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

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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) 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 protective 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 STRADA, 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 (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=tarbase8%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

H9C2 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 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% N2 and 5% CO2 at 37°C for 180 min [26]. The serum-free DMEM medium was...
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% CO₂ 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% CO₂ 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% O₂ and 2.4% sevoflurane for 20 min at the end of hypoxia [27], after which the cells were treated as 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 (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 1×10⁶ 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).
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 approximately 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
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.
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.
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
group was significantly increased by approximately 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.

**Figure 5**

A. Relative cell proliferation rate.

B. Apoptosis rate.
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.
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 cardiomyocytes, 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.

References:

1. Shao M, Ren Z, Zhang R: MYB2L2 protects against H9C2 injury induced by hypoxia via Akt and NFkB pathways. Mol Med Rep, 2018; 17: 4832–38
2. Bai S, Wu B, Yao Z et al: Development and validation of a predictive model for predicting cardiovascular morbidity in patients after pheochromocytoma surgery. Clin Endocrinol (Oxf), 2019; 91: 490–97
3. Deng T, Wang Y, Wang C, Yan H: FABP4 silencing ameliorates hypoxia reoxygenation injury through the attenuation of endoplasmic reticulum stress-mediated apoptosis by activating PI3K/Akt pathway. Life Sci, 2019; 224: 149–56
4. Saeed WK, Jun DW, Jang K et al: Does necroptosis have a crucial role in hepatic ischemia-reperfusion injury? PLoS One, 2017; 12: e0184752
5. Javadov S, Kuznetsov A: Mitochondrial permeability transition and cell death: the role of cyclophilin D. Front Physiol, 2013; 4: 76
6. Chen S, Li S: The Na+/Ca(2)+ exchanger in cardiac ischemia/reperfusion injury. Biochem, 2018; 51: 46–62
7. Parker WE, Orlova KA, Parker WH, et al. Rapamycin prevents seizures after deplination of STRADA in a rare neurodevelopmental disorder. Sci Transl Med, 2013; 5: 182ra53
8. Liu Q, Wu DH, Han L et al: Roles of microRNAs in psoriasis: Immunological functions and potential biomarkers. Exp Dermatol, 2017; 26: 359–67
9. Zhu X, Lu X: MiR-423-5p inhibition alleviates cardiomyocyte apoptosis and mitochondrial dysfunction caused by hypoxia/reoxygenation through activation of the wnt/beta-catenin signaling pathway via targeting MYB2L2. J Cell Physiol, 2019; 234: 22034–43
10. Chen F, Yang J, Li Y, Wang H: Circulating microRNAs as novel biomarkers for heart failure. Hellenic J Cardiol, 2018; 59: 209–14
11. Qin H, Ji F, Fan BY et al: MicroRNA-24-3p attenuates myocardial ischemia/reperfusion injury by suppressing RIPK1 expression in mice. Cell Physiol Biochem, 2018; 51: 46–62
12. Yang L, Wang B, Zhou Q et al: MicroRNA-21 prevents excessive inflammation and cardiac dysfunction after myocardial infarction through targeting KBTBD7. Cell Death Dis, 2018; 9: 769
13. Wen L, Wang L, Sun X et al: Sevoflurane preconditioning promotes activation of resident CSCs by transplanted BMSCs via miR-210 in a rat model for myocardial infarction. Oncotarget, 2017; 8: 114637–47
14. Wenlan L, Zhongyuan X, Shaoqing L et al: MiR-34a-5p mediates sevoflurane preconditioning induced inhibition of hypoxia/reoxygenation injury through targeting STX1A in cardiomyocytes. Biomed Pharmacother, 2018; 102: 153–59
15. Wang JX, Zhang XJ, Li Q et al: MicroRNA-103/107 regulate programmed necrosis and myocardial ischemia/reperfusion injury through targeting FADD. Cell Death Dis, 2018; 9: 769
16. Baas AF, Boudeau J, Sapkota GP et al: Activation of the tumour suppressor kinase LKB1 by the STE20-like pseudokinase STRAD. EMBO J, 2003; 22: 3062–72
17. Puffenberger EG, Strauss KA, Ramsey KE et al: Polyhydramnios, megalencephaly and symptomatic epilepsy caused by a homozygous 7-kilobase deletion in LYSK. Brain, 2007; 130: 1929–41.
18. Parker WE, Orlova KA, Parker WH, et al. Rapamycin prevents seizures after deplination of STRADA in a rare neurodevelopmental disorder. Sci Transl Med, 2013; 5: 182ra53
19. Baas AF, Boudeau J, Sapkota GP et al: Activation of the tumour suppressor kinase LKB1 by the STE20-like pseudokinase STRAD. EMBO J, 2003; 22: 3062–72
20. Puffenberger EG, Strauss KA, Ramsey KE et al: Polyhydramnios, megalencephaly and symptomatic epilepsy caused by a homozygous 7-kilobase deletion in LYSK. Brain, 2007; 130: 1929–41.
21. Parker WE, Orlova KA, Parker WH, et al. Rapamycin prevents seizures after deplination of STRADA in a rare neurodevelopmental disorder. Sci Transl Med, 2013; 5: 182ra53
22. Tan H, Qi J, Fan BY et al: MicroRNA-24-3p attenuates myocardial ischemia/ reperfusion injury by suppressing RIPK1 expression in mice. Cell Physiol Biochem, 2018; 51: 46–62
23. Yang L, Wang B, Zhou Q et al: MicroRNA-21 prevents excessive inflammation and cardiac dysfunction after myocardial infarction through targeting KBTBD7. Cell Death Dis, 2018; 9: 769
24. Wen L, Wang L, Sun X et al: Sevoflurane preconditioning promotes activation of resident CSCs by transplanted BMSCs via miR-210 in a rat model for myocardial infarction. Oncotarget, 2017; 8: 114637–47
25. Wenlan L, Zhongyuan X, Shaoqing L et al: MiR-34a-5p mediates sevoflurane preconditioning induced inhibition of hypoxia/reoxygenation injury through targeting STX1A in cardiomyocytes. Biomed Pharmacother, 2018; 102: 153–59
26. Wang JX, Zhang XJ, Li Q et al: MicroRNA-103/107 regulate programmed necrosis and myocardial ischemia/reperfusion injury through targeting FADD. Cell Death Dis, 2018; 9: 769
27. 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.

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Conflict of interest

None.
26. Cheng J, Wu Q, Lv R et al: MicroRNA-449a inhibition protects H9C2 cells against hypoxia/reoxygenation-induced injury by targeting the Notch-1 signaling pathway. Cell Physiol Biochem, 2018; 46: 2587–600
27. Yu J, Wu J, Xie P et al: Sevoflurane postconditioning attenuates cardiomyocyte hypoxia/reoxygenation injury via restoring mitochondrial morphology. Peer J, 2016; 4: e2659
28. Livak KJ, Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods, 2001; 25: 402–8
29. Shiraishi S, Cho S, Akiyama D et al: Sevoflurane has postconditioning as well as preconditioning properties against hepatic warm ischemia-reperfusion injury in rats. J Anesth, 2019; 33: 390–98
30. Wang J, Zheng H, Chen CL et al: Sevoflurane at 1 MAC provides optimal myocardial protection during off-pump CABG. Scand Cardiovasc J, 2013; 47: 175–84
31. Xie H, Zhang J, Zhu J et al: Sevoflurane post-conditioning protects isolated rat hearts against ischemia-reperfusion injury via activation of the ERK1/2 pathway. Acta Pharmacol Sin, 2014; 35: 1504–13
32. Yang L, Wu J, Xie P et al: Sevoflurane postconditioning alleviates hypoxia-reoxygenation injury of cardiomyocytes by promoting mitochondrial autophagy through the HIF-1/BNIP3 signaling pathway. Peer J, 2019; 7: e7165
33. Huang G, Hao F, Hu X: Downregulation of microRNA-155 stimulates sevoflurane-mediated cardioprotection against myocardial ischemia/reperfusion injury by binding to SIRT1 in mice. J Cell Biochem, 2019; 120: 15494–505
34. Qi Z, Li S, Su Y et al: Role of microRNA-145 in protection against myocardial ischemia/reperfusion injury in mice by regulating expression of GZMK with the treatment of sevoflurane. J Cell Physiol, 2019 [Epub ahead of print].
35. Zeqiraj E, Filippi BM, Goldie S et al: ATP and MO25alpha regulate the conformational state of the STRADalpha pseudokinase and activation of the LKB1 tumour suppressor. PLoS Biol, 2009; 7: e1000126
36. Dorfman J, Macara IG: STRADalpha regulates LKB1 localization by blocking access to importin-alpha, and by association with Crm1 and exportin-7. Mol Biol Cell, 2008; 19: 1614–26
37. Chen Y, Liu Y, Zhou Y, You H: Molecular mechanism of LKB1 in the invasion and metastasis of colorectal cancer. Oncol Rep, 2019; 41: 1035–44
38. Dogliotti G, Kulmann L, Dhumale P et al: Membrane-binding and activation of LKB1 by phosphatidic acid is essential for development and tumour suppression. Nat Commun, 2017; 8: 15747
39. Celiktas M, Tanaka I, Tripathi SC et al: Role of CPS1 in cell growth, metabolism and prognosis in LKB1-inactivated lung adenocarcinoma. J Natl Cancer Inst, 2017; 109: 1–9
40. Ma LG, Bian SB, Cui JX et al: LKB1 inhibits the proliferation of gastric cancer cells by suppressing the nuclear translocation of Yap and beta-catenin. Int J Mol Med, 2016; 37: 1039–48
41. Orlova KA, Parker WE, Heuer GG et al: STRADalpha deficiency results in aberrant mTORC1 signaling during corticogenesis in humans and mice. J Clin Invest, 2010; 120: 1591–602
42. Noga AA, Soltys CL, Barr AI et al: Expression of an active LKB1 complex in cardiac myocytes results in decreased protein synthesis associated with phenylephrine-induced hypertrophy. Am J Physiol Heart Circ Physiol, 2007; 292: H1460–69
43. Zhang J, Wang C, Yu S et al: Sevoflurane postconditioning protects rat hearts against ischemia-reperfusion injury via the activation of PI3K/AKT/mTOR signaling. Sci Rep, 2014; 4: 7317