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Sevoflurane reduces cardiomyocyte injury in a hypoxia/reoxygenation model of cardiomyocytes through the linc01278/miR-134-5pt regulatory axis

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Abstract

Background Ischemia-reperfusion leads to varying degrees of myocardial cell injury. Notably, long noncoding RNA was associated with the protective effect of sevoflurane (Sev) preconditioning against myocardial ischemic injury. Therefore, we further investigated the protective mechanism of Sev-mediated linc01278 against damaged cardiomyocytes by constructing a hypoxia/reoxygenation (HR) model of cardiomyocytes.

Methods The expression of linc01278, miR-134-5p, and apoptotic biomarkers in cardiomyocytes was detected by RT-qPCR. The proliferation was detected by CCK8; apoptosis was observed by flow cytometry; and the degree of cardiomyocyte injury and the level of oxidative stress was observed by ELISA. Dual luciferase reporter assay and RIP verified linc01278 and miR-134-5p interactions.

Results linc01278 was down-regulated in the HR group and up-regulated after Sev pretreatment. Sev markedly mitigated the HR-impaired cell proliferation, reduced apoptosis, and oxidative stress, and downregulated the expression of myocardial injury markers including cTnI, CK-MB, and LDH. However, this protection was noticeably reversed by the downregulation of the linc01278 expression. Mechanistically, linc01278 binds to miR-134-5p. miR-134-5p was highly expressed in cardiomyocytes of the HR, and lowly expressed in the Sev groups. The cardioprotective effect of Sev weakened by si-linc01278 was typically restored by miR-134-5p inhibitor.

Conclusions Sev attenuates HR-stimulated myocardial injury through linc01278/miR-134-5p axis-mediated proliferation, apoptosis, and oxidative stress.

Keywords Sevoflurane, Cardiomyocyte, linc01278, Hypoxia/reoxygenation model, miR-134-5p

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Background

Ischemic death of cardiomyocytes can cause irreversible damage to the heart, and in severe cases, even death. Acute myocardial infarction or cardiac interventions have been reported to be an unavoidable problem in medicine leading to myocardial cell damage [1]. Currently, myocardial ischemia-reperfusion (MI/R) is mainly used to restore blood flow to ischemic tissues and protect the heart and is the first line of hope for patients with heart diseases including heart failure and coronary artery disease. However, MI/R surgical injury is also one of the factors causing disability and death in cardiovascular disease patients. Therefore, it is necessary to find new ways to mitigate myocardial cell injury and to investigate the protective mechanism of myocardial cell injury, which is an important step in promoting the prevention and treatment of cardiovascular diseases.

Long-stranded noncoding RNAs (lncRNAs) are RNAs that are not less than 200 nt and do not have protein-coding capacity, taking part in the progression of several cardiovascular diseases [2]. For example, lncRNA CHKB-D is down-regulated in dilated cardiomyopathy (DCM) [3]. Linc01278, a novel lncRNA, is located on human chromosome Xq11.1 and contains five exons. Notably, Ma et al. analyzed differentially expressed lncRNAs associated with myocardial infarction and identified linc01278. However, its cardioprotective role in H/R-induced myocardial injury is unknown [4]. Mechanistically, highly heterogeneous LncRNAs usually prefer to bind to miRNAs to function, preventing post-transcriptional expression of downstream target genes and leading to the silencing of related genes to cause disease [5, 6]. Previous studies reported that linc01278 directly binding miR-134 was engaged in osteosarcoma [7] and colorectal carcinogenesis [8]. In addition, it has been reported in the literature that miR-134-5p is also able to participate in the regulation of cardiomyocyte injury mechanisms. Jibin Y et al. [9] found that miR-134-5p ameliorated myocardial infarction symptoms through Creb1. miR-134-5p/XIAP expression was elevated in acute myocardial infarction (AMI) and HR-induced cardiomyocytes (AC16 and HCM), which were involved in the regulation of cardiomyocyte injury in AMI and HR [10]. Currently, it is unknown whether linc01278 is involved in myocardial injury by targeting miR-134-5p.

Sevoflurane (Sev) is a clinical inhalational anesthetic used for general anesthesia in children [11], which is safe and efficient. Investigations have found that Sev has a protective effect on the nervous system [12] and the treatment of cardiac patients [13], and can somewhat improve the prognosis of patients with cardiac hypoxia/reperfusion. Sevoflurane was found to protect HR cardiomyocytes from injury through the miR-30a-5p/LINC01133 regulatory axis [14] to improve the prognosis of patients undergoing double valve replacement surgery [15]. Duo Q [16] reported that Sev preconditioning attenuated HR rat cardiomyocyte injury. This shows that sevoflurane is particularly important for reducing the risk of cardiac surgery and improving the prognosis of patients.

Therefore, in this study, Sev pretreatment was first used to assess the protective effect of hypoxia/restimulation on AC16 in cardiomyocytes. A hypoxia/reoxygenation (HR) cell model was constructed to explore the expression of LncRNA linc01278 in Sev pretreated cardiomyocytes, and the reciprocal miRNAs of linc01278 were predicted by the LncRBase database, to reveal the potential regulatory mechanism of linc01278-miRNA axis in Sev pretreated cardiomyocytes.

Methods

Establishment of cardiomyocyte HR models

Human cardiomyocytes AC16 were grown in a DMEM complete medium (containing 1% cyan/chain and 10% fetal bovine serum). The HR model was constructed using logarithmic growth phase cells with a confluence of 70% or more. Control cells were left untreated and grown in an incubator with 5% CO_2 and 95% O_2. According to the previous study [14], the HR cells were first cultured in 5% CO_2, 95% N_2 for 6 h, and then replaced with new DMEM complete medium in 5% CO_2 for another 6 h. The Sev cells needed to be pretreated with drugs before receiving H/R stimulation.

Sevoflurane pretreatment and cell transfection

Non-control cells were treated differently. Among them, the cells in the HR group were treated with 6 h hypoxia and 6 h reoxygenation; the cells in the Sev group were treated with 2% sev for 1 h [14, 17], followed by 6 h hypoxia and 6 h reoxygenation; the cells in the control group were cultured under normal conditions. Small interfering RNA targeting linc01278 and its negative control (si-NC) as well as miR-134-5p inhibitor or inhibitor NC were purchased from RiboBio (Guangzhou, China). cardiomyocyte was inoculated in 6-well plates in advance and cultured for 24 h. Cells were transfected with Lipofectamine™ 3000 (L3000001, Thermo, U.S.) at a density of about 70%. Opti-MEM + *Lipo* 3000 was added to tube A at the recommended ratio and left to stand, and Opti-MEM + si- was added to tube B at the recommended ratio and left to stand. The liquid in tube B was mixed with tube A and added to the corresponding well plate, and the medium was changed to complete medium after 6 h in the incubator, and the experiment was carried out after 12–18 h.

RNA extraction and real-time fluorescence quantitative PCR technique (RT-qPCR)

Total RNA from different treated cells was extracted using Trizol reagent (Invitrogen, Madison, U.S.) according to the manufacturer's instructions and stored temporarily in an ice bath. The RNA concentration was determined by NanoDrop 2000 Bio-spectrometer (Thermo, U.S.) and RNA was reverse transcribed to cDNA according to the manufacturer's instructions of the cDNA Synthesis Kit (E6300L; New England Biolabs, U.S.). Subsequently, RNA was transcribed into cDNA using the SYBR Premix Ex Taq II kit (RR820L, TaKaRa, Japan) on an AriaMx real-time PCR system (G8830A, Santa Clara, U.S.) for RT-qPCR reactions under the following conditions: 95℃ for 10 min, 95℃ for 15 s, and 60℃ for 1 min for a total of 40 cycles. LncRNA and miRNA expression were normalized by GAPDH and small RNA U6, respectively. The results were quantified by the $2^{-\Delta\Delta Ct}$ calculation method to calculate the relative gene expression. RT-qPCR primer sequences for the genes involved are shown in Supplementary Table S1.

CCK-8 examination of cell proliferation ability

AC16 cells with different treatments after transfection were inoculated into 96-well plates (1×10^3 cells/well) for continuous culture. Cell proliferation was detected by the CCK-8 method. Every 24 h, 8 µl of CCK-8 reagent was added to the cells and incubated in a 37 ℃ thermostat for 1 h. The absorbance values at 450 nm were then recorded using a Spectra-Max Plus 384 microplate reader (Molecular Devices, San Jose, U.S.). The average of 3 assays was taken to count the proliferative capacity of cardiomyocytes.

Flow cytometry to detect apoptotic ability

Apoptosis was detected using the Annexin V-FITC/R-Propidium Apoptosis Kit (K101; BioVision, Milpitas U.S.) according to the manufacturer's instructions. No less than 1×10^6 cells were precipitated by centrifugation and then resuspended twice by rinsing with cold PBS. To the precipitate, 200 µl of staining buffer was added to resuspend the cells, and then 10 µl Annexin V-FITC was dropped into the reaction for 15 min protected from light. 200 µl of staining buffer containing 5 µl of PI was added to the above cell suspension and incubated for 1 h. The cells were resuspended by filtration through a 200-mesh filter and detected by flow cytometry (APO006; Bio-Rad, U.S.) to detect the effects of different treatments on the apoptotic ability of cells.

Myocardial injury and oxidative stress marker assays

Changes in cardiac troponin l (cTnl) and lactate dehydrogenase (LDH) expression in cardiomyocytes after different treatments were detected by chemiluminescence. The fluorescence values of the treated groups were analyzed by Atellica IM fully automated chemiluminescence immunoassay analyzer according to the manufacturer's instructions of Troponin I Assay Kit (Chemiluminescence) (10995697, Siemens, U.S). AC16 cells (1×10^4) were inoculated into 96-well plates and grown for 24 h according to the manufacturer's instructions of Cyto Tox 96® Non-Radioactive Cytotoxicity Assay (G1780, Promega, Beijing, China). 10× lysates were added sequentially, and after 45 min, 50 µl of Cyto Tox96 reaction was added. After 45 min, 50 µl of Cyto Tox96 was added to the reaction for 30 min, and finally, 50 µl of termination solution was added to end the reaction. Measured the absorbance at 490 nm (Hidex Sense Enzyme Labeler) [18] and calculated the LDH release ratio. Biochemical indicators of residual cardiomyocyte injury markers were detected by enzyme-linked immunosorbent assay (ELISA). The supernatants of the cells in each group were collected according to the recommended concentration, and were analyzed by creatine kinase isoenzyme (CK-MB) assay kit (H197-1-1, Nanjing Jiancheng, China), human MDA assay kit (ml950271, ELISA, Shanghai, China), human superoxide dismutase (SOD) Elisa assay kit (ml063052, ELISA, Shanghai, China), Glutathione Peroxidase (GSH-Px) Assay Kit (ml095262, ELISA, Shanghai, China) were used to detect the changes in the expression of CK-MB, MDA, SOD, and GSH-Px in the different treatment groups at 450 nm, respectively.

Dual luciferase reporter assay

It was predicted by LncRBase (https://ngdc.cncb.ac.cn/databasecommons/database/id/4637) that miR-134-5p might be a target gene that interacts with linc01278. Thus, we constructed WT-linc01278 (5'- CACCACGU AUUAAGUCAGUCACA-3') and MUT-llin01278 (5'-C ACCACGUAUUAAGUUC AGACAC- 3') recombinant luciferase reporter plasmids. Two plasmids were co-transfected with mimic NC, miR-134-5p mimic, inhibitor NC, or miR-134-5p inhibitor, respectively, into AC16 using Lipofectamine™ 3000 (L3000001, Therm, U.S.). Cells were lysed after 48 h, and the supernatant was taken and reacted by adding fluorescent dye to avoid light, and then the relative intensity of fluorescence was immediately detected by a multifunctional enzyme labeling instrument in which the light value of sea renal fluorescence enzyme was used as an internal reference.

RNA Immunoprecipitation (RIP) assay

RIP is an important method to detect the interaction of linc01278 with the target gene miR-134-5p. A sufficient amount of cardiomyocyte AC16 (1×10^6) was cured with formaldehyde to fully cross-link RNA and bound protein; cells were precipitated by centrifugation and then resuspended with RIP buffer and lysate to break up the cells

and release intracellular RNA and protein; 2 μg Ago2/IgG antibody was added to 10 mg of supernatant and incubated at 4℃ overnight, followed by the addition of 40 μl protein A /G beads for 1 h. Wash the mixture thoroughly to remove non-specific binding and impurities, and detect them by RT-qPCR.

Western blotting

Cardiomyocytes after different treatments were collected in centrifuge tubes, and strong RIPA lysate (Beyotime, Shang, China) containing protease inhibitor (PMSF, Sigma, Germany) was added to extract cardiomyocyte proteins in an ice bath environment. The protein content of different groups of cell extracts was monitored by the Beyotime BCA protein concentration assay kit. Western blot experiments were performed by wet transfer using a 15% PAGE gel rapid preparation kit (Epizyme, Shanghai, China). Antibodies used in this study were Anti-Bax antibody (ab53154, 1: 500), Anti-Bcl-2 antibody (ab196495, 1: 1000), Anti-beta Actin antibody (ab8226, 1: 1000), goat anti-rabbit IgG H&L (HRP) (ab205718), goat anti-mouse IgG H&L (HRP) (ab205719) were purchased from Abcam, U.K. Experimental results were acquired by ChemiDoc MP chemiluminescent gel imaging system.

Data analysis

GraphPad Prism 9.0 was used to statistically analyze the results of the relevant data in the experiment. The statistical results were expressed as mean ± SD. It was tested that our data conformed to normal distribution and the assumption of normality was satisfied. Therefore, one-way ANOVA and t-test were chosen to assess the between-group differences of the samples. A post hoc test was also conducted on the ANOVA results by Tukey's HSD. All experiments were replicated three times, stipulating that differences were statistically significant when $P < 0.05$.

Results

Silencing of linc01278 reversed the effect of sevoflurane pretreatment on H/R-induced cardiomyocyte proliferation and apoptosis

HR induction noticeably reduced the expression of linc01278 in AC16 cells ($P < 0.001$) compared to controls ($P < 0.0001$, Fig. 1A). Conversely, Sev administration elevated linc01278 levels; however, this upregulation was markedly attenuated by the silencing of linc01278 ($P < 0.0001$, Fig. 1A). Additionally, HR also slowed down the growth of AC16 cells, but the proliferative capacity of cells was significantly enhanced after Sev treatment, however, this enhancement was notably reversed by silencing of linc01278 ($P < 0.0001$, Fig. 1B). From the results of flow cytometry, it can be seen that HR stimulation increased the number of apoptotic cells, and the percentage of apoptotic cells in the HR model after Sev pretreatment was significantly reduced, but the percentage of apoptotic cells was increased after transfection with si-linc01278 ($P < 0.01$, Fig. 1C). Detection of apoptosis-related proteins revealed that both HR stimulation and linc01278 silencing promoted the expression of the apoptotic protein Bax and inhibited the expression of the anti-apoptotic protein Bcl-2, but Sev pretreatment reversed this phenomenon ($P < 0.01$, Fig. 1D-F, Supplemental Figure S1).

Silencing of linc01278 reverses sevoflurane pretreatment impairing cardiomyocyte injury and oxidative stress

Cardiomyocyte injury markers cTnI, CK-MB, and LDH expression were up-regulated after HR stimulation but significantly down-regulated after Sev pretreatment; however, this downregulation was notably reversed by silencing of linc01278 ($P < 0.01$, Fig. 2A-C). Similar results were confirmed in oxidative stress indicators. After HR, MDA levels were significantly increased, and the expression of the anti-free radical enzymes SOD and GSH-Px was inhibited, they were notably reversed by Sev; however, this reversal was typically attenuated by silencing of linc001278 ($P < 0.001$, Fig. 2D-F).

miR-134-5p is a direct reciprocal target gene of linc01278

miR-134-5p was predicted to be a possible target gene of linc01278 using the LncRBase database. The putative binding sequences are presented in Fig. 3A. Dual luciferase reporter assay revealed that the luciferase activity of cardiomyocytes transfected into the linc01278 vector (WT-linc01278) was notably reduced ($P < 0.01$) by the addition of miR-134-5p, whereas the luciferase activity in the cardiomyocytes was well enhanced ($P < 0.01$) by the addition of the miR-134-5p inhibitor, but the fluorescence intensity detected in MUT-linc01278 cardiomyocytes (Fig. 3B). The results of RIP experiments showed that both linc01278 and miR-134-5p were significantly enriched to the RNA-binding protein Argonaute 2 (Ago2) antibody ($P < 0.01$, Fig. 3C) than the IgG, which indicated that miR-134-5p was a target gene of linc01278 and that the two had a direct interaction.

Sev pretreatment with linc01278 antagonizes miR-134-5p to increase proliferative capacity and reduce apoptosis in H/R cardiomyocytes

To verify the effects of linc01278 with miR-134-5p on HR stimulation and Sev pretreatment of cardiomyocytes, we used si-linc01278 with miR-134-5p inhibitor to detect changes in mRNA expression of miR-134-5p. As depicted in Fig. 4A, Sev mitigated the HR-induced upregulation of miR-143-5p expression, and this reduction was significantly restored by si-LINC01278. Nonetheless, the miR-134-5p inhibitor suppressed the restored effect of

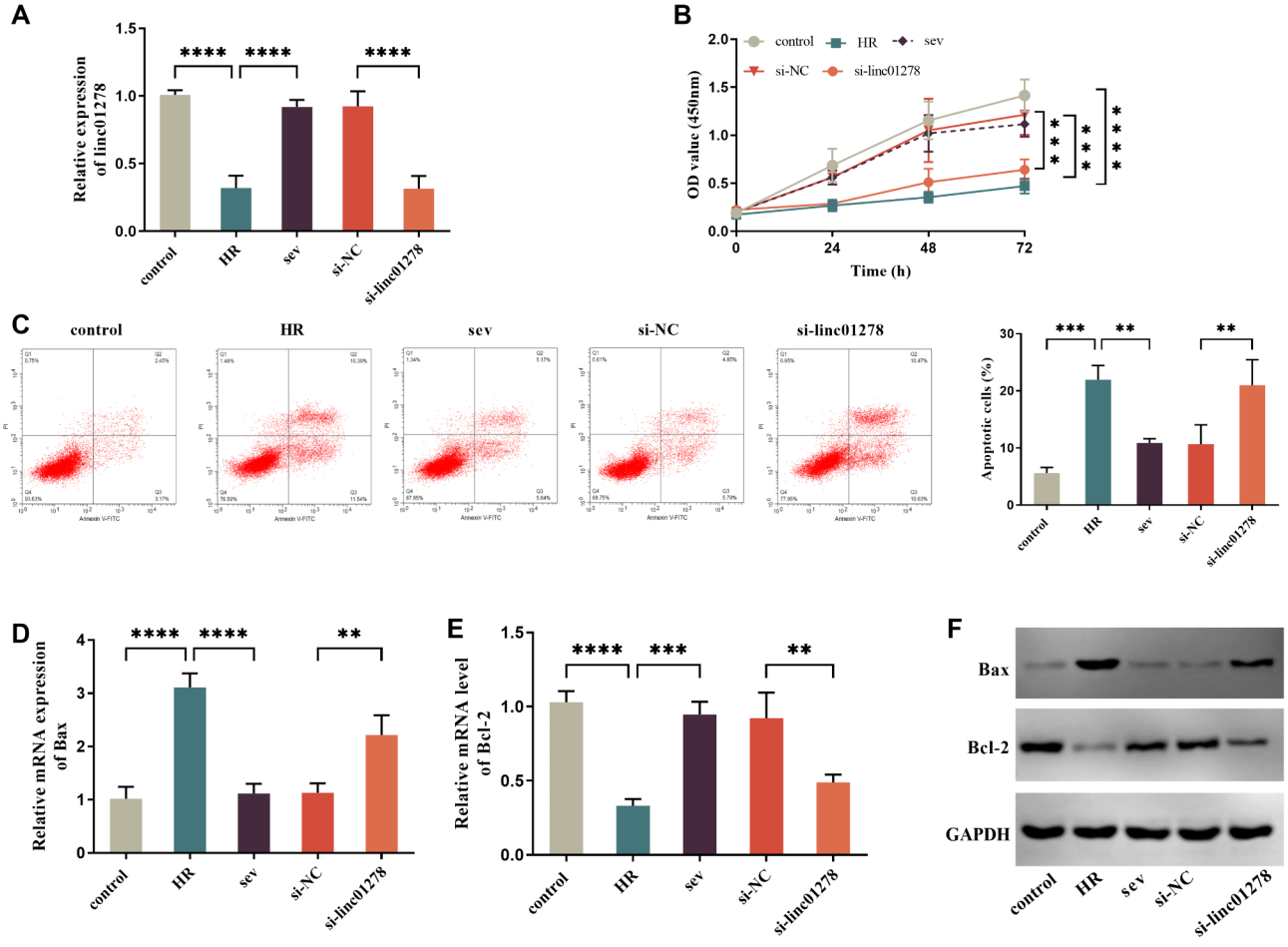

Fig. 1 linc01278 expression and cell proliferation and apoptosis in HR and Sev pretreated cardiomyocytes. **(A)** RT-qPCR observation of linc01278 mRNA expression in human cardiomyocytes AC16 from control, HR, Ser, si-NC, and si-linc01278 groups. **(B)** Cell cultures of control, HR, Ser, si-NC, and si-linc01278 groups were monitored at 0 h, 24 h, 48 h, and 72 h OD values at 450 nm. **(C)** Flow cytometry to observe the number of apoptosis in cardiomyocytes of control, HR, Ser, si-NC, and si-linc01278 groups. **D-E** RT-qPCR to detect the apoptosis-related protein genes Bax, and Bcl – 2 mRNA expression. F: Western blot plot of apoptosis-related protein (Bax, Bcl-2) expression after different treatments. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$

si-lnc01278 on miR-134-5p ($P< 0.001$, Fig. 4A). Next, the proliferation of Sev-treated cells was suppressed by si-lnc01278, but this suppression was noticeably restored by miR-134-5p inhibitor ($P<0.01$, Fig. 4B). Furthermore, si-linc01278 significantly increased apoptosis, and the expression of Bax and inhibited Bcl_2 expression; however, miR-134-5p inhibitor sharply impaired the effect of si-linc01278 on apoptosis ($P<0.05$, Fig. 4C-F, and Supplementary Figure S2).

Inhibition of miR-134-5p expression by linc01278 after Sev pretreatment attenuates H/R cardiomyocyte injury

Finally, we then explored the effects of linc01278/miR-134-5p axis regulation on cardiomyocytes and the level of oxidative stress in groups of cells pretreated with Sev. After Sev pretreatment, silencing linc01278 upregulated the expression of myocardial fine damage markers, but the simultaneous use of inhibition of miR-134-5p significantly reduced the expression of cardiomyocyte damage markers (Fig. 5A-C) and attenuated cardiomyocyte damage. Similarly, si-linc01278 promoted the metabolism of oxidative stress product MDA and inhibited the secretion of antioxidant enzymes SOD and GSH-Px, but the use of miR-134-5p inhibitor resulted in a substantial reduction of oxidative stress products ($P< 0.01$), an increase in the secretion of antioxidant enzymes, and a reduction in the level of cellular oxidative stress in cardiomyocytes (Fig. 5D-F).

Discussion

In the present study, we confirmed the involvement of linc01278 in the protective mechanism of sevoflurane on HR-affected cardiomyocytes. linc01278 was down-regulated in cardiomyocytes with HR, and the number of slow-growing and apoptotic cells increased, whereas Sev preconditioning rescued the down-regulation of linc01278 induced by HR stimulation, and it was capable of significantly restoring the proliferative capacity and

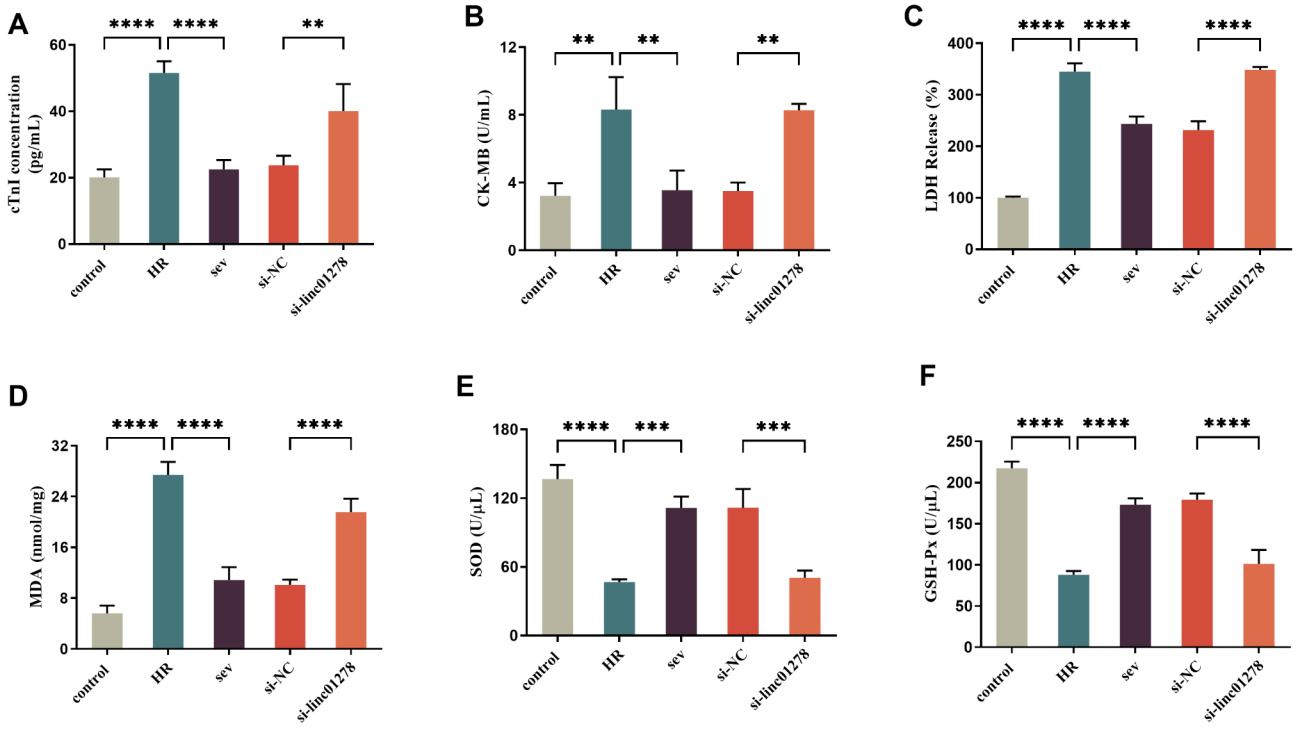

Fig. 2 Cardiomyocyte injury and oxidative stress markers after HR and sevoflurane pretreatment. **A-C** Detection of markers of cardiac troponin I (cTnI), creatine kinase isoenzyme (CK-MB), and lactate dehydrogenase (LDH) in cardiomyocyte injury after different treatments. **(A)** cTnI. **(B)** CK-MB. **(C)** LDH. **D-F** Detection of oxidative stress metabolite malondialdehyde (MDA), antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) expression in cardiomyocytes after different treatments. **(D)** MDA. **(E)** SOD. **(F)** GSH-Px. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$

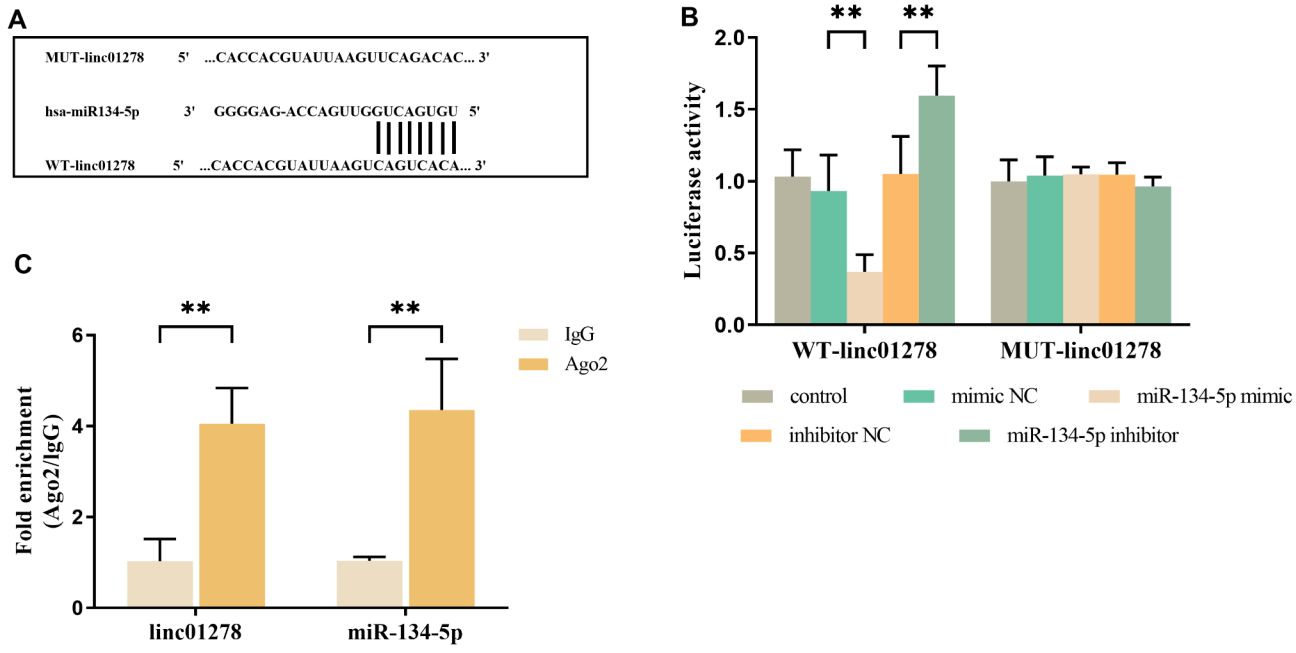

Fig. 3 Luciferase assay with RIP to analyze the targeting relationship between linc01278 and miR-134-5p. **A** The putative binding sequences between linc01278 and miR-134-5p. **B-C** Dual luciferase report assay and RIP assay verified the targeting binding of linc01278 to miR-134-5p. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$

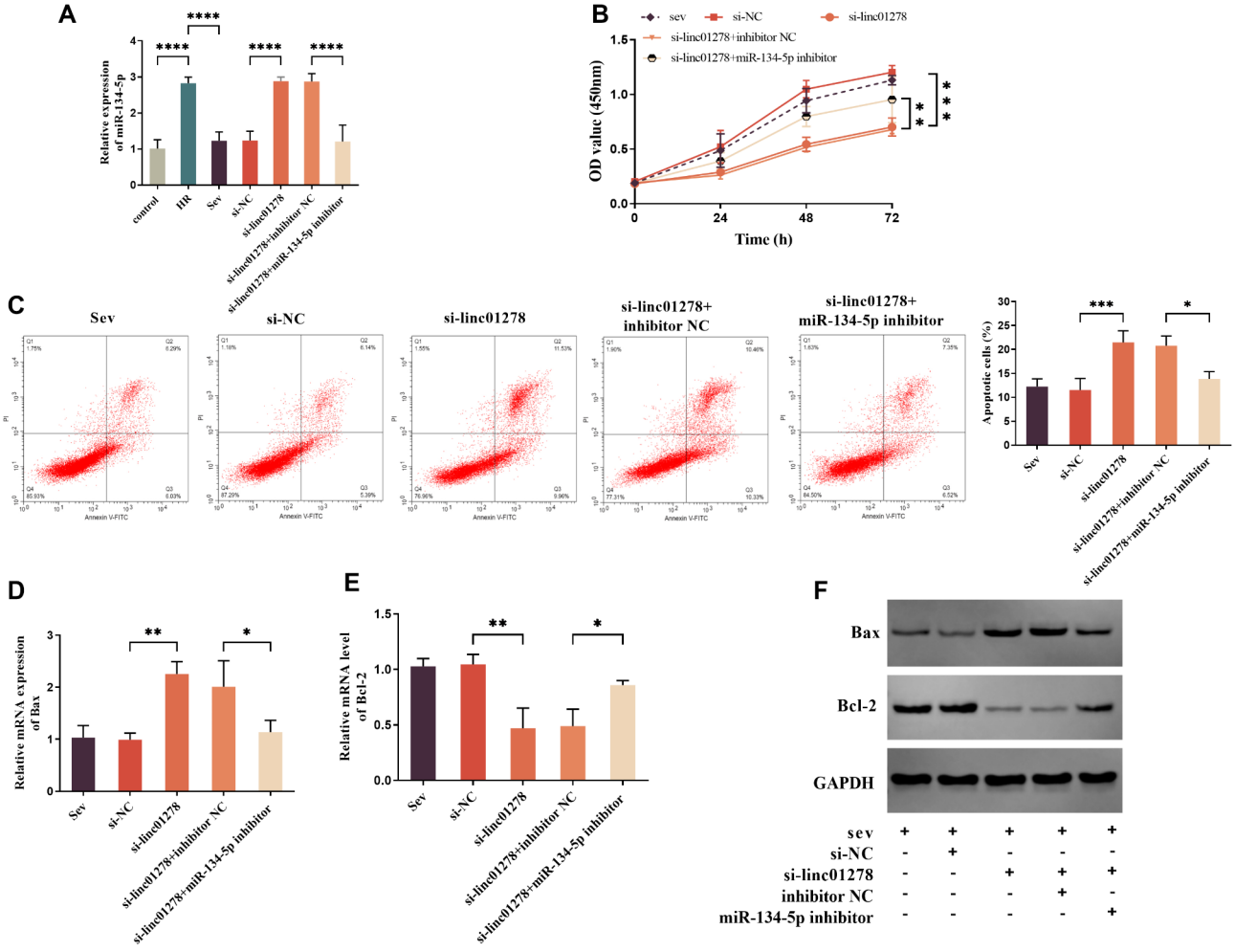

Fig. 4 Effect of Sev pretreatment on the proliferation and apoptosis ability of linc0278/miR-134-5p regulated HR cardiomyocytes. **(A)** Changes in miR-134-5p mRNA expression were detected by RT-qPCR. **(B)** Effect of Sev pretreatment on the proliferative capacity of HR cardiomyocytes (OD = 450 nm). **(C)** Changes in the amount of apoptosis in Sev-pretreated HR cardiomyocytes observed by flow cytometry. **D-E** RT-qPCR to detect changes in the expression of apoptosis-related protein genes Bax and Bcl-2 mRNA in Sev-pretreated HR cardiomyocytes. F: Effect of Sev pretreatment on the expression of apoptosis-related proteins after transfection. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$

reducing apoptosis in HR cardiomyocytes. In addition, we found that the predicted miR-134-5p was able to bind directly to linc01278, and the two were jointly involved in the regulation of HR-induced cardiomyocyte injury by Sev. When transfected with si-linc01278 in HR cardiomyocytes, miR-134-5p down-regulation was significantly suppressed in the cells, whereas the opposite result was observed after the addition of miR-134-5p. Exploration of the regulatory role of the linc01278/miR-134-5p axis in Sev-pretreated HR cardiomyocytes revealed that low expression of linc01278 reduced the proliferative capacity of Sev-rescued injured cardiomyocytes and apoptosis of cells rescued by Sev pretreatment. However, this situation would be improved by the simultaneous use of miR-134-5p inhibitor in si-linc0127, which was mainly manifested in the significant increase in proliferation and decrease in apoptosis of Sev-saved injured cardiomyocytes, significant down-regulation of myocardial injury markers cTnl, CK-MB, and LDH, and decrease in the oxidative stress metabolite MD after miR-134-5p inhibition, and decrease in antioxidant enzymes SOD, GSH-PH, and LDH, which are the most important components of myocardial injury. SOD, GSH-Pxb expression increased. Therefore, we hypothesized that the protective effect of Sev on cardiomyocytes damaged by hypoxia/reoxygenation is regulated through the linc01278/miR-134-5p axis.

Sevoflurane is a proven protective agent for the treatment of ischemia-reperfusion myocardial injury in many reports [19]. However, myocardial ischemic injury, including heart failure and myocardial infarction, is a prerequisite for consideration of anesthesia procedures in the elderly [20]. It has been shown that the inhalational anesthetic sevoflurane improves blood flow stability and

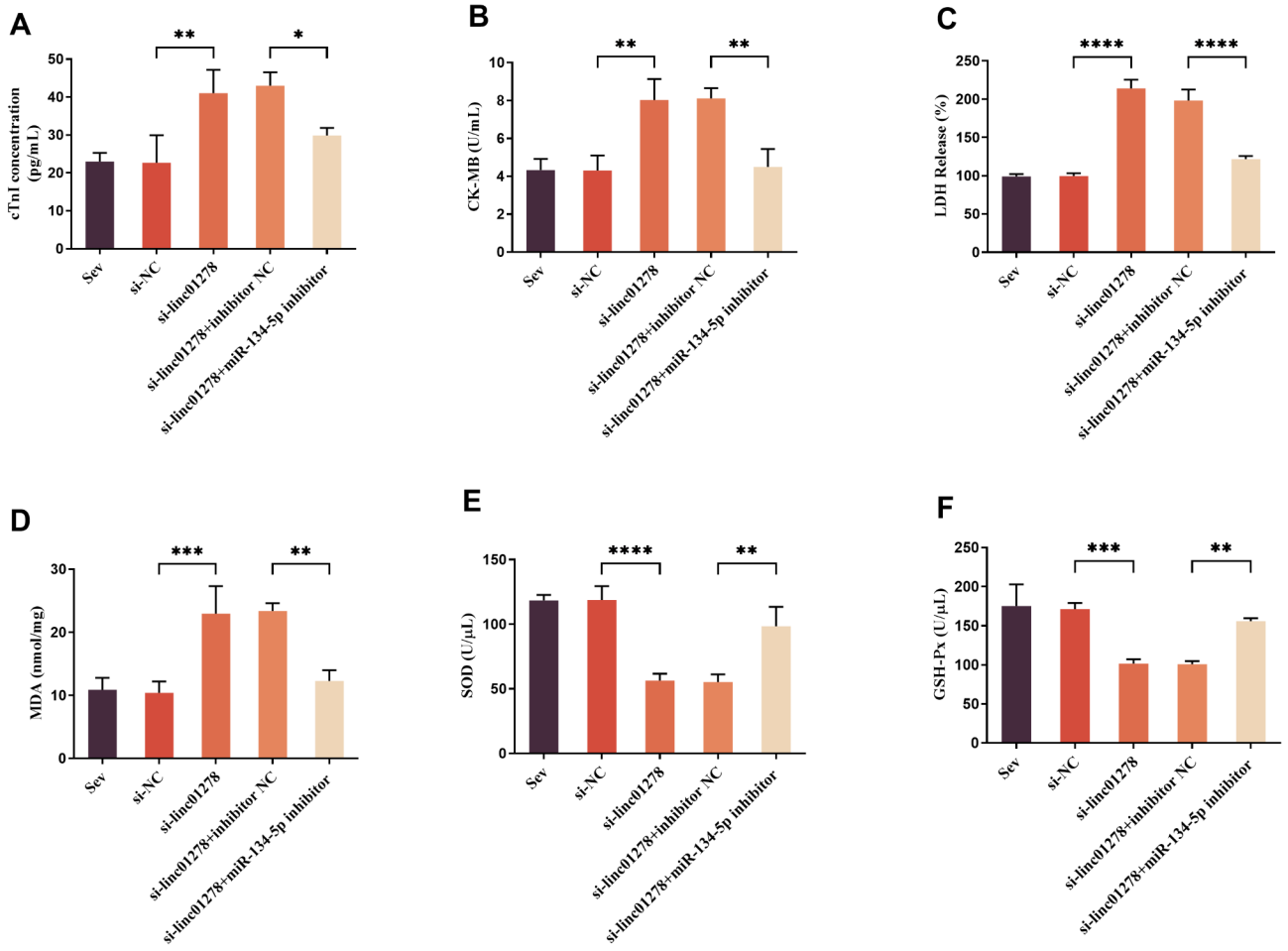

Fig. 5 Effects of Sev preconditioning on linc0278/miR-134-5p-regulated HR myocyte injury and oxidative stress levels. **A-C** Expression of cardiomyocyte injury markers cTnI, CK-MB, and LDH in Sev-preconditioned HR myocytes under normal conditions, after silencing of linc01278, and after concomitant use of miR-134-5p inhibitor. **(A)** cTnI. **(B)** CK-MB. **(C)** LDH. **D-F** The levels of oxidative stress markers MDA, SOD and GSH-Px in cardiomyocytes co-regulated by miR-134-5p and linc01278 were measured in Sev-treated H/R cells. **(D)** MDA. **(E)** SOD. **(F)** GSH-Px. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$

reduces the risk of surgery-induced cardiac disease in elderly diabetic patients undergoing noncardiac surgery compared with propofol [21]. In addition, sevoflurane has been reported to be protective against heart failure caused by several diseases [22] (e.g., pulmonary hypertension). For example, Sev prevents right ventricular dysfunction in rats with pulmonary arterial hypertension [23]; protects against ventricular remodeling in rats with myocardial infarction (MI) [24]; and attenuates cardiomyocyte apoptosis in murine myocardial ischemia/reperfusion (I/R) injury [9]. Specifically, the protective effect of the anesthetic agent sevoflurane against ischemic cardiomyocyte damage is mainly reflected in the ability of sevoflurane to promote cell proliferation, reduce cell death, and decrease the inflammatory response of damaged cardiomyocytes in a pro-cellular manner [25, 26]. The results of our study showed that sevoflurane pretreatment significantly improved the proliferative capacity of HR-injured cardiomyocytes and reduced cardiomyocyte apoptosis, which is consistent with the reported results. In addition, the degree of recovery from ischemic cardiomyocyte injury by sevoflurane was assessed mainly based on myocardial injury markers and oxidative stress levels. The heart is the hub of blood circulation in the human body, whereas mitochondria are the main source of energy in the organism and the main site of enzymatic reactions including inflammatory reactions. Sevoflurane has been reported to reduce the level of oxidative stress during cardiac ischemia-reperfusion and protect cardiomyocytes through mitochondrial effects [27]. Similarly, our results showed decreased expression of cardiomyocyte injury markers cTNI, CK-MB, LDH, decreased secretion of oxidative stress metabolites, and increased expression of antioxidant enzymes SOD and GSH-Px after sevoflurane preconditioning compared to HR. The results of this experiment suggest that sevoflurane pretreatment can attenuate ischemia-reperfusion injury to cardiomyocytes by promoting the proliferation of damaged cells and

reducing apoptosis and oxidative stress levels. However, according to previous experience [28–30], when evaluating the use of anesthetics in the treatment of disease, the dose of the drug and blood concentration should also be considered [31–33]. In follow-up experiments we will focus on and record the changes in blood indices of animals in the state of general anesthesia with different concentrations of Sev through in vivo animal experiments.

Although non-coding RNAs are not involved in the process of protein synthesis in the organism, non-coding RNAs are involved in the prevention and treatment of a variety of diseases [34, 35], whereas long-chain non-coding RNAs play a key role in the treatment of cardiomyocyte injury [36, 37]. It has been shown that linc01278 is down-regulated in aortic dissection (AD) disease and is involved in vascular smooth muscle cell phenotypic transition [38–40]. Similarly, we showed that linc01278 expression was significantly decreased in HR-treated cardiomyocytes, and in agreement with the results of the si-linc01278 group, cells in the HR group showed inhibition of proliferation, increased secretion of apoptotic proteins, apoptosis, and more severe markers of damage and oxidative stress in damaged cardiomyocytes. However, in regulating cell function and participating in pathogenesis Lnc RNAs often do not act alone but generally regulate disease progression by directly binding to target genes or interacting with other miRNAs [41]. Therefore, in this study, we predicted the downstream target genes of linc01278 by the LncRBase database, and as a result, miR-134-5p was captured by us. To further validate the relationship, we constructed WT-linc01278 and MUT-linc01278 cardiomyocyte cell lines and proved by dual luciferase reporter assay and RIP that the fluorescence value of WT-linc01278 cardiomyocytes was significantly elevated by HR treatment with miR-134-5p inhibitor, but MUT-linc01278 cardiomyocytes fluorescence values did not change significantly after the same treatment. There was a direct interaction between linc01278 and miR-134-5p. It has been shown that linc01278 is involved in the pathogenic process of diseases through interactions with miRNAs [42, 43], and linc01278/miR-134-5p also regulates the development of a variety of diseases [7, 8]. After the RT-qPCR assay, we found that miR-134-5p was significantly up-regulated in HR cardiomyocytes, but miR-134-5p remained low in normal cells versus Sev-pretreated cardiomyocytes. We therefore further explored the role of the linc01278/miR-134-5p axis in Sev-pretreated cardiomyocytes using miR-134-5p inhibitors. RESULTS FINDINGS: In Sev pretreated cells, in contrast to the results of si-linc01278, miR-134-5p inhibitor promoted cardiomyocyte proliferation apoptotic protein secretion was inhibited, and increased apoptosis was significantly prevented. In addition, detection of markers of myocardial injury and oxidative stress revealed that inhibition of miR-134-5p expression reduced the secretion of markers of myocardial injury and decreased the level of oxidative stress in damaged cardiomyocytes. Similar results have been demonstrated in other studies, such as linc01278 dependence on miR-1258 to mediate hepatocellular carcinoma (HCC) metastasis, i.e., linc01278/miR-1258 interactions are involved in the development and metastasis of HCC [43]. linc01278's oncogenic effect on osteosarcoma was mediated through linc01278/miR- 133a-3p was realized [44]. Through the present study, we finalized that Sev pretreatment mediates the role of lncRNA linc01278 in the repair of ischemic cardiomyocyte injury and found that modulation of cellular hypoxia/reoxygenation injury through the linc01278/miR-134-5p axis may be one of the mechanisms by which sevoflurane protects damaged myocardium. Specifically, we found that linc01278 was down-regulated in hypoxic/reoxygenated cardiomyocytes and significantly up-regulated in sevoflurane-pretreated cardiomyocytes, and that linc01278/miR-134-5p could directly bind to each other and act antagonistically to each other in attenuating myocyte injury.

Studies have shown that miR-134-5p is involved in the regulation of adverse cardiomyopathies [45]. For example, miR-134-5p is involved in oxidative stress and apoptosis in damaged cardiomyocytes by down-regulating and targeting XIAP [10]; miR-134-5p inhibits the reduction of apoptosis in infarcted cardiomyocytes through up-regulation of Creb1 [46]; and miR-134-5p also regulates angiogenesis through direct targeting of KDM2A in myocardial infarction patients [47]. In the present study, we preliminarily found that Sev was involved in the repair process of ischemic myocardial injury through linc01278/miR-134-5p, but the downstream regulatory mechanism of miR-134-5p was not clear. Subsequently, the regulatory mechanism of Sev protection against myocardial injury needs to be explored in depth in conjunction with the results of raw letter analysis. In addition, unfortunately, we have only verified the protective mechanism of Sev in attenuating myocardial damage by constructing the H/R cell model, and the number of sample replicates will be enlarged as well as animal experiments in the in vivo environment. Anyway, there are the potential limitations of the current study, and we will focus on solving these problems in the further.

Conclusions

This study further explored the role of linc01278 in sevoflurane-pretreated HR cardiomyocytes based on previous studies. We demonstrated that sevoflurane preconditioning mediates HR cardiomyocyte damage repair via the linc01278/miR-134-5p regulatory axis may be one of the mechanisms by which it protects the myocardium. However, due to force majeure factors, this experiment

did not continue to explore in depth through which or which target genes, and in what way, linc01278/ miR-134-5p was involved in the protection of ischemic cardiomyocytes after sevoflurane pretreatment. Therefore, subsequent studies will be continued as experimental conditions allow to provide a theoretical basis for clinical treatment of myocardial injury and reduction of cardiac burden.

Abbreviations

AD	Aortic dissection
Ago2	Argonaute 2
AMI	Acute myocardial infarction
Bax	BCL2-associated X
Bcl-2	B-cell lymphoma-2
CK-MB	Creatine kinase isoenzyme
CTnI	Cardiac troponin I
DCM	Dilated cardiomyopathy
ELISA	Enzyme-linked immunosorbent assay
GSH-Px	Glutathione Peroxidase
HCC	Hepatocellular carcinoma
HR	Hypoxia/reoxygenation
LDH	Lactate dehydrogenase
lncRNAs	Long-stranded noncoding RNAs
MI/R	Myocardial ischemia-reperfusion
NC	Negative control
RIP	RNA immunoprecipitation
RT-qPCR	RNA extraction and real-time fluorescence quantitative PCR technique
Sev	Sevoflurane
SOD	Superoxide dismutase

Supplementary Information

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Supplementary Material 1

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Not applicable.

Author contributions
YG S and R C designed this study. H L, L W and CH Z conducted the experiment and analyzed the data. L W and CH Z wrote the manuscript. R C revised the manuscript. All authors reviewed and approved for publication.

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Data availability
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
An ethics statement was not required for this study type since no human or animal subjects or materials were used.

Competing interests
The authors declare no competing interests.

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