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Sevoflurane Postconditioning Reduces Hypoxia-Reoxygenation Injury in H9C2 Embryonic Rat Cardiomyocytes and Targets the STRADA Gene by Upregulating microRNA-107

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Sevoflurane as a widely used inhalational general anesthetic that also has a cardioprotective role in hypoxia-reoxygenation (H/R) injury. This study aimed to investigate the effects of microRNA-107 (miR-107) on sevoflurane postconditioning (SpostC) in H9C2 embryonic rat cardiomyocytes and to use bioinformatics analysis to identify the molecular basis of cardioprotection from sevoflurane in human cardiac tissue.

Material/Methods: The STRADA gene was identified from the Gene Expression Omnibus (GEO) database. H9C2 embryonic rat cardiomyocytes were cultured with sevoflurane. Quantitative real-time polymerase chain reaction (qRT-PCR) and Western blot were used to measure the mRNA expression and protein expression of STRADA and miR-107 in H9C2 cells. TargetScanHuman version 7.2 was used to identify the target gene of miR-107 and to predict the STRADA 3'-UTR binding site of miR-107. The dual-luciferase reporter assay measured the relative luciferase activity. The cell proliferation rate and cell apoptosis were measured using the MTT assay and flow cytometry, respectively.

Results: H/R injury in H9C2 cells following SpostC resulted in increased expression of miR-107 and reduced expression of STRADA. Specific binding of miR-107 was identified to STRADA 3'-UTR. Upregulation of the miR-107 in SpostC H/R injured H9C2 cells promoted cell proliferation, reduced cell apoptosis, and downregulating the protein expression of caspase-3. STRADA overexpression reduced the effects of a miR-107 mimic on SpostC.

Conclusions: SpostC reduced H/R injury in H9C2 embryonic rat cardiomyocytes by targeting the STRADA gene and by upregulating the expression of microRNA-107.

MeSH Keywords: Cell Hypoxia • Ischemic Postconditioning • Myocytes, Cardiac

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Background

Worldwide, ischemic heart disease is a leading cause of morbidity and mortality [1]. Patients with coronary artery atherosclerosis who undergo cardiac ischemia and infarction can undergo further myocardial damage following revascularization due to ischemia-reperfusion (I/R) injury, which can increase patient mortality rates [2]. The mechanisms of I/R injury include changes in mitochondrial permeability, the disruption of ionic homeostasis, apoptosis-induced cell death, and necrosis [3–6]. Hypoxia-reoxygenation (H/R) is the *in vitro* model that is used to investigate the mechanism of I/R injury [1,7–9].

Sevoflurane is a widely used volatile general anesthetic, which has cardioprotective effects on I/R injury [10]. Sevoflurane preconditioning and sevoflurane postconditioning (SpostC) can reduce cardiac ischemic injury, but SpostC is more widely used than sevoflurane preconditioning in clinical therapy [11]. SpostC protects the heart from I/R injury by multiple mechanisms that involve the nuclear factor-erythroid-2-related factor-2 (NRF2) pathway, the reduction of reactive oxygen species (ROS) levels, and extracellular signal-regulated kinase (ERK) phosphorylation [12–14]. However, the key genes and molecular mechanism involved the protective effects of SpostC in cardiomyocytes remain to be determined.

STE20-related kinase adaptor α (STRADA), which is also known as LYK5, belongs to the STE20-like kinase family and is considered to be a pseudo-kinase due to the lack of essential residues for intrinsic enzyme activity [15]. Recently, STRADA was identified in cerebral tissue in patients with polyhydramnios, megalencephaly, and symptomatic epilepsy syndrome, and was associated with AMP-activated protein kinase (AMPK) signaling [16]. Also, silencing the STRADA gene resulted in the activation of the mammalian target of rapamycin (mTOR) signaling pathway involved in cell migration *in vitro* [17]. However, the role of STRADA in cardiac I/R injury *in vivo*, or in H/R injury in cardiomyocytes *in vitro* remains to be investigated.

MicroRNAs (miRNAs), belonging to the non-coding RNAs, contributed to mediating target gene expression via binding to the 3'-untranslated region (UTR) [18]. Recently, increasing evidence has proved that miRNAs are correlated with cardiovascular diseases [19,20]. To name a few, Tan et al. demonstrated that the upregulation of miR-24-3p reduced myocardial infarct size and I/R injury [21]. miR-21 protected cardiomyocytes from I/R injury through preventing cell apoptosis, inflammation, and autophagy [22]. Sevoflurane preconditioning increased miR-210 expression to protect bone marrow mesenchymal stem cells from hypoxia injury [23]. Sevoflurane was found attenuated H/R injury in cardiomyocytes via downregulating miR-34a-5p [24]. Also, microRNA-107 (miR-107) it is reported that its expression had a significant role in myocardial necrosis [25]. However, the effects of miR-107 on SpostC in H/R injured cardiomyocytes are still not clear. Both TarBase and TargetScan predicted the binding relationship between miR-107 and STRADA. As stated in the previous paragraph, it is of significance to study the role of STRADA in the protection mechanism of sevoflurane during H/R, thus miR-107 was chosen as our interested miRNA.

Therefore, this study aimed to investigate the effects of miR-107 on SpostC in H9C2 embryonic rat cardiomyocytes. Bioinformatic analysis showed that STARDA, a key mediator of mTOR signaling, responded to sevoflurane treatment in human cardiac tissue and the effects of this gene on SpostC were investigated to determine the molecular basis for cardiac protection by sevoflurane.

Material and Methods

Bioinformatics analysis

The Gene Expression Omnibus (GEO) datasets of GSE4386 were acquired from the National Center for Biotechnology Information (NCBI) (*https://www.ncbi.nlm.nih.gov/*) and included nine atrial tissue specimens from patients undergoing coronary artery bypass graft (CABG) surgery and sevoflurane anesthesia and 19 atrial tissue specimens from patients undergoing CABG surgery before sevoflurane anesthesia. The differentially expressed genes (DEGs) were selected (at $P<0.05$). FunRich version 2.1.2 software, an enrichment analysis tool for interaction network analysis, was used to analyze the key biological pathways of the DEGs (Figure 1A). TarBase (*http://carolina.imis.athena-innovation.gr/diana_tools/web/index.php?r=tarbasev8%2Findex*) and TargetScan (*http://www.targetscan.org/vert_71/*) online tools were used to predict the binding site of the gene 3'UTR for microRNAs (miRNAs) (Figure 1B).

Experimental groups and establishment of the rat cardiomyocyte model of H/R

HPC2 rat (*Rattus norvegicus*) embryonic myocardial cells (CRL-1446) were purchased from American Type Culture Collection (ATCC) (Manassas, VA, USA). The cells were randomly divided into the following groups: the control group, the H/R model group, the SEVO group, the SEVO+miR-107 mimic group, the SEVO+negative control (NC) group, the SEVO+miR-107 inhibitor group, and the SEVO+miR-107 mimic+STRADA overexpression group. In the H/R cell model, the H9C2 cells were cultured in serum-free Dulbecco's modified Eagle's medium (DMEM) (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) and placed into a hypoxic chamber (Thermo Fisher Scientific, Waltham, MA, USA) in an atmosphere of 95% N_2 and 5% CO_2 at 37°C for 180 min [26]. The serum-free DMEM medium was

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replaced with DMEM medium containing 10% fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA, USA) was replenished in DMEM medium, and the cells were re-oxygenated in 95% air and 5% CO_2 in an incubator for a further 180 min.

Cell treatment

The microRNA-107 (miR-107) mimic and inhibitor, negative control, and STRADA overexpression were designed and purchased from Shanghai GenePharma Co., Ltd. (Shanghai, China). Sevoflurane was obtained from Sigma-Aldrich (Shanghai, China). The H9C2 cells were cultured in DMEM medium at 37°C and 5% CO_2 until the cells reached 90% confluency, after which the cells were divided into seven groups. The cells were cultured without any treatment in the control group; the cells in the model that underwent hypoxia-reoxygenation (H/R) injury were the H/R group. In the SEVO group, the cells were placed in a Vapor sevoflurane vaporizer (Draeger, Lübeck, Germany) with 97.6% O_2 and 2.4% sevoflurane for 20 min at the end of hypoxia [27], after which the cells were treated as for the H/R group. The cells in the SEVO+NC group, SEVO+miR-107 mimic group, SEVO+miR-107 inhibitor group, and the SEVO+miR-107 mimic+STRADA overexpression group, were transfected with negative control (NC), miR-107 mimic, miR-107 inhibitor, and the co-expression plasmid of miR-107 mimic and STRADA overexpression, respectively, after which, the cells were treated as for the SEVO group (Figure 1C).

Expression of miR-107 and STRADA

The miR-107 mRNA expression and STRADA mRNA expression were detected using quantitative real-time polymerase chain reaction (qRT-PCR). Total RNA was isolated from the seven experimental groups using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The cDNA was synthesized using the PrimeScript RT reagent Kit with gDNA Eraser (Takara Biomedical Technology, Beijing, China). The mRNA relative expression was measured using the SYBR Premix Ex Taq II kit (Takara Biomedical Technology, Beijing, China) and the ABI 7500 Fluorescent Quantitative PCR system (Applied Biosystems, Foster City, CA, USA). The expression of U6 and GAPDH were used to normalize miRNA and mRNA expression, respectively. The gene expression was analyzed using the $2^{-\Delta\Delta CT}$ method [28].

Dual-luciferase reporter assay

Wild-type (WT) fragments of STRADA 3' UTR were amplified by PCR, which contained the miR-107 binding site. The PCR primers sequences of WT-STRADA were forward, 5'-CCCCTCTCCTTTCACGTTGG-3' and reverse, 5'-ACGAAGGGGCTACGATGCCT-3'. The mutant-type (MUT) fragments of STRADA 3' UTR were designed and purchased from RiboBio Ltd. (Guangzhou, China). WT-STRADA and MUT-STRADA were inserted into the pGL4-control vector (Promega, Madison, WI, USA). The H9C2 cells were seeded into 24-well plates and cotransfected with 100 ng of pGL4-STRADA or pGL4-STRADA-MUT, the Renilla vector, and miR-107 mimic or NC. After co-transfection for two days, the dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) was used to measure the relative activity of luciferase.

Western blot for protein expression

Western blot was performed to detect STRADA and caspase-3 protein expression, as previously described [24]. Briefly, the cells were harvested and lysed in cold RIPA cell lysis buffer (Beyotime Biotechnology, Inc., Shanghai, China) for 20 min. The mixture was then centrifuged to collect the supernatant containing the total protein. A BCA Protein Assay Kit (Beyotime Biotechnology, Inc., Shanghai, China) was used to measure the concentration of total protein. A total of 20 μg of protein was separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and incubated at 4°C overnight with the primary antibodies to STRADA (1: 1000) (ab230118; Abcam, Cambridge, MA, USA), caspase-3 (1: 1000) (ab197202; Abcam, Cambridge, MA, USA), and GAPDH (1: 1000) (ab9485; Abcam, Cambridge, MA, USA). The membranes were incubated with the secondary anti-mouse or anti-rabbit antibody as 1: 5000 dilution for 90 min (Abcam, Cambridge, MA, USA). The ECL Substrate Kit (Abcam, Cambridge, MA, USA) was used to visualize the protein bands.

MTT assay for cell proliferation

MTT was purchased from Sigma-Aldrich (St. Louis, MO, USA) and used to detect the cell proliferation rate of the H9C2 cells in the seven study groups. Briefly, the cells were treated as described above and were placed in a 96-well plate and cultured overnight in DMEM and 10% FBS containing 10% MTT. An automated microplate reader was used to measure the absorbance at 490 nm (Biobase, Jinan, Shandong, China).

Flow cytometry for cell apoptosis

The cells in the seven study groups were plated in six-well plates and treated using the protocol described above. When the cell concentration was 1×10^6 cells/ml, the medium was replaced with cold 75% ethanol (Sinopharm Chemical Reagent Co., Ltd, Beijing, China) to fix the cells at 4°C overnight. Then, 10 μL of Annexin V- fluorescein isothiocyanate (FITC) binding buffer (Sigma-Aldrich, St. Louis, MO, USA) and 5 μL of propidium iodide (PI) (Sigma-Aldrich, St. Louis, MO, USA) were added to each well to incubate cells for 20 min avoiding light. The apoptosis rate of each well was detected using a flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA).

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Statistical analysis

Data were expressed as the mean±standard deviation (SD) from three independent experiments. Statistical analysis was performed using SPSS version 19.0 software (IBM Corp., Armonk, NY, USA). Data between multiple groups were compared with one-way analysis of variance (ANOVA), and data between two groups were compared using Student's t-test to validate the establishment of the H/R *in vitro* cell model. A P-value <0.05 was considered to be statistically significant.

Results

Identification of the STRADA gene and microRNA-107 (miR-107) in sevoflurane postconditioning (SpostC)

Gene microarray analysis of GSE4386 from the Gene Expression Omnibus (GEO) database identified 816 significantly upregulated differentially expressed genes (DEGs) involving sevoflurane anesthesia ($P<0.05$). By uploading the identified 816 DEGs to FunRich version 2.1.2, mTOR signaling was identified as the key biological pathway, which included four DEGs (STRADA, PRKAG2, RHEB, and MTOR) (Figure 1A). Due to the limited number of studies on STRADA in sevoflurane anesthesia and the close relationship between STRADA and the mTOR signaling pathway, STRADA was selected as the gene of interest to investigate its effect on sevoflurane anesthesia. TarBase and TargetScan were used to select the miRNA that could bind to the STRADA gene mRNA 3'UTR. The results showed that miR-616-5p, miR-1-3p, miR-107, and miR-27a-3p were the overlapping miRNAs (Figure 1B). Together with a review of the published literature, the effect of miR-107 on sevoflurane was not found to have been previously reported, but its expression was associated with ischemia-reperfusion (I/R) injury. Therefore, miR-107 was selected as miRNA for further study.

Sevoflurane postconditioning (SpostC) resulted in miR-107 overexpression and down-regulation of STRADA

The *in vitro* H9C2 rat cardiomyocyte model of hypoxia-reoxygenation (H/R) injury was investigated using the MTT assay to determine the mechanism of the protective effect of SpostC. As shown in Figure 2A, the cell proliferation rate was significantly reduced by approximately 25% and 50% in the H/R injury group at 6 h and 12 h, respectively, compared with the control group. Then, the expression levels of miR-107 and STRADA were measured in the control group, the H/R injury group, and the SEVO group using quantitative real-time polymerase chain reaction (qRT-PCR). Compared with the H/R injury group, miR-107 expression was upregulated by more than 4-fold in the SEVO group, although there was no difference between the control group and the H/R injury group (Figure 2B). Also, H/R injury resulted in a 2.5-fold increase in STRADA mRNA expression compared with the control group. SpostC resulted in a 40% reduction in STRADA mRNA expression compared with the H/R injury group (Figure 2C). The target relationship between miR-107 and STRADA might indicate an association in the protective mechanism of sevoflurane against H/R injury in cardiomyocytes *in vitro*.

The targeting relationship between miR-107 and STRADA

The binding sites of miR-107 on STRADA mRNA 3'UTR was predicted by TargetScanHuman version 7.2, and the findings are shown in Figure 3A. The 3'UTR reporter assay results showed that the relative luciferase activity was significantly reduced when the H9C2 cells were transfected with the miR-107 mimic together with STRADA overexpression plasmids (Figure 3B).

Transfection efficiency and the inhibition of STRADA by miR-107

The transfection efficiency of molecules by the H9C2 cells was confirmed before H/R stimulation or SpostC. Briefly, compared with the SEVO group, miR-107 expression was upregulated by more than 2-fold in the SEVO+miR-107 mimic group, while miR-107 expression was significantly downregulated by approximatively 95% in the SEVO+miR-107 inhibitor group (Figure 4A). The use of the miR-107 mimic significantly inhibited the expression of STRADA mRNA and protein, while the miR-107 inhibitor significantly increased the expression (Figure 4B, 4C). The protein level of STRADA in the SEVO group was lower than that in H/R group (Figure 4C). Also, the mRNA expression of STRADA in the SEVO+STRADA overexpression group was increased by more than 2.6-fold compared with the SEVO group (Figure 4D). All molecules were successfully transfected into H9C2 cells after SEVO preconditioning, and the expression of STRADA was inhibited by miR-107 upregulation and SpostC.

miR-107 expression increased cell proliferation and inhibited cell apoptosis by regulating STRADA expression

The miR-107 mimic, the miR-107 inhibitor, and co-transfection with the miR-107 mimic and STRADA overexpression plasmids were transfected into H9C2 cells before SEVO treatment and H/R stimulation. The cell proliferation rate and cell apoptosis of the seven study groups were measured by the MTT assay and flow cytometry, respectively. The transfection with the miR-107 mimic significantly increased the cell proliferation rate compared with H/R injury group or the SEVO group (1.8-fold increase versus the H/R group, a 1.47-fold increase versus the SEVO group). Transfection of miR-107 inhibitor significantly decreased the cell proliferation rate (40% decrease versus the SEVO group) (Figure 5A). Transfection with the miR-107 mimic resulted in mild suppression of cell apoptosis, while transfection

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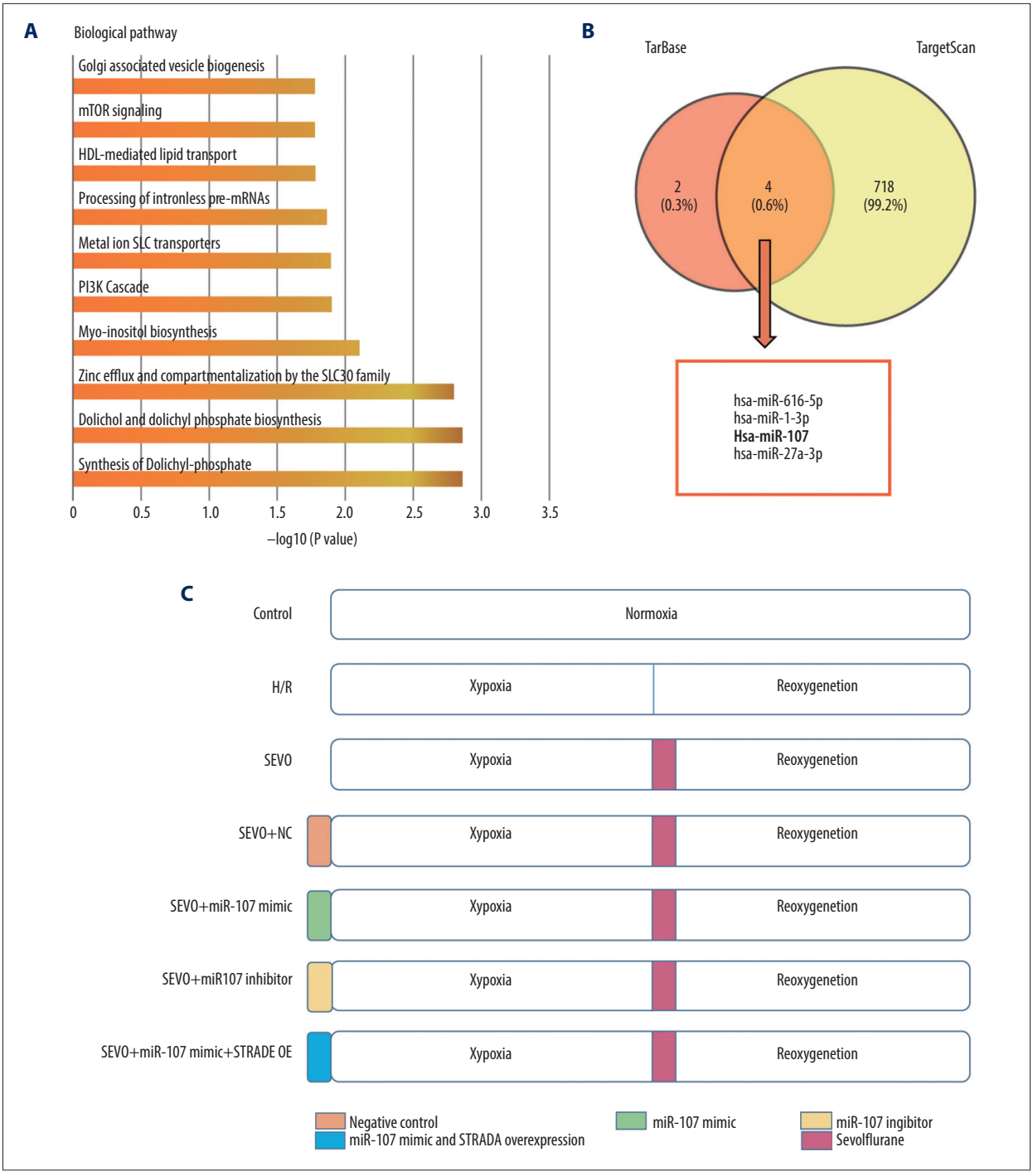

Figure 1. Bioinformatics analysis and the construction of the sevoflurane postconditioning (SpostC) model and the hypoxia-reoxygenation (H/R) injury model in H9C2 embryonic rat cardiomyocytes. (**A**) The biological pathway of differentially expressed genes (DEGs) was enriched using FunRich version 2.1.2 software. (**B**) The overlapping microRNAs (miRNAs) were selected from the TarBase and TargetScan online tools to predict the binding site of the gene and miRNA. (**C**) The experimental groups and the corresponding cell treatment groups are shown. H9C2 embryonic rat cardiomyocytes were randomly divided into six groups: the control group, the H/R group, the SEVO group, the SEVO+NC group, the SEVO+miR-107 mimic group, the SEVO+miR-107 inhibitor group, and the SEVO+miR-107 mimic+STRADA OE group. NC – negative control; SEVO – sevoflurane; OE – overexpression.

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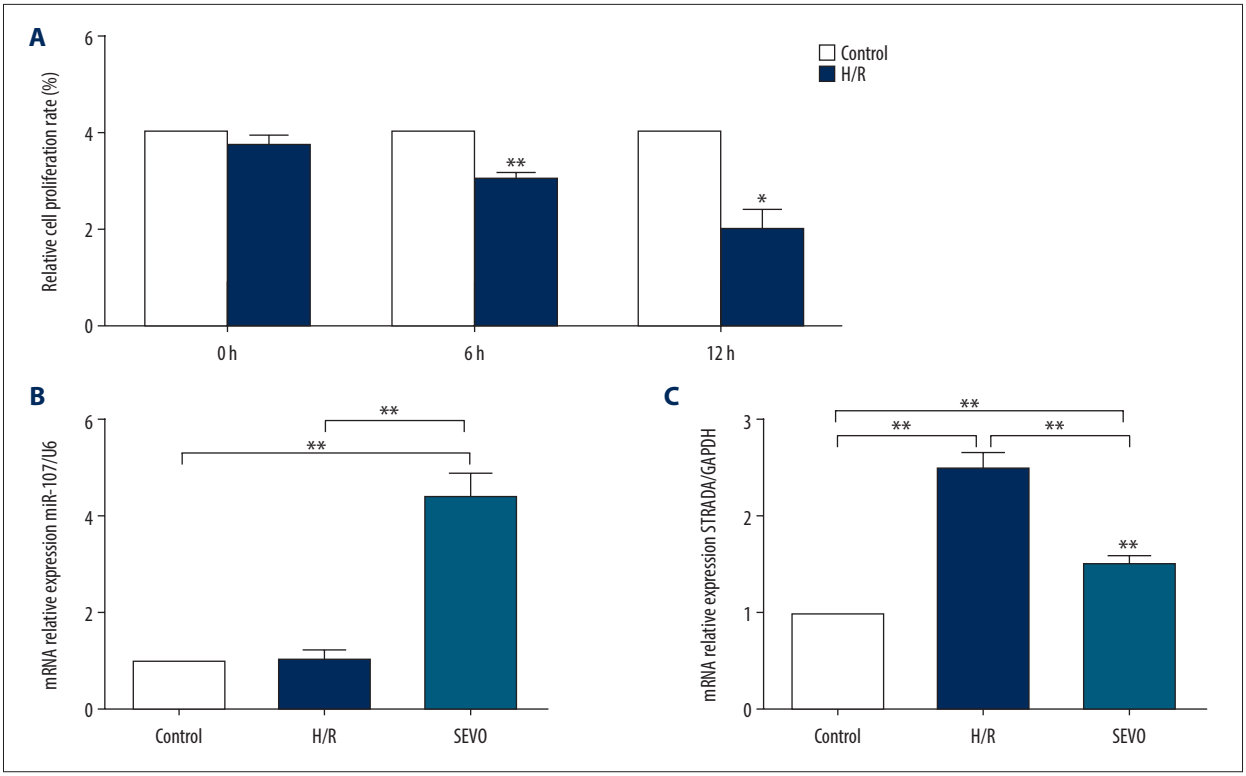

Figure 2. Sevoflurane preconditioning increased the expression of microRNA-107 (miR-107) and reduced STRADA mRNA expression in hypoxia-reoxygenation (H/R) injury in H9C2 embryonic rat cardiomyocytes. (**A**) The *in vitro* hypoxia-reoxygenation (H/R) injury model was assessed by detecting the cell proliferation rate using the MTT assay. (**B**) The expression of miR-107 was detected using quantitative real-time polymerase chain reaction (qRT-PCR). (**C**) The mRNA expression of STRADA was also detected using qRT-PCR. The H9C2 cells were randomly divided into three groups: the control group (cells without the establishment of the H/R model), the H/R group (cells in the H/R model), and the SEVO group (cells in the H/R model with sevoflurane preconditioning). Data are presented as the mean±standard deviation (SD) of three independent experiments. ** P<0.001 versus the H/R group.

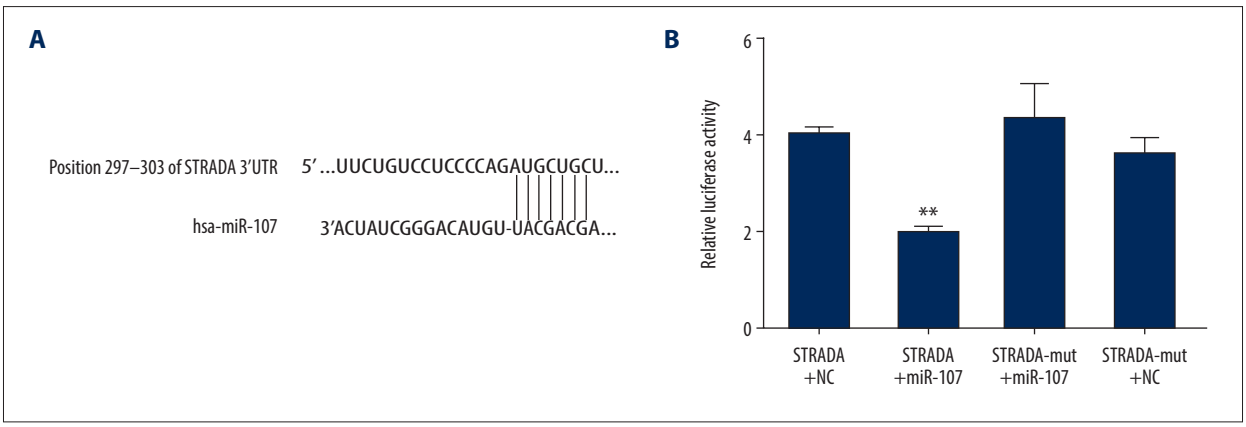

Figure 3. STRADA was the target gene of microRNA-107 (miR-107). (**A**) The target gene of miR-107 was predicted by TargetScanHuman version 7.2. (**B**) The targeting relationship between miR-107 and STRADA mRNA 3'UTR was identified using the dual-luciferase reporter assay. Data are presented as the mean±standard deviation (SD) of three independent experiments. ** P<0.001 versus the other three groups.

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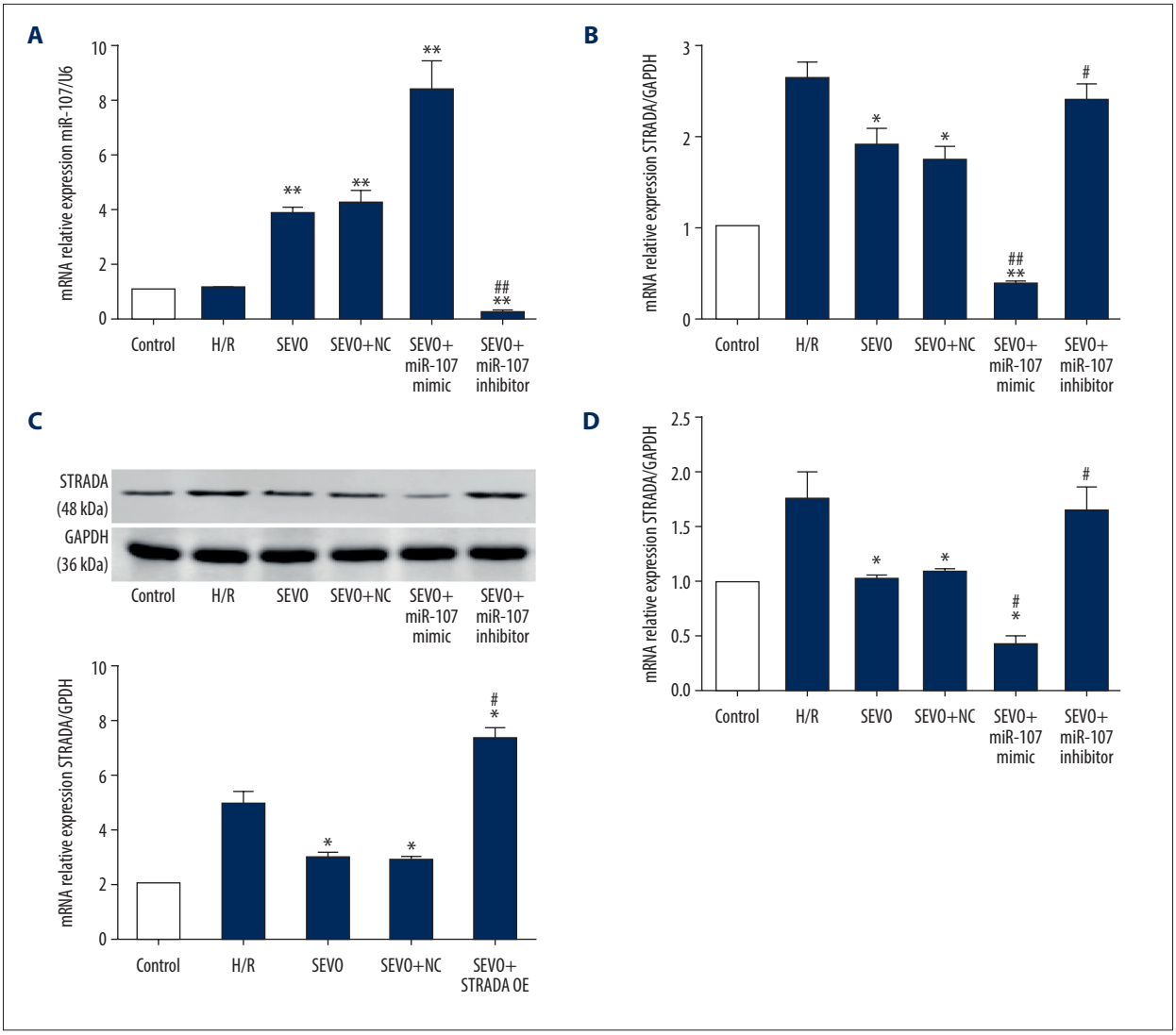

Figure 4. microRNA-107 (miR-107) inhibited STRADA expression. (**A**) The transfection efficiency of the miR-107 mimic and inhibitor was measured by quantitative real-time polymerase chain reaction (qRT-PCR) in H9C2 cells. (**B**) miR-107 expression downregulated STRADA mRNA expression in H9C2 embryonic rat cardiomyocytes. (**C**) The protein expression level of STRADA was detected by Western blot in H9C2 cells. (**D**) The transfection efficiency of STRADA overexpression plasmids was confirmed by qRT-PCR in H9C2 cells. The H9C2 cells were divided into six groups: the control group (cells without the establishment of hypoxia-reoxygenation [H/R] injury), the H/R group (cells with establishment of the H/R model), the SEVO group (cells with establishment of the H/R model and sevoflurane preconditioning), the SEVO+NC group (cells with establishment of the H/R model, sevoflurane preconditioning, and negative control), the SEVO+miR-107 mimic group (cells with establishment of the H/R model, sevoflurane preconditioning, and miR-107 mimic), the SEVO+miR-107 inhibitor group (cells with establishment of the H/R model, sevoflurane preconditioning, and miR-107 inhibitor), and the SEVO+STRADA OE group (cells with establishment of the H/R model, sevoflurane preconditioning, and STRADA overexpression). The data are presented as the mean±standard deviation (SD(of three independent experiments. * P<0.05, ** P<0.001 versus the H/R groups. # P<0.05, ## P<0.001 versus the SEVO group.

with the miR-107 inhibitor promoted cell apoptosis by 2.17-fold compared with the SEVO group (Figure 5B). However, the co-transfection of the miR-107 mimic and STRADA overexpression significantly reduced the effect of the miR-107 mimic on cell proliferation and cell apoptosis (64% decrease in cell proliferation rate versus the SEVO+miR-107 mimic group, 1.74-fold increase in the cell apoptosis rate versus the SEVO+miR-107 mimic) (Figure 5A, 5B).

Compared with the SEVO group, the caspase-3 protein level in the SEVO+miR-107 mimic group was slightly reduced, while the caspase-3 protein level in the SEVO+miR-107 inhibitor

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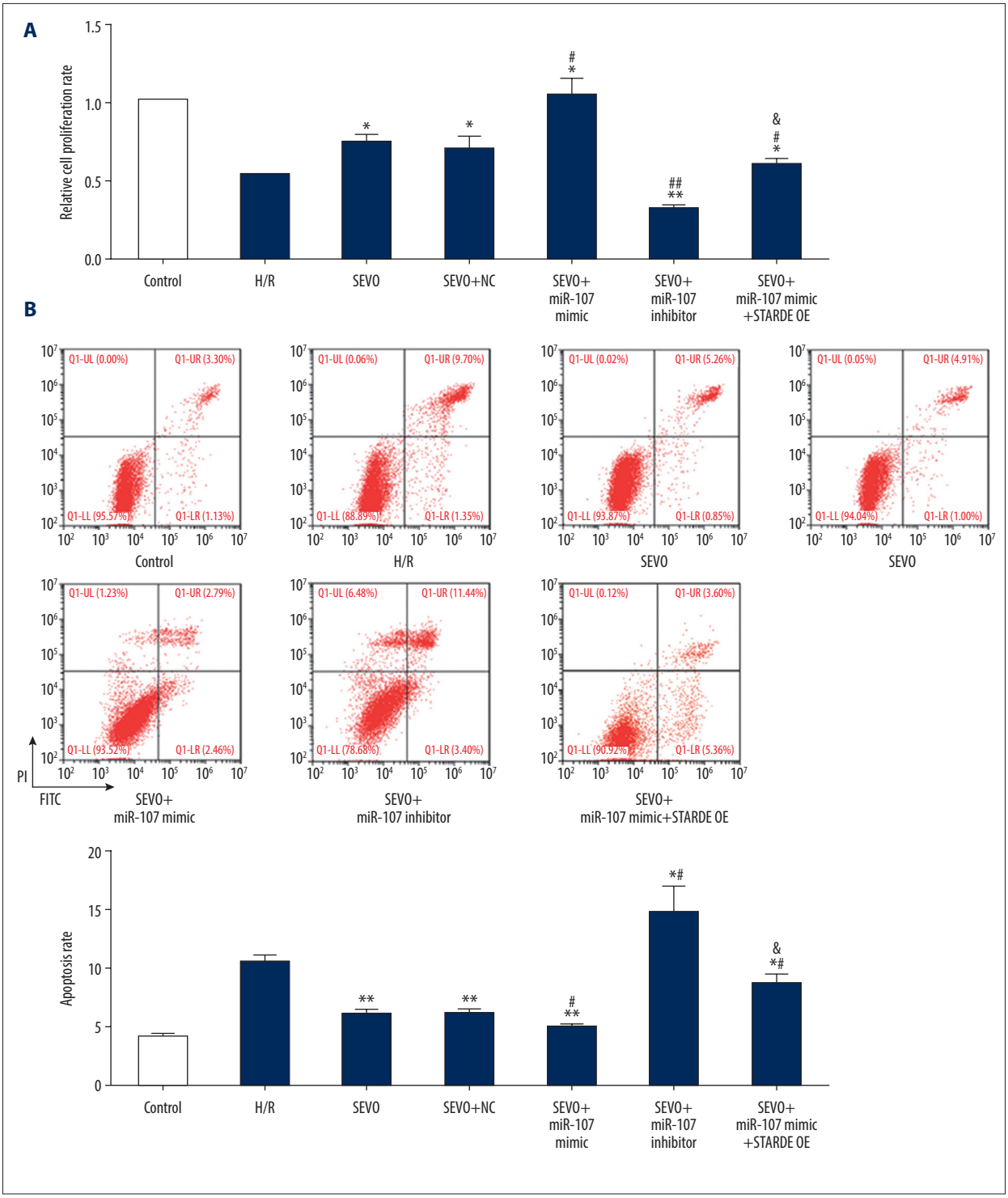
group was significantly increased by approximatively 3.7-fold (Figure 5C). The caspase-3 protein level in the SEVO+miR-107 mimic+STRADA overexpression group was significantly increased compared with the SEVO + miR-107 mimic group (Figure 5C). These findings showed that miR-107 enhanced the effects of SpostC on cell proliferation and cell apoptosis by regulating STRADA expression.



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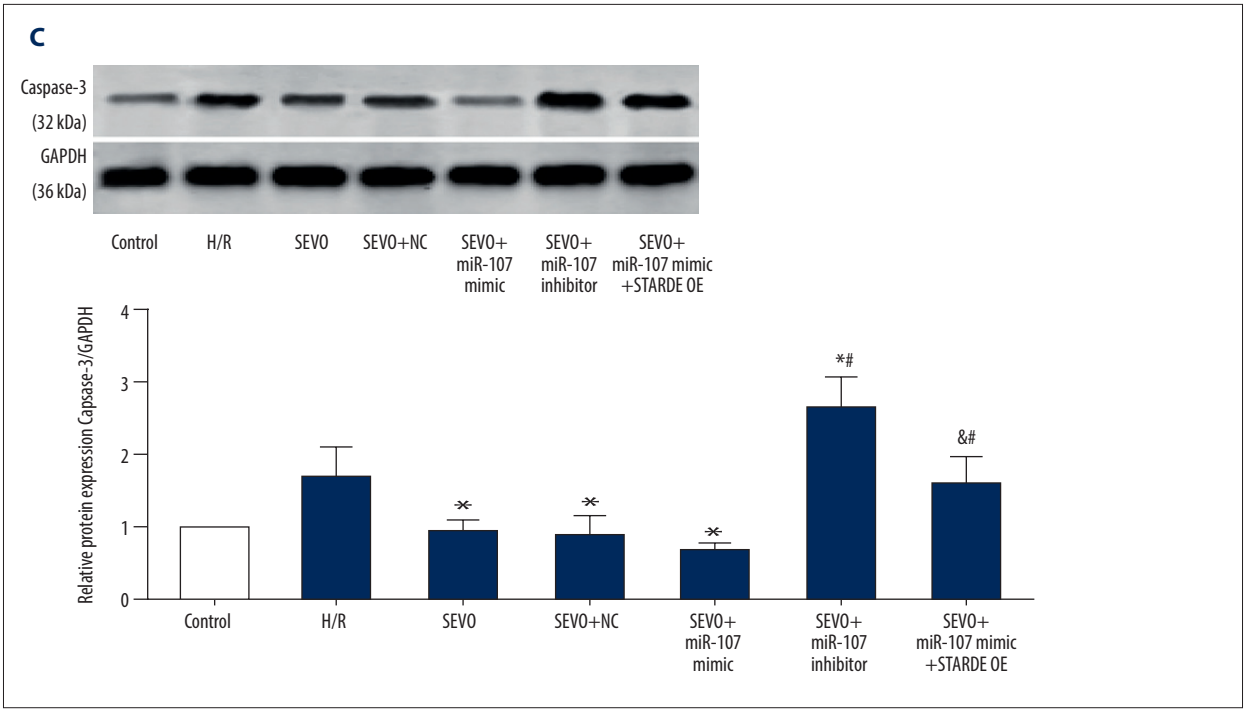

Figure 5. The effects of microRNA-107 (miR-107) expression, and co-expression of miR-107 and STRADA, on cell proliferation and apoptosis in H9C2 embryonic rat cardiomyocytes with sevoflurane preconditioning. (**A**) The cell proliferation rate was measured by the MTT assay. (**B**) The cell apoptosis rate was detected by flow cytometry. (**C**) Caspase-3 protein expression was detected using Western blot. The H9C2 cells were randomly divided into seven groups: the control group (cells without the establishment of hypoxia-reoxygenation [H/R] injury), the H/R group (cells with establishment of the H/R model), the SEVO group (cells with establishment of the H/R model and sevoflurane preconditioning), the SEVO+NC group (cells with establishment of the H/R model, sevoflurane preconditioning, and negative control), the SEVO+miR-107 mimic (cells with establishment of the H/R model, sevoflurane preconditioning, and miR-107 mimic), the SEVO+miR-107 inhibitor (cells with establishment of the H/R model, sevoflurane preconditioning, and miR-107 inhibitor), and the SEVO+miR-107 mimic+STRADA OE (cells with establishment of the H/R model of sevoflurane preconditioning, the miR-107 mimic and STRADA overexpression plasmids). Data are presented as the mean±standard deviation (SD) of three independent experiments. * P<0.05, ** P<0.001 versus the H/R groups. # P<0.05, ## P<0.001 versus the SEVO group. & P<0.05 versus the SEVO+miR-107 mimic group.

Discussion

Sevoflurane postconditioning (SpostC) has previously been shown to enhance cardiac resistance to ischemia-reperfusion (I/R) injury in experimental and clinical studies [29,30]. Hong et al. used SpostC to treat Sprague-Dawley rats with I/R injury, and showed that SpostC significantly reduced the infarct size and improved cardiac function by activation of the ERK pathway [31]. SpostC promoted autophagosome clearance *in vitro*, reduced cell damage, and enhanced cell viability to reduce hypoxia-reoxygenation (H/R) injury in H9C2 cells [32]. The findings from the present study showed that cell proliferation increased and cell apoptosis was inhibited when SpostC was used to treat the H/R injured H9C2 embryonic rat cardiomyocytes, indicating that SpostC played a cardioprotective role in H/R injury. Also, SpostC treatment resulted in an increase in microRNA-107 (miR-107) expression in H/R injured H9C2 cells. H/R injured H9C2 cells that were treated with miR-107 mimic, and SpostC showed increased cell proliferation and reduced cell apoptosis.

Previous studies have shown that microRNAs (miRNAs) participated in the cardioprotective mechanism of SpostC on I/R injury. In the I/R injured mice, miR-155 overexpression reduced cardiac functions, and increased infarct size in the sevoflurane-treated I/R mice, indicating that miR-155 inhibited the cardioprotective role of sevoflurane in myocardial I/R injury [33]. Qi et al. showed that SpostC protected the myocardium from I/R injury by upregulating miR-145 in a mouse model of I/R injury *in vivo* [34]. In a previously reported study, in H/R injured H9C2 cells, sevoflurane preconditioning resulted in reduced expression of miR-34a-5p and miR-34a-5p mimic significantly increased cell apoptosis and reduced cell proliferation in the sevoflurane preconditioning H/R injured H9C2 cells by targeting STX1A [24]. These previous studies suggested that different miRNAs had different roles in the protective mechanism

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of sevoflurane on I/R injury. The data from the present study showed that miR-107 had a protective role in SpostC on H/R injured H9C2 cells.

STRADA binds to and regulates the subcellular localization and activity of Ser/Thr kinase 11 (LKB1), leading to the nuclear export of LKB1 and activation of LKB1 catalytic activity [35,36]. LKB1 regulates cell mobility and polarization in human carcinomas [37–40]. Previous studies have shown that the STRADA/LKB1 heterodimer inhibited mTOR signaling through activation of phosphorylated AMPK [17,41]. In cardiac myocytes, the formation of the STRADA/LKB1 complex resulted in reduced phosphorylation of p70S6 kinase that was the key protein of the mTOR pathway via activating AMPK [42]. Zhang et al. showed that the SpostC-induced cardioprotection in I/R injury in Sprague-Dawley rats was modulated by the activation of the PI3K/Akt/mTOR pathway, and SpostC protected mitochondrial functions and had an anti-apoptotic role in I/R injured rats [43]. These findings suggested that STRADA might participate in the protection of sevoflurane on H/R injury. The findings from the present study showed that STRADA was the target gene of miR-107, and its expression was inhibited in SpostC-induced H/R injured H9C2 cells. STRADA overexpression significantly reduced the effect of the miR-107 mimic on the cardioprotective role of SpostC. Cardioprotection by SpostC resulted from the interaction between miR-107 and STRADA, which may be enhanced by the activation of mTOR signaling. These preliminary findings require validation with further *in vitro* and *in vivo* studies.

Conclusions

This study aimed to investigate the effects of microRNA-107 (miR-107) on sevoflurane postconditioning (SpostC) in H9C2 embryonic rat cardiomyocytes and to use bioinformatics analysis to identify the molecular basis of cardioprotection from sevoflurane in human cardiac tissue. SpostC reduced H/R injury in H9C2 embryonic rat cardiomyocytes by targeting the STRADA gene and upregulating the expression of microRNA-107.

Conflict of interest

None.

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