Upregulated microRNA-210-3p improves sevoflurane-induced protective effect on ventricular remodeling in rats with myocardial infarction by inhibiting ADCY9

Yahui Wu1 · Taofu Wang1 · Liang Qiao2 · Hongqi Lin1

Received: 12 July 2021 / Revised: 15 October 2021 / Accepted: 19 October 2021 / Published online: 6 January 2022 © The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2021

Abstract
Myocardial infarction (MI) is a significant cause of death and disability, and sevoflurane (sevo) can protect myocardium in clinic. We aim to assess the effects of miR-210-3p on MI rats undergoing sevo treatment with the involvement of adenylyl cyclase type 9 (ADCY9). Rat MI models were constructed by ligation of the left anterior descending, and the modeled rats were respectively treated with sevo, miR-210-3p agomir, antagomir, or overexpressed ADCY9. Then, miR-210-3p and ADCY9 expression, cardiac function, myocardial injury and fibrosis, and cardiomyocyte apoptosis in rats were evaluated. Target relation between miR-210-3p and ADCY9 was detected. miR-210-3p was downregulated while ADCY9 was upregulated in MI rats. Sevo was able to promote cardiac function and attenuate myocardial injury and fibrosis, as well as cardiomyocyte apoptosis in MI rats. These effects of sevo were strengthened by miR-210-3p elevation but abolished by miR-210-3p inhibition in MI rats. The role of elevated miR-210-3p in MI rats was reversed by overexpression of ADCY9. Upregulated miR-210-3p improves sevo-induced protective effect on ventricular remodeling in rats with MI through inhibiting ADCY9.

Keywords Myocardial infarction · Ventricular remodeling · Sevoflurane · MicroRNA-210-3p · ADCY9

Introduction
Myocardial infarction (MI) is mostly caused by rupture of atherosclerotic plaques leading to thrombus formation in lumen of a coronary vessel, which in turn blocks the blood flow to distal myocardium (Curley et al. 2018). MI is a life-threatening disease with a high mortality rate that appears not only in developed countries but also in industrialized developing countries (Michaud et al. 2020). In pathology, MI is defined as cardiomyocyte apoptosis resulting from the prolonged ischemia (Thygesen et al. 2007). Stents were used to treat MI, which open the occluded coronary artery. Nevertheless, when stents are insufficient, coronary bypass is performed by cardiac pulmonary bypass surgery to maintain regular nourishment of the heart (Aydin et al. 2019). Although 30-day mortality rate of MI has reduced in the last two decades, it still stays at 7.8% (Montrief et al. 2019). Therefore, it is urgent to explore novel biomarkers for the treatment of MI.

Sevoflurane (sevo, 1,1,1,3,3,3-hexafluoro-2-[fluoromethoxy] propane), a highly fluorinated methyl isopropyl ether, was utilized as an inhalational anesthetic for induction and maintenance of general anesthesia (Montrief et al. 2019). Although basic and clinical researches have suggested that the anesthetic postconditioning of sevo is an effective strategy to ameliorate myocardial injury (Qiao et al. 2019; Cao et al. 2015), the detailed mechanism has not been completely explored. MicroRNAs (miRNAs) are small non-coding RNAs that constrain gene expression through binding the 3’-untranslated region (3’-UTR) and then influence mRNA stability or protein translation (Yang et al. 2019). Previous studies have shown that sevo treatment was able to alter the expression of miRNAs in cardiovascular diseases (Wen et al. 2017; Qi et al. 2019).
miR-210-3p is one of the two mature forms for miR-210 which is the most prominent hypoxia-regulated miRNAs (Pasculli et al. 2019). miR-210-3p has been elucidated to show a decreased level in MI mice (Ma et al. 2018), while the underlying mechanisms of miR-210-3p on MI as well as the regulatory relation between sevo and miR-210-3p remain rarely explored. Adenylyl cyclase type 9 (ADCY9) is an enzyme producing the ubiquitous second messenger cyclic AMP. In the absence of cholesteryl ester transfer protein activity, ADCY9 inactivation is found to protect against atherosclerosis (Rautureau et al. 2018).

This study focused on the effects of sevo treatment and miR-210-3p on ventricular remodeling following MI. We inferred that miR-210-3p may affect the sevo-induced effect on ventricular remodeling in MI rats with the involvement of ADCY9.

Materials and methods

Ethics statement

Animal experiments were strictly in accordance with the Guide to the Management and Use of Laboratory Animals issued by the National Institutes of Health. The protocol of animal experiments was approved by the Institutional Animal Care and Use Committee of Henan Provincial People’s Hospital.

Experimental animals

Clean and healthy adult male Sprague Dawley rats (aged 8–12 weeks and weighed 200–300 g) obtained from Henan Experimental Animal Center (Zhengzhou, Henan, China) were fed in standard rat cages capable of ventilation in a quiet environment (natural light, 23–25 °C, relative humidity of 55–60%). The paddings in cages were changed every day.

Establishment of MI rat models

Rats were anesthetized by intraperitoneal injection of 2.5% pentobarbital sodium (50 mg/kg), fixed on an operating table in a supine position, and intubated with a small animal artificial ventilator (respiratory rate at 50–60 times/min, tidal volume = 2 mL/mg based on body weight). A longitudinal incision was made 1 cm from the left side of rat sternum to open the thorax and pericardium. With portal coronary vein as the mark, 5/0 suture was inserted 2 mm below root of the left atrial appendage and reached the arterial cone (3–4 mm from the origin, depth of 1.5–2 mm). A piece of plastic tube (length of 1 cm) was pressed down along the double lines to block the blood flow of the left anterior descending branch of the coronary artery (LAD). The gray color of the left ventricular wall and the ST segment elevation of electrocardiogram indicated complete ligation. Then, the rats were subjected to 30-min ischemia and 24-h reperfusion (Ferdinandy et al. 2007).

Animal grouping

The modeled rats were classified into the following groups (n = 10): the MI, sevo, sevo + antagomir negative control (NC), sevo + miR-210-3p antagomir, sevo + agomir NC, sevo + miR-210-3p agomir, sevo + miR-210-3p agomir + overexpressed (oe)-NC, and sevo + miR-210-3p agomir + oe-ADCY9 groups. Rats in the MI group performed under the MI model establishment, and those in the sevo group were subjected to inhalation of 3.4% sevo for 5 min before reperfusion. Rats in other groups were, respectively, intramyocardially injected with lentivirus (5 × 10^7 TU/mL) carrying NC of miR-210-3p antagomir, miR-210-3p antagomir, NC of miR-210-3p agomir, miR-210-3p agomir + empty vector, and miR-210-3p agomir + 10 μg (100 μL) overexpressed ADCY9 plasmid 30 min before the surgery. Next, rats were subjected to inhalation of 3.4% sevo for 5 min before reperfusion. Rats in the sham group (LAD was threaded but not ligated) were taken as the controls (Qi et al. 2019).

Echocardiography

Transthoracic echocardiography was performed 14 days after surgery using a SONOS-7500 echocardiography system (Philips Medical Systems, Andover, MA, USA) equipped with a 15-MHz transducer. The long axis and short axis sections of the left ventricle beside the sternum were obtained by gently touching the precordial area with an ultrasonic probe. The left ventricle internal dimensions at systole (LVIDs) and diastole (LVIDd) were detected, and the left ventricle ejection fraction (LVEF) and fractional shortening (FS) were calculated (n = 10).

Collection of blood samples and myocardial tissues

Blood from celiac artery (n = 5) was collected before euthanasia. The heart tissue was then harvested. All the samples were stored at −20°C before analysis. Blood was used for testing the contents of creatine kinase (CK) and lactate dehydrogenase (LDH). Tissues were used for the detection of malondialdehyde (MDA) and superoxide dismutase (SOD) levels, protein and RNA extraction, hematoxylin–eosin (HE) staining, Masson staining, and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling (TUNEL) staining.
Evaluation of creatine kinase (CK), lactate dehydrogenase (LDH), malondialdehyde (MDA), and superoxide dismutase (SOD) levels

Serum samples were extracted from rats to assess the activities of CK and LDH using the kits (JianCheng Bioengineering Institute, Jiangsu, China). Tissue homogenate was appropriately diluted, and protein concentration was measured with bicinchoninic acid (BCA) kits (Sigma-Aldrich Chemical Company, MO, USA). Then, SOD activity and MDA content were measured based on the kits (JianCheng Bioengineering Institute).

HE staining

Sections were normally dehydrated with gradient ethanol, permeabilized with xylene, rinsed by deionized water, and stained by hematoxylin for 3–5 min, and then differentiated by 1% hydrochloric alcohol (20 s), blued by 1% ammonium hydroxide (30 s), and counterstained with 1% eosin dye solution (5 min). Next, the sections were normally dehydrated, permeabilized, dried, sealed, and observed and photographed through a microscope.

Masson staining

Sections were normally dehydrated with gradient ethanol, permeabilized with xylene, stained by hematoxylin for 30 s, blued with clear water for 3 min, and soaked in ponceau S acid fuchsin for 3 min, and then differentiated by 1% phosphomolybdic acid (twice, 3 min/time), counterstained with 1% aniline blue for 5 min, and differentiated by 1% glacial acetic acid for 10 s. Then, the sections were permeabilized, sealed, and conducted with microscopy observation. An image analysis system was used for semi-quantitative analysis of myocardial collagens, and the collagen volume fraction (CVF) was calculated. Five random fields of view were selected for the calculation of percentage of collagen tissues in each field, and the mean value was adopted.

TUNEL staining

Paraffin sections were normally dewaxed and hydrated and then TUNEL staining performed with the TUNEL kits (Roche, CA, USA). The sections were developed with diamobenzidine, counterstained with hematoxylin for 3 min, and soaked in ethanol for 1–2 s, and then were dehydrated and permeabilized with xylene and sealed with neutral balsam. Positive standard: nuclei of apoptotic cardiomyocytes were brownish yellow under a light microscope.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Trizol kits were used to extract total RNA in myocardial tissues, and the RNA was reversely transcribed into cDNA according to the instructions of the kits (TaKaRa Biotechnology Co., Ltd., Liaoning, China). The transcribed cDNA was used for PCR amplification. Data were analyzed using 2−ΔΔCt method, and the PCR primers (Supplementary Table 1) were synthesized by TaKaRa. U6 was used as the internal reference for miR-210-3p, and glyceraldehyde phosphate dehydrogenase (GAPDH) was taken as the internal reference for ADCY9, collagen I, and collagen III.

Western blot analysis

Total protein in myocardial tissues was extracted. The proteins were treated with 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis for 2 h, and transferred onto membranes, which were blocked with 5% skim milk powder for 2 h. Afterwards, the membranes were incubated with primary antibodies ADCY9 (1:1000) and GAPDH (1:500; all from Abcam) and then incubated with relative secondary antibody (1:1000). Enhanced chemiluminescence method was used for development, and the optical density values of bands were measured with GAPDH as the loading control. The ratio of OD value of target band to that of GAPDH was the relative expression of target proteins.

Dual luciferase reporter gene assay

ADCY9 was predicted as a target gene of miR-210-3p by using TargetScan (http://www.targetscan.org). The 3′-UTR of ADCY9 containing the complementary sequences of miR-210-3p or mutation sequences was amplified and subcloned into the pmirGLO plasmid (Promega, Madison, WI, USA), which were named as ADCY9-3′-UTR wild-type (WT) and ADCY9-3′-UTR mutant (MUT), respectively. Lipidosome method was used to respectively co-transfect the report gene vectors and miR-210-3p mimic and mimic NC into 293 T cells (American Type Culture Collection, VA, USA). Upon 48 h incubation at 37 °C, the luciferase activity was tested using a Dual-Luciferase Reporter Assay system (Promega). Renilla luciferase activity was selected for normalization.

Statistical analysis

All data analyses were conducted using SPSS 21.0 software (IBM Corp. Armonk, NY, USA). The data were expressed as mean ± standard deviation. The t test was performed for comparisons between two groups; one-way or two-way analysis of variance (ANOVA) was used for comparisons.
Results

Sevo improves cardiac function of MI rats

The role of sevo has been identified in myocardial ischemia–reperfusion (I/R) injury (Liu et al. 2019). In order to explore the effect of sevo on MI, we firstly evaluated rat cardiac function using an electrocardiograph, and the results (Fig. 1A–D) indicated that LVIDs and LVIDd were heightened, while LVEF and FS were lowered in the MI group versus those in the sham group (all \( P < 0.05 \)); rats in the sevo group had suppressed LVIDs and LVIDd and elevated LVEF and FS when compared with the MI group (all \( P < 0.05 \)). The activities of serum CK and LDH as well as myocardial MDA and SOD were determined, and we found that (Fig. 1E–H) versus the sham group, the MI group exhibited increased levels of CK, LDH, and MDA and decreased level of SOD (all \( P < 0.05 \)). In comparison with the MI group, levels of CK, LDH, and MDA were reduced, and SOD level was increased in the sevo group (all \( P < 0.05 \)).

Collagen I and collagen III expressions in rats were detected to investigate the role of sevo on myocardial collagen expression in MI rats. The results suggested that (Fig. 1I) collagen I and collagen III were upregulated in the MI group rather than those in the sham group (both \( P < 0.05 \)), while the upregulated collagen I and collagen III were suppressed in rats of the sevo group compared with those in the MI group (both \( P < 0.05 \)). Masson staining results (Fig. 1J, K) showed that CVF was significantly increased in the MI group versus the sham group; CVF was significantly decreased in the sevo group compared with the MI group. Results of HE staining (Fig. 1L) implied that in the sham group, myocardial fibers were arranged orderly, and there existed a clear striation structure without myocardial fiber rupture and inflammatory cell infiltration. In the MI group, myocardial fibers were arranged disorderly, and there exhibited myocardial fiber rupture, fuzzy striation structure, and a large number of inflammatory cell infiltration. In the sevo group, the myocardial injury was improved. TUNEL staining was used to detect the apoptosis of cardiomyocytes, and the results (Fig. 1M) showed that versus the sham group, the apoptosis rate of cardiomyocytes in the MI group was significantly increased; in contrast to the MI group, the apoptosis rate of cardiomyocytes in the sevo group was significantly decreased (all \( P < 0.05 \)). These results suggested that sevo can reduce the collagen fibers and cardiomyocyte apoptosis in myocardial tissue of MI rats.

miR-210-3p downregulation eliminates sevo-induced effect on improving MI in a rat model

miR-210-3p has been identified to be lowly expressed after MI (Ma et al. 2018; Zhong et al. 2020a). Here, we detected miR-210-3p expression in MI rat myocardial tissues to explore its regulatory role in MI. It was found via RT-qPCR that (Fig. 2A) miR-210-3p was downregulated in the MI group versus the sham group; in contrast to the MI group, miR-210-3p was upregulated in the sevo group (all \( P < 0.05 \)).

As reported, the upregulation of miR-203 by sevo protected rats from myocardial I/R injury (Tan et al. 2020a). Sevo attenuated myocardial I/R injury in mice by inhibiting CotII and upregulating miR-204 (Tan et al. 2020b). This suggests that sevo may regulate miRNAs as its anti-MI molecular mechanism. In order to detect the effect of sevo on miR-210-3p, we downregulated miR-210-3p in sevo-treated MI rats, and it was verified by RT-qPCR that (Fig. 2B) compared with the sevo + antagomir NC group, the expression level of miR-210-3p in myocardial tissue of the sevo + mir-210-3p antagonist group was significantly decreased (\( P < 0.05 \)). Moreover, the cardiac function of rats was evaluated, and the results showed that versus the sevo + antagonist NC group, LVIDs and LVIDd in the sevo + miR-210-3p antagonist group were significantly increased, while LVEF and FS were significantly decreased (Fig. 2C–F) (all \( P < 0.05 \)), indicating that downregulating miR-210-3p reverses the effect of sevo on improving cardiac function in MI rats.

Serum activities of CK and LDH, as well as myocardial MDA content and SOD activity of the rats, were determined. The results suggested that (Fig. 2G–J) in relation to the sevo + antagomir NC group, the levels of CK, LDH, and MDA were decreased, while the SOD level was increased in the sevo + miR-210-3p antagonist group (all \( P < 0.05 \)). Collagen I and collagen III expressions were determined, and we found that (Fig. 2K) versus the sevo + antagomir NC group, collagen I and collagen III expressions were increased in the sevo + miR-210-3p antagonist group (both \( P < 0.05 \)). The Masson staining, HE staining, and TUNEL staining revealed that (Fig. 2L–O) compared with the sevo + antagomir NC group, the CVF, inflammatory cell infiltration, and cardiomyocyte apoptosis were increased in the sevo + miR-210-3p antagonist group (all \( P < 0.05 \)).

The above data implied that miR-210-3p downregulation could eliminate sevo-induced effect on improving MI in a rat model.

miR-210-3p upregulation strengthens sevo-induced effect on improving MI in a rat model

We upregulated miR-210-3p in MI rats to observe its role. Results of RT-qPCR suggested that (Fig. 3A) relative to the
sevo + agomir NC group, miR-210-3p was upregulated in the sevo + miR-210-3p agomir group ($P < 0.05$). We detected the cardiac function of rats, and it was found that (Fig. 3B–E) versus the sevo + agomir NC group, LVIDd and LVIDs in the sevo + miR-210-3p antagomir group were reduced, while LVEF and FS were enhanced, implying that upregulating miR-210-3p improves the effect of sevo on promoting cardiac function in MI rats (all $P < 0.05$).

CK, LDH, MDA, and SOD levels of the rats were assessed, and we found that (Fig. 3F–I) compared with the sevo + agomir NC group, the levels of CK, LDH, and MDA were decreased, while the SOD level was increased in the sevo + miR-210-3p agomir group (all $P < 0.05$). Collagen I and collagen III expressions were detected, and the results showed that (Fig. 3J) versus the sevo + agomir NC group, collagen I and collagen III expressions were
suppressed in the sevo + miR-210-3p agomir group (both $P < 0.05$). Results of Masson staining, HE staining, and TUNEL staining reflected that (Fig. 3K–N) versus the sevo + agomir NC group, the CVF, inflammatory cell infiltration, and cardiomyocyte apoptosis were inhibited in the sevo + miR-210-3p agomir group (all $P < 0.05$).

These results mirrored that miR-210-3p upregulation could promote sevo-induced effect on improving MI in a rat model.

ADCY9 upregulation reverses the therapeutic effects of elevated miR-210-3p on sevo-induced MI rats

In order to study the effect of ADCY9 on MI in rats treated with sevo + miR-210-3p agomir, we upregulated ADCY9 and verified the alteration using RT-qPCR and Western blot analysis (Fig. 4A, B). It came out that compared with the sevo + miR-210-3p group, ADCY9 expression was enhanced in the sevo + miR-210-3p agomir + oe-ADCY9 group ($P < 0.05$). Next, cardiac function was evaluated,
and our results indicated that in contrast to the sevo + miR-210-3p agomir group, LVIDs and LVIDd were significantly increased, while LVEF and FS were significantly decreased in the sevo + miR-210-3p agomir + oe-ADCY9 group (Fig. 4C–F) (all \( P < 0.05 \)), showing that ADCY9 elevation abolishes the effect of sevo and miR-210-3p on improving cardiac function in MI rats.

Serum activities of CK and LDH, as well as myocardial MDA content and SOD activity of the rats, were assessed. We discovered that (Fig. 4G–J) versus the sevo + miR-210-3p agomir group, the levels of CK, LDH, and MDA were enhanced, while SOD level was reduced in the sevo + miR-210-3p agomir + oe-ADCY9 group (both \( P < 0.05 \)). Results of collagen I and collagen III expression detection reflected that (Fig. 4K) the sevo + miR-210-3p agomir + oe-ADCY9 group had higher collagen I and collagen III in rat myocardial tissues was detected using RT-qPCR; K representative images of Masson staining (50 μm); L CVF of rat in each group; M representative images of HE staining (50 μm); N cardiomyocyte apoptosis in rat myocardial tissues was observed through TUNEL staining (50 μm); *\( P < 0.05 \) vs the sevo + agomir NC group; the data were expressed as mean ± standard deviation; and the unpaired \( t \) test was performed for comparisons between two groups (in A–E, \( n = 10 \); in F–N, \( n = 5 \)).

Our results showed that ADCY9 upregulation reversed the therapeutic effects of sevo and elevated miR-210-3p on MI rats.
ADCY9 is a direct target gene of miR-210-3p

To further study the effect of ADCY9 on miR-210-3p, we detected ADCY9 expression in myocardial tissue of rats using RT-qPCR and Western blot analysis. Results showed that (Fig. 5A–D) compared with that in the sevo + antagomir NC group, ADCY9 was upregulated in the sevo + miR-210-3p antagomir group (\(P < 0.05\)). In relation to that in the sevo + agomir NC group, ADCY9 was downregulated in the sevo + miR-210-3p agomir group (\(P < 0.05\)).

As predicted by a bioinformatic software (http://www.targetscan.org/vert_72/), there existed a binding relationship between miR-210-3p and ADCY9 (Fig. 5E). Outcomes of dual luciferase report gene assay indicated that (Fig. 5F) in ADCY9-3’UTR-WT, the luciferase activity of cells in the mimic NC group was lower than that in the mimic NC group (\(P < 0.01\)), while in ADCY9-3’UTR-MUT, there showed no apparent difference in luciferase activity of cells between the mimic NC group and the miR-210-3p mimic group (\(P > 0.05\)), indicating that ADCY9 is a direct target gene of miR-210-3p and miR-210-3p upregulation could inhibit ADCY9 expression.

Discussion

Adverse left ventricular remodeling after MI is the structural basis for ischemic heart failure and involves complex short- and long-term changes in size, shape, function, and cellular and molecular composition in the left ventricular (Prabhu and Frangogiannis 2016). We aimed to assess the role of sevo treatment regulating miR-210-3p in ventricular...
remodeling after MI with the involvement of ADCY9, and we found that sevo upregulated miR-210-3p to serve a protective role in ventricular remodeling after MI by inhibiting ADCY9.

In our study, MI rats were treated with sevo to observe its effects. The results reflected that sevo treatment was able to promote cardiac function and repress myocardial injury and fibrosis and cardiomyocyte apoptosis in MI rats. Consistently, Zhang et al. have elucidated that sevo contributed to stabilizing the cardiopulmonary function and preventing myocardial injury in patients undergoing coronary artery bypass (Zhang and Wang 2016). A recent literature has confirmed that sevo improved pulmonary fibrosis in rats with pulmonary arterial hypertension (Zhang and Wang 2016).

Fig. 5 ADCY9 is a direct target gene of miR-210-3p. A ADCY9 mRNA expression in the sevo+antagomir NC group and the sevo+miR-210-3p antagomir was detected using RT-qPCR; B ADCY9 protein expression in the sevo+antagomir NC group and the sevo+miR-210-3p antagomir was detected using Western blot analysis; C ADCY9 mRNA expression in the sevo+agomir NC group and the sevo+miR-210-3p agomir was detected using Western blot analysis; D ADCY9 protein expression in the sevo+agomir NC group and the sevo+miR-210-3p agomir was detected using Western blot analysis; E an online software was used to predict the binding sites of miR-210-3p and ADCY9; F target relationship between miR-210-3p and ADCY9 was confirmed by dual luciferase report gene assay; $P<0.05$ vs the sevo+antagomir NC group, $\&P<0.05$ vs the sevo+agomir NC group, *$P<0.05$ vs the mimic NC group; the data were expressed as mean±standard deviation; and the unpaired t test was performed for comparisons between two groups (in F, $N=3$; in A–E, $n=5$); in A–D, data were analyzed using one-way ANOVA; in E and F, data were analyzed using two-way ANOVA; Tukey’s post hoc test was used for pairwise comparisons after ANOVA.
It has been previously identified that sevo exposure prevented diaphragmatic oxidative stress during mechanical ventilation in a rat model (Breuer et al. 2015). Pasqualin et al. have affirmed that sevo preconditioning reduced infarct size in rats after myocardial I/R (Pasqualin et al. 2016), and Lu et al. have recently demonstrated that sevo prevented hypoxia/reoxygenation-induced cardiomyocyte apoptosis (Lu et al. 2019). Moreover, sevo treatment has been verified to have a positive or negative impact on the expression of some particular RNAs (Zhong et al. 2020b). For instance, sevo has been clarified to upregulate miR-203 in colorectal cancer (Fan et al. 2019), and it has also been revealed that sevo anesthesia positively regulated the expression of miR-34a-3p to increase viability and inhibit apoptosis of lung cells in acute lung injury (Yuan and Zhang 2020). However, the mechanisms of sevo treatment affecting expression of miR-210-3p remain unclear.

miR-210-3p expression in rat myocardial tissues was assessed in our research, and it was found that miR-210-3p was lowly expressed in myocardial tissues from MI rats, and the sevo treatment was able to upregulate miR-210-3p in MI rats. In line with this finding, Ma et al. have discovered that miR-210-3p expression was reduced in fat-1 transgenic mice in comparison to WT mice (Ma et al. 2018), and a former document has unraveled that miR-210 expression was lower in MI rats (Wang et al. 2016). Furthermore, sevo preconditioning has been demonstrated to elevate the expression of miR-210 to promote activation of resident cardiac stem cells in a MI rat model (Wen et al. 2017). As for the protective role of miR-210-3p, we have found that sevo-induced upregulation of miR-210-3p was capable of promoting cardiac function and repressing myocardial injury and fibrosis and cardiomyocyte apoptosis in MI rats. Similarly, Wang et al. have affirmed that miR-210, enriched by mesenchymal stem cells-derived extracellular vesicles, had the ability to promote cardiac function recovery (Wang et al. 2017); a study has unearthed that miR-210 protected cardiomyocytes against oxygen–glucose deprivation/reperfusion (Bian et al. 2018); and it has been uncovered that the elevation of mesenchymal stem cells-derived exosomal miR-210 reduced fibrosis of cardiac progenitor cells in the infarcted heart (Zhu et al. 2018). In addition, Arif et al. have mentioned that rodent hearts with miR-210 upregulation performed a decrease in cardiomyocyte apoptosis after myocardial infarction (Arif et al. 2017), and a recent research has identified that the elevated miR-210 alleviated endothelial oxidative stress (Liu et al. 2017). Additionally, the miRNAs were known to bind to the 3’UTR of mRNAs of their target genes (Zhang et al. 2020). The targeting relationship between miR-210-3p and ADCY9 was confirmed in our study. We also measured ADCY9 expression in rat myocardial tissues, and it came out that ADCY9 was highly expressed in myocardial tissues from MI rats and overexpression of ADCY9 reduced the effects of miR-210-3p on MI rats as well. Consistently, Yohann Rautureau et al. have identified that the ADCY9 inactivation is protective on atherosclerosis in the absence of cholesterol ester transfer protein (Rautureau et al. 2018).

In conclusion, we demonstrated that sevo treatment upregulated miR-210-3p to protect from ventricular remodeling after MI through reducing ADCY9 expression. This research may deepen the understanding of molecular mechanisms of MI. However, we did not conduct the in vitro experiment for investigating the detailed mechanisms due to the limited time and fund; thus, more efforts are needed for further investigations.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s10142-021-00816-6.

**Declarations**

**Conflict of interest** The authors declare no competing interests.

**References**

Arif M, Pandey R, Alam P, Jiang S, Sadayappan S, Paul A, Ahmed RPH (2017) MicroRNA-210-mediated proliferation, survival, and angiogenesis promote cardiac repair post myocardial infarction in rodents. J Mol Med (Berl) 95(12):1369–1385

Aydin S, Uğur K, Aydin S, Sahin I, Yardım M (2019) Biomarkers in acute myocardial infarction: current perspectives. Vasc Health Risk Manag 15:1–10

Bian WS, Shi PX, Mi XF, Sun YY, Yang DD, Gao BF, Wu SX, Fan GC (2018) MiR-210 protects cardiomyocytes from OGD/R injury by inhibiting E2F3. Eur Rev Med Pharmacol Sci 22(3):743–749

Breuer T, Maes K, Rossaint R, Marx G, Scheers H, Bergs I, Bleilevens C, Gayan-Ramirez G, Brueells CS (2015) Sevoflurane exposure prevents diaphragmatic oxidative stress during mechanical ventilation but reduces force and affects protein metabolism even during spontaneous breathing in a rat model. Anesth Analg 121(1):73–80

Cao J, Xie H, Sun Y, Zhu J, Ying M, Qiao S, Shao Q, Wu H, Wang C (2015) Sevoflurane post-conditioning reduces rat myocardial ischemia reperfusion injury through an increase in NOS and a decrease in phosphorylated NHE1 levels. Int J Mol Med 36(6):1529–1537

Curley D, Lavin Plaza B, Shah AM, Botnar RM (2018) Molecular imaging of cardiac remodelling after myocardial infarction. Basic Res Cardiol 113(2):10

Fan L, Wu Y, Wang J, He J, Han X (2019) Sevoflurane inhibits the migration and invasion of colorectal cancer cells through regulating ERK/MMP-9 pathway by up-regulating miR-203. Eur J Pharmacol 850:43–52

Ferdinandy P, Schulz R, Baxter GF (2007) Interaction of cardiovascular risk factors with myocardial ischemia/reperfusion injury, preconditioning, and postconditioning. Pharmacol Rev 59(4):418–438

Liu H, Wang J, Chen Y, Chen Y, Ma X, Bihl JC, Yang Y (2017) NPC-EVs alleviate endothelial oxidative stress and dysfunction through the miR-210 downstream Nox2 and VEGFR2 pathways. Oxid Med Cell Longev 2017:9397631

Liu AJ, Pang CX, Liu GQ, Wang SD, Chu CQ, Li LZ, Dong Y, Zhu DZ (2019) Ameliorative effect of sevoflurane on endoplasmic

© Springer
reticulum stress mediates cardioprotection against ischemia-reperfusion injury (1). Can J Physiol Pharmacol 97(5):345–351
Lu Y, Bu M, Yun H (2019) Sevoflurane prevents hypoxia/reoxygenation-induced cardiomyocyte apoptosis by inhibiting PI3KC3-mediated autophagy. Hum Cell 32(2):150–159
Ma H, Chen P, Sang C, Huang D, Geng Q, Wang L (2018) Modulation of apoptosis-related microRNAs following myocardial infarction in fat-1 transgenic mice vs wild-type mice. J Cell Mol Med 22(11):5698–5707
Michaud K, Basso C, d’Amati G, Giordano C, Kholova I, Preston SD, Rizzo S, Sabatasso S, Sheppard MN, Vink A et al (2020) Diagnosis of myocardial infarction at autopsy: AECVP reappraisal in the light of the current clinical classification. Virchows Arch 476(2):179–194
Montrief T, Davis WT, Koyfman A, Long B (2019) Mechanical, inflammatory, and embolic complications of myocardial infarction: an emergency medicine review. Am J Emerg Med 37(6):1175–1183
Pasculli B, Barbano R, Rendina M, Fontana A, Copetti M, Mazza T, Valori VM, Morritti M, Maiello E, Graziano P et al (2019) Hsa-miR-210-3p expression in breast cancer and its putative association with worse outcome in patients treated with Docetaxel. Sci Rep 9(1):14913
Pasquale RC, Mostarda CT, Souza LE, Vane MF, Sirvente R, Otsuki DA, Torres ML, Irigoyen MC, Auler JO Jr (2016) Sevoflurane preconditioning during myocardial ischemia-reperfusion reduces infarct size and preserves autonomic control of circulation in rats. Acta Cir Bras 31(5):338–345
Prabhu SN, Frangogiannis NG (2016) The biological basis for cardiac repair after myocardial infarction: from inflammation to fibrosis. Circ Res 119(1):91–112
Qi Z, Li S, Su Y, Zhang J, Kang Y, Huang Y, Jin F, Xing Q (2019) 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 234(9):16526–16539. https://doi.org/10.1002/jcp.28323
Qiao SG, Sun Y, Sun B, Wang A, Qiu J, Hong L, An JZ, Wang C, Zhang HL (2019) Sevoflurane postconditioning protects against myocardial ischemia/reperfusion injury by restoring autophagic flux via an NO-dependent mechanism. Acta Pharmacol Sin 40(1):35–45
Rautureau Y, Deschambault V, Higgins D, Rivas M, Mecteau M, Geoffroy P, Miquel G, Uy K, Sanchez R, Lavoie V et al (2018) ADCY9 (adenylate cyclase type 9) inactivation protects from atherosclerosis only in the absence of CETP (cholesteryl ester transfer protein). Circulation 138(16):1677–1692
Tan J, Wu Z, Liu J, Zhang W, Yuan W, Peng H (2020a) MicroRNA-203-mediated inhibition of doublecortin confers cardioprotection conferred by sevoflurane in rats after myocardial ischaemia-reperfusion injury. J Cell Mol Med 24(17):9825–9838
Tan DX, Chen XX, Bai TZ, Zhang J, Li ZF (2020) Sevoflurane up-regulates microRNA-204 to ameliorate myocardial ischemia/reperfusion injury in mice by suppressing Cotl1. Life Sci 259:118162
Thygesen K, Alpert JS, White HD, E.S.C.A.A.H.A.W.F.T.F.f.t.R.o. M.I. Joint (2007) Universal definition of myocardial infarction. J Am Coll Cardiol 50(22):2173–95
Wang J, Zhang Y, Liu YM, Guo LL, Wu P, Dong Y, Wu GJ (2016) Huoxue anxin recipe (1) promotes myocardium angiogenesis of acute myocardial infarction rats by up-regulating miR-210 and vascular endothelial growth factor. Chin J Integr Med 22(9):685–690
Wang N, Chen C, Yang D, Liao Q, Luo H, Wang X, Zhou F, Yang X, Yang J, Zeng C et al (2017) Mesenchymal stem cells-derived extracellular vesicles, via miR-210, improve infarcted cardiac function by promotion of angiogenesis. Biochim Biophys Acta Mol Basis Dis 1863(8):2085–2092
Wen T, Wang L, Sun XJ, Zhao X, Zhang GW, Li-Ling J (2017) Sevoflurane preconditioning promotes activation of resident CSCs by transplanted BMSCs via miR-210 in a rat model for myocardial infarction. Oncotarget 8(70):114637–114647
Yang M, Tang X, Wang Z, Wu X, Tang D, Wang D (2019) miR-125 inhibits colorectal cancer proliferation and invasion by targeting TAZ. Biorxiv Rep 39(12). https://doi.org/10.1042/BSR20190193
Yuan J, Zhang Y (2020) Sevoflurane reduces inflammatory factor expression, increases viability and inhibits apoptosis of lung cells in acute lung injury by microRNA-34a-3p upregulation and STAT1 downregulation. Chem Biol Interact 322:109027
Zhang J, Wang S (2016) Effects of sevoflurane on cardiopulmonary function in patients undergoing coronary artery bypass. J Biol Regul Homeost Agents 30(4):1079–1083
Zhang X, Li M, Sun G, Bai Y, Lv D, Liu C (2020) MiR-563 restraints cell proliferation via targeting LIN28B in human lung cancer. Thorac Cancer 11(1):55–61
Zhong L, Jia, Ye G (2020a) Rian/miR-210-3p/NFkB1 feedback loop promotes hypoxia-induced cell apoptosis in myocardial infarction through deactivating the PI3K/Akt signaling pathway. J Cardiovasc Pharmacol 76(2):207–215
Zhong H, Chen H, Gu C (2020b) Sevoflurane post-treatment upregulated miR-203 expression to attenuate cerebral ischemia-reperfusion-induced neuroinflammation by targeting MyD88. Inflammation 43(2):651–663
Zhu J, Lu K, Zhang N, Zhao Y, Ma Q, Shen J, Lin Y, Xiang P, Tang Y, Hu X et al (2018) Myocardial reparative functions of exosomes from mesenchymal stem cells are enhanced by hypoxia treatment of the cells via transferring microRNA-210 in an nSMase2-dependent way. Artif Cells Nanomed Biotechnol 46(8):1659–1670

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.