure 6.

Discussion

Worldwide, coronary artery disease (CAD) results in ischemic heart disease and is a leading cause of morbidity and

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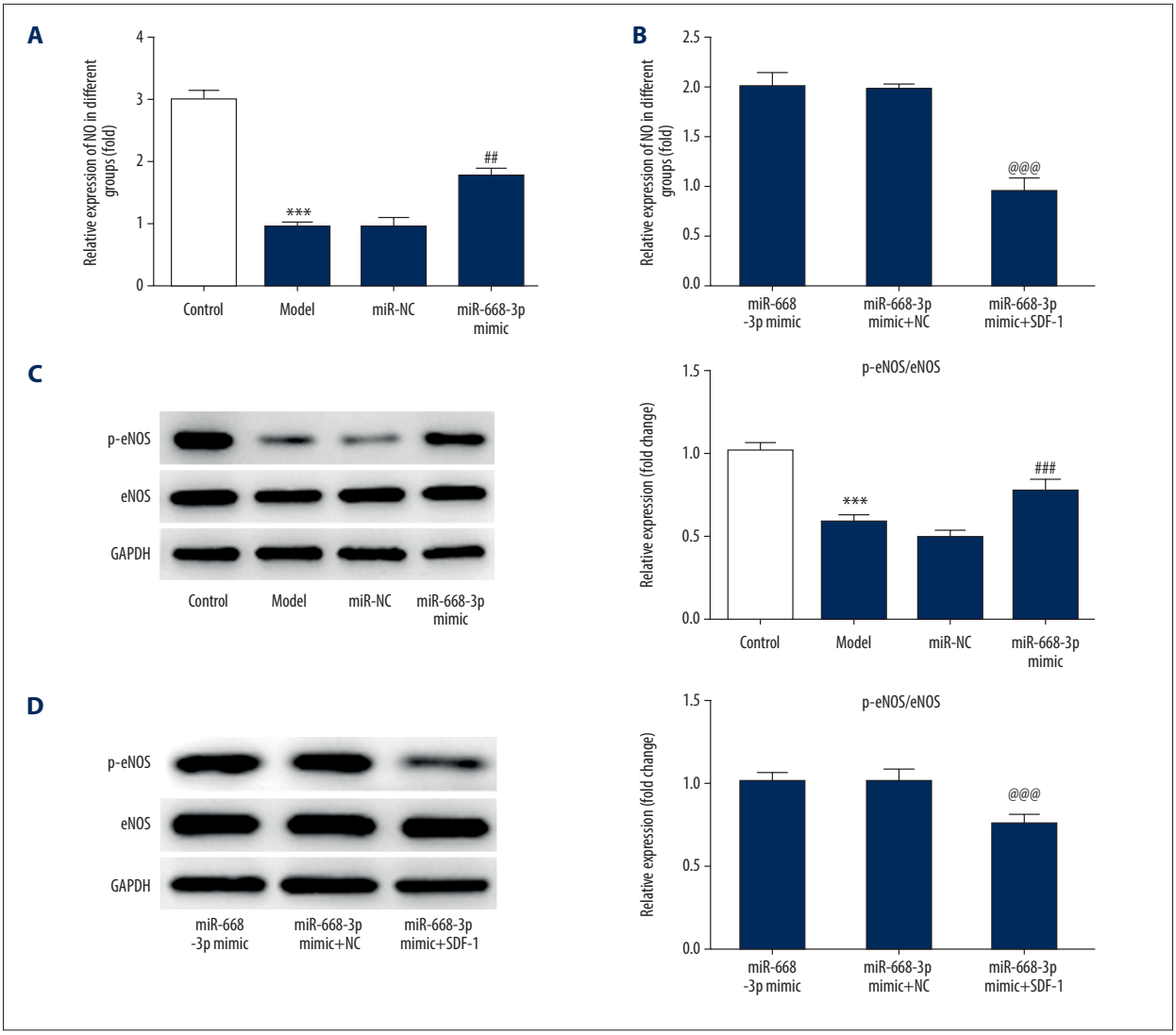

Figure 4. (**A–D**) Stromal cell-derived factor-1 (SDF-1) reversed the effects of miR-668-3p on the expression of nitric oxide (NO) and endothelial nitric oxide synthase (eNOS). (**A**) Commercial test kits measured the level of NO. (**B**) The expression level of eNOS was measured by Western blot. The data are expressed as the mean±standard deviation (SD). *** P<0.001 *vs.* the control. ## P<0.001, ### P<0.001 *vs.* the model. @@@ P<0.001 *vs.* miR-668-3p+NC.

mortality [22]. Ischemia-reperfusion injury (IRI) significantly contributes to cardiac damage due to cell damage, inflammation, and the generation of reactive oxygen species (ROS), resulting in myocardial dysfunction [23,24]. In the present study, the role of microRNA-668-3p (miR-668-3p) was studied in oxygen-glucose deprivation (OGD) in rat H9c2 cardiomyocytes in an *in vitro* model of IRI. A previous study showed that miR-668-3p was a protective factor in acute kidney injury, reduced mitochondrial fragmentation, and had protective effects on renal function [16]. Recent studies showed that miR-668-3p had a protective role and increased renal tubular cell survival by down-regulating the expression of mitochondrial protein 18 kDa (MTP18) [17].

Studies have shown that an increased body mass index (BMI) and abdominal adiposity are associated with an increased risk of acute myocardial infarction and reduce the prognosis of patients with cardiovascular disease [25]. Also, there is an association between obesity and inflammation [12]. A recent study showed that light-emitting diode (LED) therapy protected against myocardial IRI-induced ventricular arrhythmia by suppressing microglial activation and the expression of inflammatory cytokines in the ischemic myocardium [26]. Salahshoor et al. showed that royal jelly had antioxidant and anti-inflammatory properties that reduced IRI-induced renal injury [27].

In the present study, the increased levels of inflammatory cytokines and factors associated with oxidative stress were significantly

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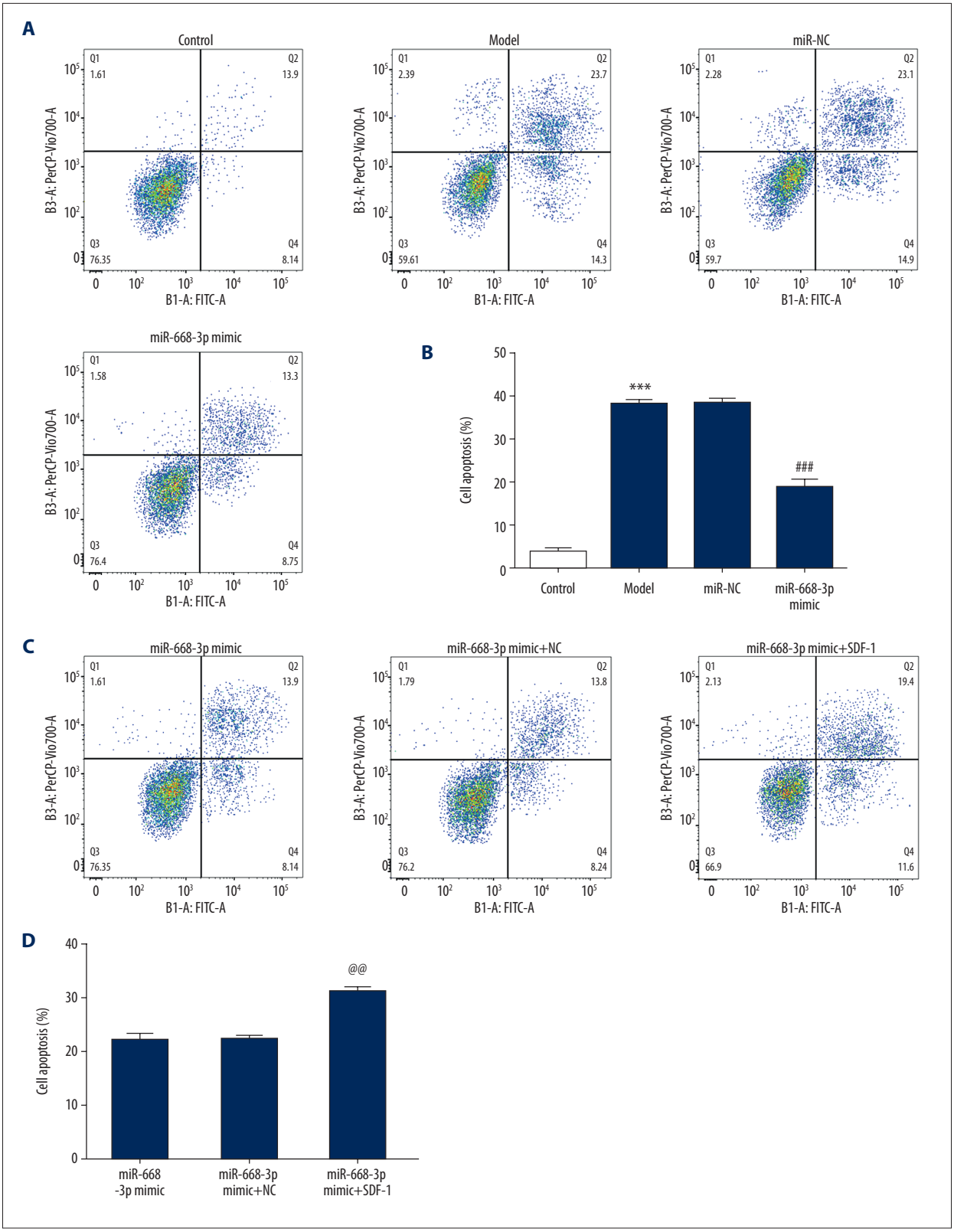

Figure 5. (**A–D**) Stromal cell-derived factor-1 (SDF-1) reversed the effects of miR-668-3p on cell apoptosis assayed by flow cytometry. The data are expressed as the mean±standard deviation (SD). *** P<0.001 *vs.* the control. ### P<0.001 *vs.* the model. @@ P<0.01 *vs.* miR-668-3p+NC.

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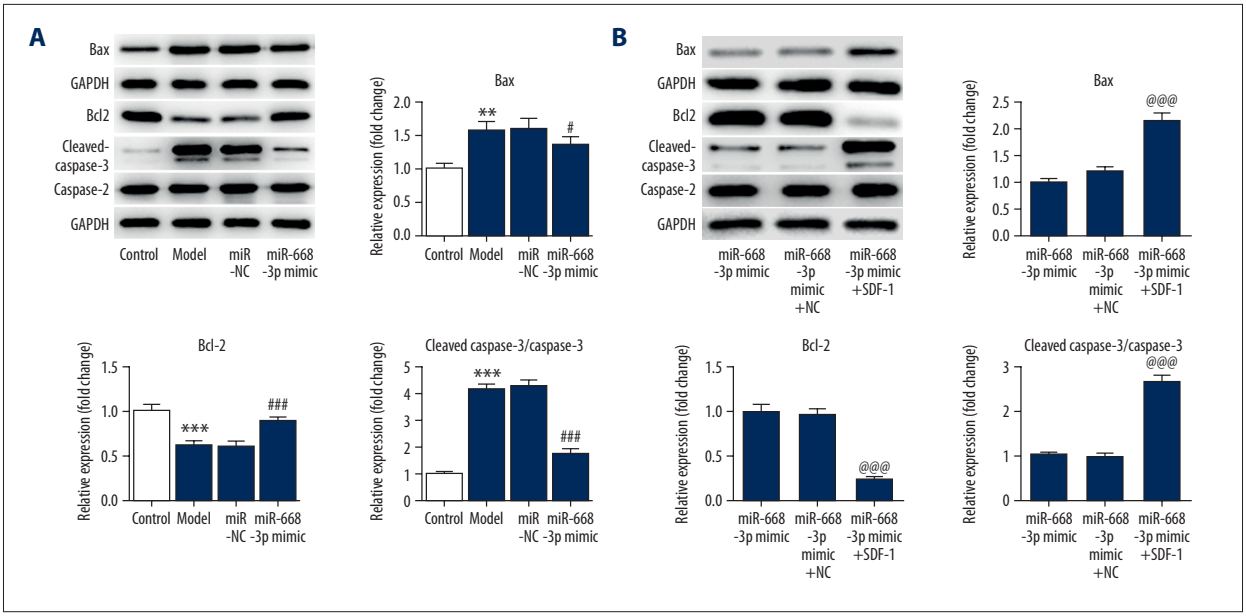

Figure 6. (**A, B**) Stromal cell-derived factor-1 (SDF-1) reversed the effects of miR-668-3p on the expressions levels of proteins involved in apoptosis. The expression level of endothelial nitric oxide synthase (eNOS) measured by Western blot. The data are expressed as the mean±standard deviation (SD). ** P<0.01, *** P<0.001 *vs.* the control. # P<0.05, ### P<0.001 *vs.* the model. @@@ P<0.001 *vs.* miR-668-3p+NC.

increased in the OGD rat H9c2 cardiomyocyte model of IRI, which could be inhibited by the miR-668-3p mimic. Also, the stromal derived factor-1 (SDF-1)/CXC chemokine receptor 4 (CXCR4) axis has previously been shown to have a major role in hematopoiesis and immunity, and the SDF-1/CXCR4 signaling pathway has been shown to have regulatory roles in cell homing and angiogenesis of endothelial progenitor cells in ischemic myocardium [19]. Compared with the control group, the expression level of SDF-1 protein was increased in the OGD rat H9c2 cardiomyocyte model of IRI. This finding is supported by the findings from a study by Jin et al., who also showed that levels of SDF-1 were increased in the mouse liver with IRI [28]. However, in the present study, the miR-668-3p mimic significantly reduced the increased levels of SDF-1 and CXCR4 proteins. These findings showed that the miR-668-3p mimic effectively suppressed the inflammatory response through regulation of the SDF-1 and CXCR4 axis.

Endothelial NO synthase (eNOS) is a dimeric protein associated with NO synthesis. Studies have shown that eNOS-derived NO improved cardiomyocyte function and maintained vascular homeostasis [29]. Reduced NO bioavailability contributes to the onset and development of endothelial dysfunction in pathological conditions. The activation of the PI3K/Akt/eNOS signaling pathway has been identified as an underlying mechanism in vasorelaxation [30]. The inhibition of eNOS induces and enhances cardiac fibrosis through the activation of the SDF-1/CXCR4 pathway [31]. The findings from the present study were consistent with previous studies and showed reduced levels of NO and eNOS in the OGD rat H9c2 cardiomyocyte model of IRI. Also, the miR-668-3p mimic restored the balance of the NO systems. SDF-1 overexpression increased the reduced bioavailability of NO. These findings support that further studies should be performed to further investigate the potential role of miR-668-3p in IRI in cardiomyocytes with *in vivo* models.

Increased levels of SDF-1 have previously been shown to induce TNFα-mediated apoptosis in cardiac myocytes under conditions of hypoxic stress [32], which is consistent with the findings from the present study. Also, the miR-668-3p mimic increased the viability of the rat H9c2 cardiomyocytes in the OGD model of IRI. However, SDF-1 overexpression promoted cardiac myocytes apoptosis induced by OGD and IRI. These study findings are supported by those from a previous study by Song et al., who showed that SDF-1 reduced IRI by suppressing caspase-3/caspase-9-related cell apoptosis [33]. The findings from the present study showed that miR-668-3p protected against OGD and IRI-induced rat cardiomyocyte injury *in vitro* by targeting the SDF-1/CXCR4 signaling pathway.

Conclusions

This study aimed to investigate the role of miR-668-3p in oxygen-glucose deprivation (OGD) in a rat H9c2 cardiomyocyte model of ischemia-reperfusion injury (IRI). In the OGD rat H9c2 cardiomyocyte model of IRI, miR-668-3p suppressed mediators of inflammation and oxidative stress and enhanced cell viability through the SDF-1/CXCR4 signaling pathway.

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Conflicts of interest

None.

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