Long non-coding NEAT1 weakens the protective role of sevoflurane on myocardial ischemia/reperfusion injury by mediating the microRNA-140/RhoA axis

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Research article

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Abstract

Background: The inhaled Sevoflurane (Sev) has been implicated to protect myocardial tissues against ischemia/reperfusion (I/R)-evoked injury. Nevertheless, the detailed mechanisms of Sev-mediated cardioprotection remain basically unknown. This study intends to examine the roles of nuclear enriched abundant transcript 1 (NEAT1), a long non-coding RNA (lncRNA) during the cardioprotection exerted by Sev.

Methods: We initially performed I/R surgery in mice, and Sev treatment was given during the perfusion, followed by the determination of cardiac function, myocardial infarction area and myocardial apoptosis for the validation of the model. Then, we conducted lncRNA microarray analysis on Sev-treated hearts to find out lncRNAs with significant differences in expression. The changes of myocardial I/R injury were evaluated by echocardiography, TTC staining and TUNEL assay after establishing mice with NEAT1 upregulation. Afterwards, the targeting miRNA of NEAT1 and the targeting mRNA of the miRNA were screened out through StarBase and microarray analyses to carry out rescue experiments.

Results: NEAT1 was downregulated in Sev-treated cardiomyocytes. Overexpression of NEAT1 weakened cardiac function, increased infarct size and increased apoptosis in the presence of Sev. NEAT1 bound to microRNA (miR)-140, which was significantly upregulated in Sev-treated cardiomyocytes. Inhibition of miR-140 expression weakened the cardiac function of Sev-treated mice, increased infarct size and cardiomyocyte apoptosis. miR-140 mediated the RhoA pathway, and the RhoA activity inhibition attenuated miR-140 effects on Sev protection.

Conclusion: Sev mitigates myocardial I/R injury by restoring NEAT1 expression and regulating the miR-140/RhoA axis.

Background

Ischemia/reperfusion (I/R) is characterized by deficiency of oxygen supply and the following restoration of blood flow, resulting in irreversible injuries to tissues [1]. Acute myocardial infarction is one of the leading causes of death and disability in the world range, and the treatment option for easing myocardial injury and reducing myocardial infarction size is timely and operative reperfusion using thrombolytic therapies or primary percutaneous coronary intervention [2]. Sevoflurane (Sev) is a volatile anesthetic agent that offers hypnosis, analgesia and akinesia during surgical and procedural interventions [3]. The role of Sev during I/R injury has been underscored in the lung [4], brain [5] as well as heart [6]. Interestingly, LINC00652, a kind of long non-coding RNA (lncRNA), was observed to work with Sev to exert cardioprotective role against myocardial I/R injury [7]. Hence, we postulated that lncRNAs may engage in the Sev-mediated myocardial I/R injury alleviation.

Noncoding RNAs, a group of RNAs that do not encode proteins, can be divided into lncRNAs, microRNAs (miRNAs) as well as circular RNAs and are closely related to cardiovascular disease by regulating cell proliferation, apoptosis and other vital biological events [8]. For example, downregulation of nuclear

enriched abundant transcript 1 (NEAT1) acted as a protector against the apoptosis of cardiomyocytes under hypoxia/reoxygenation [9]. It has been proposed that competing endogenous RNAs (ceRNAs) modulate the distribution of miRNAs on their target mRNAs, thereby exerting a post-transcriptional regulation [10]. For instance, NEAT1 enhanced myocardial I/R injury by competing with mitogen-activated protein kinase 6 to regulate miR-495-3p [11]. While miR-140, which is predicted a binding miRNA of NEAT1 by a bioinformatics website in the current study, was found to attenuate myocardial I/R injury via inhibiting mitochondria-mediated apoptosis by interacting with YES1 [12]. Also, lncRNA SNHG1 protected SH-SY5Y cells from injury caused by oxygen glucose deprivation through the miR-140-5p/Bcl-XL axis [13]. The additional activity of Ras-associated guanosine triphosphatase RhoA is monitored to promote oxidative stress and the progression of cardiovascular diseases [14]. More specifically, the protective role of Ginsenoside Rb1 against I/R-induced myocardial injury was achieved by mediating the RhoA signaling pathway [15]. In addition, RhoA was found to share binding sites with miR-140 in the present study by Starbase. Based on the aforementioned reports, we carried out this experiment to probe the function of NEAT1 during injury evoked by myocardial I/R in a developed mouse model by regulating the miR-140/RhoA axis with the administration of Sev.

Methods

Mouse treatment

Our experiments were carried out under the approval of the Ethics Committee of Liyang People's Hospital of Jiangsu Province on the basis of the Guide for the Care and Use of Laboratory Animals formulated by the National Institutes of Health. Substantial efforts were made to minimize the suffering and numbers of the animals used during the experiment. All mice used in this study were from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Twenty-seven C57BL/6N male mice (aged 6-8 weeks, weight 15-25 g) were allowed to acclimatize for one week in the animal facility (at a constant temperature of 22°C with 12 h light and dark cycles) before any intervention was initiated. Mice were allocated into sham (sham-operated mice), I/R (mice subjected to I/R surgery only), I/S (mice inhaled with Sev during the reperfusion of I/R surgery), NEAT1-negative control (NC) (mice injected with mock 48 h before I/R treatment), NEAT1-overexpression (OE, mice injected with NEAT1-OE 48 h before I/R treatment), miR-140 control (mice injected with miR-mock 48 h before I/R treatment), miR-140 inhibitor (mice injected with miR-140 inhibitor 48 h before I/R treatment), miR-140 inhibitor + dimethylsulfoxide (DMSO, mice injected with miR-140 inhibitor and DMSO 48 h before I/R treatment), miR-140 inhibitor + C3 (mice injected with miR-140 inhibitor and extracellular enzyme C3 48 h before I/R treatment) groups. NEAT1-OE and miR-140 inhibitor fragments and NCs were synthesized by GenePharma (Shanghai, China). The lentiviral vector pLenti6 (Thermo Fisher Scientific Inc., Waltham, MA, USA) was transfected with RNA fragments by BLOCK-iT lentiviral RNAi expression system (Thermo Fisher Scientific). The mice were then anesthetized with 1% pentobarbital sodium through an intraperitoneal injection at 60 mg/kg. A longitudinal incision was made between the 2nd, 3rd and 4th ribs to expose the heart. The corresponding

transfection solution (10 µL) was slowly injected at the anterior wall of the left ventricle of the mouse. After the injection, the mouse chest was sutured.

Development of a myocardial I/R mouse model

On the day of I/R experiment, all mice were injected intraperitoneally with atropine at 0.04 mg/kg and with pentobarbital sodium at 60 mg/kg after 5 min. Once the respiratory frequency of mice reached 90 times/min, the mice were connected to an animal breathing apparatus, which was set at the frequency 90-105 times/min with a tidal volume at mL. A longitudinal incision was made between the second and fourth ribs to expose the heart and pericardium. The left anterior descending coronary artery was ligated from the tip of the left auricle. An electrocardiogram (ECG) was applied to assess the ischemic condition. After 30 min, blood flow was restored for reperfusion for 15 min, during which mice in the IS groups were inhaled with 2.4% Sev continuously, and mice in the sham group was inhaled with common air. The intercostal and pectoral muscles were sutured to close the chest after heart rhythm stabilization. After the suture, excess gas was extracted with needles from thoracic cavities. When the mice had mild spontaneous respiration, the apparatus was removed. Following the skin was sutured, the mice were put back into 25°C animal rooms with a 0.5 mL saline intraperitoneal injection.

ECG

A small animal ultrasound imaging system was purchased from VisualSonics (FUJIFILM VisualSonics, Inc., Toronto, ON, Canada). The left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV), left ventricular end-diastolic diameter (LVDd) and left ventricular end-systolic diameter (LVDs) were measured to obtain left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS). $LVEF (\%) = (LVEDV - LVESV) / (LVEDV) \times 100\%$, $LVFS (\%) = (LVDd - LVDs) / LVDd \times 100\%$.

Triphenyl tetrazolium chloride (TTC) staining

After testing the cardiac cycle of mice, we performed euthanasia by intraperitoneal injection of 1% sodium pentobarbital at 150 mg/kg. Once observing the lack of heartbeat, respiratory arrest, pupil dilation, and lack of nerve reflex, the mouse heart tissues were removed and fixed in 4% paraformaldehyde immediately, frozen and cut into 5 µm slices. Afterwards, sliced fresh tissues were cultured in a petri dish containing TTC staining solution (Solabio, China). After a 30-min incubation at 37°C, the stained sections were removed with a pipette, rinsed with 4°C phosphate buffered saline, observed by naked eyes and photographed. The red part represents normal non-ischemic myocardial tissues, the gray part represents ischemic but non-infarct myocardial tissues, and the white part represents myocardial tissues with ischemic infarction. Image-Pro Plus 6.0 software was applied to determine the percentage of myocardial infarction area.

Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) staining

The cells in logarithmic growth of each group were plated at 1×10^6 cells/mL on the cover glasses in the 6-well plate. TUNEL apoptosis was detected using In Situ Cell Death Detection Kit-peroxidase (Roche, Basel, Switzerland). The cell suspension was coated on the cover glasses, reacted with Proteinase K for 15 min and incubated with 50 μ L TUNEL solution in a dark chamber for 1 h at 37°C. Following a 10-min treatment with 50 μ L diaminobenzidine, the results were recorded using an M-60 microscope (Olympus Optical Co., Ltd., Tokyo, Japan) equipped with BH2 system with attached 35 mm camera (Olympus). The number of TUNEL-positive cells was counted under five randomly-selected high magnification views.

Microarray analysis

Total RNA was extracted by ice-lysed mouse tissue homogenate in Trizol (Beijing Solabio Life Sciences Co., Ltd., Beijing, China). A total of 0.5 μ g total RNA was reversely transcribed into cDNA through PrimeScript™ IV 1st strand cDNA Synthesis Mix (Takara Holdings Inc., Kyoto, Japan). The cDNA was then fragmented and hybridized with a mouse lncRNA expression array V6.0 (Shanghai Biotechnology Corporation), and then the array was screened using GeneChip™ Scanner 3000 7G system (Thermo Fisher Scientific Inc., Waltham, MA, USA). Scanning results were subjected to quality assessment and data correction. R language package was used to plot the heatmap using processed data. $p < 0.01$ and $|\log_2 \text{FoldChange}|$ were used as the screening criteria, and the top ten RNAs were selected for analysis.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Total RNA was used for RNA extraction from tissues using Trizol. Totally 5 μ g total RNA was collected for reverse transcription and RT-qPCR through the SuperScript™ IV One-Step RT-PCR System. U6 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the internal normalized references. The primers are displayed in Table 1. Relative expression of targets was determined using $2^{-\Delta\Delta C_t}$.

Luciferase reporter assay

HEK293T cells (Cell Bank of Shanghai Institute of Cells, Chinese Academy of Science, Shanghai, China) were grown in Dulbecco's modified Eagle's medium. When the density reached 80%-90%, the cells were detached with 0.25% trypsin and passaged in a 5% CO₂ incubator at 37°C, and the cells in logarithmic growth phase were harvested for further experiments. The targets of NEAT1 and miR-140 were analyzed using StarBase and verified by luciferase reporter assays. The miRNA 3'untranslated region (3'UTR) sequence and the lncRNA and mRNA 3'UTR sequence were inserted into pGL4.18 luciferase reporter vectors (Promega Corporation). HEK293T cell co-transfection was carried out using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). After 24 h, the cells were lysed, and the luciferase activity was assessed by a dual-luciferase reporter assay system (Promega). The ratio of firefly luciferase activity to renilla luciferase activity reflects the relative luciferase activity.

Western blot

After 48 h of transfection, the cells were lysed on ice with radio immunoprecipitation assay lysis buffer. The samples were centrifuged at 20000 g for a period of 10 min at 4°C to collect the supernatant. Following the protein quantification using a bicinchoninic acid kit (Sigma-Aldrich Chemical Company, St Louis, MO, USA). We then separated 15 µg protein lysates using 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Afterwards, the proteins were transferred to polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA) and sealed with 5% skim milk for 2 h. Blots were probed with the primary antibody against RhoA (ab187027, Abcam Inc., Cambridge, UK) at 4°C overnight and with the secondary antibody (ab205718, Abcam) for 3 h at room temperature. At last, the immunoblot was visualized by Superstar electrochemiluminescence Plus. Gel imaging system (Bio-Rad Laboratories, Hercules, CA, USA) was applied to determine protein expression.

Statistics

Statistical analysis was validated by SPSS 22.0 (IBM Corp. Armonk, NY, USA). All data are expressed as mean ± standard derivation (SD). Statistical significance was determined using unpaired *t* test and one-way or two-way analysis of variance (ANOVA), as appropriate. If ANOVA was significant, individual differences were assessed using the Tukey's post-test. For all studies, values of $p < 0.05$ were considered statistically significant.

Results

Reduction of myocardial I/R injury is found in mice treated with Sev

To test whether the injury following I/R was successfully induced and the relevance of Sev in the biochemical parameters related to cardiac function were measured. The LVEF and LVFS of I/R-treated mice were lower than that of sham-operated mice, and the those of Sev-treated mice were higher than that of the I/R-treated mice without inhalation (Figure 1A). We later found that the myocardial infarction area of Sev-treated mice was significantly smaller than that of mice subjected to I/R alone by heart observation (Figure 1B). Subsequently, it was further exhibited that the apoptosis of cardiomyocytes in mice after Sev treatment was significantly reduced (Figure 1C), which implied that the I/R model was effectively developed and that Sev treatment could reduce the occurrence of injury caused by myocardial I/R in mice.

Decreased NEAT1 expression is identified in mice with myocardial I/R following Sev treatment

We then performed a lncRNA microarray analysis on myocardial tissues of I/R mice treated with Sev or without any other treatment. The microarray analysis results showed that NEAT1 was significantly downregulated after Sev inhalation (Figure 2A), which was verified by RT-qPCR (Figure 2B). We then injected NEAT1-NC and NEAT1-OE into the nude mice, and the success of model construction was established by RT-qPCR analysis of cardiomyocytes (Figure 2C).

NEAT1 inhibits the role of Sev in myocardial I/R injury

The LVEF and LVFS indices of NEAT1-OE-injected mice were notably decreased versus compared with NEAT1-NC-injected mice (Figure 3A). Meanwhile, NEAT1-OE increased significantly the myocardial infarction area of the heart compared to the NEAT1-NC (Figure 3B), and overexpression of NEAT1 also resulted in an increased rate of apoptosis in mouse cardiomyocytes (Figure 3C). Here it can be inferred that NEAT1 could reduce the alleviating role of Sev in injury caused by myocardial I/R.

miR-140 promotes the role of Sev in myocardial I/R injury

We then performed a miRNA microarray analysis on myocardial tissues of I/R mice treated with Sev or without any other treatment to screen the significantly upregulated miRNAs (Figure 4A), which was intersected with the binding miRNAs of NEAT1 predicted by StarBase (Figure 4B). miR-140 was remarkably upregulated in I/R mice treated with Sev and had a targeting relationship with NEAT1. The results of dual-luciferase reporter gene assays showed that in terms of the miR-140 mimic group, the luciferase activity of NEAT1-wild type (WT) was profoundly lower than that of the miR-140 NC group (Figure 4C). RT-qPCR validated that miR-140 was significantly upregulated in mice subjected to I/R after Sev treatment (Figure 4D). Then we analyzed the expression of miR-140 in mice injected with NEAT1-NC or NEAT1-OE and found that the miR-140 expression in NEAT1-OE-injected mice was decreased (Figure 4E). As a consequence, we administrated miR-140 inhibitor or control into nude mice, which was verified by RT-qPCR (Figure 4F). miR-140 inhibitor resulted in significantly lower LVEF and LVFS indices than the miR-140 control (Figure 4G). Meanwhile, miR-140 inhibitor led to increased myocardial infarction area in the mouse heart compared with miR-140 control (Figure 4H). Poor expression of miR-140 also caused increased apoptosis rate in mouse cardiomyocytes (Figure 4I).

The inhibition of RhoA partially restores the effect of miR-140 on Sev

StarBase predicted that RhoA was a possible target of miR-140, which was further corroborated by dual-luciferase reporter assays (Figure 5A). The expression of RhoA in mice injected with NEAT1-NC, NEAT1-OE, miR-140 control or miR-140 inhibitor was analyzed, and its expression was increased in mice with NEAT1-OE and miR-140 inhibitor (Figure 5B). To further verify the effect of RhoA on I/R injury, we treated mice with RhoA inhibitor Exoenzyme C3 or DMSO in the presence of miR-140 inhibitor, and the success of which was authenticated by western blot analysis (Figure 5C). In mice treated with miR-140 inhibitor + C3, the LVEF and LVFS were increased (Figure 5D), myocardial infarction area was diminished (Figure 5E) and the apoptosis rate of mouse cardiomyocytes (Figure 5F) was also decreased significantly relative to the miR-140 inhibitor + DMSO treatment. This proves that the weakening of RhoA activity partially restored the effect of miR-140 on Sev.

Discussion

In spite of the fact that substantial advances have been made in antithrombotic therapy, the mortality of patients with myocardial I/R injury remains dismal; consequently, it is an urgency to improve the efficacy of cardioprotective agents to ameliorate myocardial function and limit the total area of infarction during injury caused by I/R [16]. Besides, the emerging relevance of lncRNAs in I/R injury of heart caught our

attention [17]. In view of this, we performed this study to decipher the potential mechanism behind myocardial I/R injury, and our obtained data indicate that poor expression of NEAT1 is able to attenuating myocardial I/R injury after Sev treatment in mice by mediating the miR-140/RhoA axis.

Excessive apoptosis is associated with myocardial ischemia, I/R injury as well as postischemia cardiac remodeling [18], while infarct size is an independent indicator effective in predicting mortality after infarction [19]. In the current work, we observed that Sev worked as a cardio-protector against injury induced by myocardial I/R by reducing LVEF, LVFS, myocardial infarct size and apoptosis rate, indicating the beneficial role of Sev on I/R injury [20, 21]. Moreover, Sev was found to diminish NEAT1, which was enhanced in mice with myocardial I/R, by microarray analysis. NEAT1 has been most frequently studied in various cancer as an oncogene, including cervical cancer [22], colon cancer [23] as well as glioma [24] by regulating cell migration, proliferation along with drug resistance. Additionally, NEAT1 was upregulated in Parkinson's disease and conferred drug-inducible neuroprotection against oxidative stress [25]. Also, NEAT1 expression was enhanced in patients suffered from acute ischemic stroke versus controls, which could predict unsatisfactory recurrence-free survival [26]. Furthermore, NEAT1 overexpression repressed the alleviating role of Sev in mice with injury induced by myocardial I/R. Consistently, NEAT1 was overexpressed in cells with myocardial I/R injury relative to normal myocardial cells, and declines in NEAT1 expression contributed to enhanced cell proliferation and inhibited cell apoptosis [27]. Meanwhile, silencing of NEAT1 promoted LVEF and LVFS to normal levels, implying a protective role of NEAT1 knockdown in diabetic rats with myocardial I/R injuries [28].

The relevance of lncRNA-miRNA-mRNA network in cardiovascular diseases has been highlighted recently [29]. After determination of the stimulative role of NEAT1 on myocardial I/R injury, we next sought to its target miRNA. Through a miRNA microarray analysis of significantly upregulated miRNAs in mice after Sev and Starbase prediction, miR-140 was substantiated as our target. In line with our finding, miR-370 was reduced in mice exhibited IR injuries, but Sev anesthetic preconditioning promoted the miR-370 expression pattern [30]. Previously, circRNA_000203 aggravated cardiac hypertrophy by lowering the expression of miR-140-3p [31]. Additionally, miR-140-5p was corroborated as a promising therapeutic target for alleviating brain injury induced by intracerebral hemorrhage [32]. Our data presented that with the treatment of miR-140 inhibitor, the effects of Sev were inhibited. Moreover, Catalpol improved myocardial damage by regulating NEAT1/miR-140-5p in mice with diabetic cardiomyopathy *in vivo* [33]. Lastly, we screened out RhoA as a putative target of miR-140. Besides, RhoA shares a positive correlation with NEAT1 and an inverse one with miR-140. RhoA activity in myocardium of the Sprague-Dawley rats (0.35 ± 0.06) enhanced remarkably compared with that in the sham-operated rats (0.18 ± 0.03) [34]. Suppression of RhoA prevented propofol-regulated hippocampal neurotoxicity and following cognitive deficits [35]. The TLR4/RhoA/ROCK pathway was indicated in blood-brain barrier impairment after cerebral I/R injury [36]. Furthermore, Forgione *et al.* showed that C3 transferase, which could selectively inhibit Rho expression without disturbing other guanine triphosphatases, has the potency to augment neuroprotection and axonal regeneration following spinal cord injury [37]. Nevertheless, the cardioprotection role of exoenzyme C3 was rarely probed. Here we reported that C3 could rescue the inhibitory role of miR-140 on Sev-mediated injury alleviation in mice.

Conclusion

Overall, the present study established that NEAT1 positively regulates RhoA expression by binding to miR-140, by which the mechanism of action of the cardioprotection of Sev is weakened against myocardial I/R in the mouse model (Figure 6). NEAT1 silencing may act as an attractive target for promoting Sev-induced myocardial protection. Further works are warranted to obtain more information about the NEAT1/miR-140/RhoA axis *in vitro*, and to explore new treatment options for myocardial I/R injury.

Abbreviations

ceRNAs, competing endogenous RNAs; DMSO, dimethylsulfoxide; ECG, electrocardiogram; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; I/R, ischemia/reperfusion; lncRNA, long non-coding RNA; LVDd, left ventricular end-diastolic diameter; LVDs, left ventricular end-systolic diameter; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; LVFS, left ventricular fractional shortening; miR, microRNA; NC, negative control; NEAT1, nuclear enriched abundant transcript 1; OE, overexpression; RT-qPCR, reverse transcription quantitative polymerase chain reaction; SD, standard derivation; Sev, Sevoflurane; TTC, 2,3,5-Triphenyl tetrazolium chloride; TUNEL, terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling; 3'UTR, 3'untranslated region;

Declarations

Ethics approval and consent to participate

Our experiments were carried out under the approval of the Ethics Committee of Liyang People's Hospital of Jiangsu Province on the basis of the Guide for the Care and Use of Laboratory Animals formulated by the National Institutes of Health. Substantial efforts were made to minimize the suffering and numbers of the animals used during the experiment.

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

All authors declare no conflict of interest.

Funding

Not applicable.

Authors' contributions

PFR designed the experiments. PFR, JHW and JX performed each of the tests and collated the data. All authors analysed the results and prepared the manuscript and they all read and approved the manuscript.

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Tables

Table 1 Primers for reverse transcription quantitative polymerase chain reaction

Targets Primer sequence (5'-3')	
NEAT1	Forward: CAAATTCGTGAAGCGTTCCATA Reverse: GTGCCGAGTCCGGACGATATTC
miR-140	Forward: TACCACAGGGTAGAACCACGG Reverse: TGCTTGGCAATAACAGACCAAC
RhoA	Forward: GATTGGCGCTTTTGGGTACAT Reverse: AGCAGCTCTCGTAGCCATTTC
U6	Forward: CTCGCTTCGGCAGCACA Reverse: AACGCTTCACGAATTTGCGT
GAPDH	Forward: GGAGCGAGATCCCTCCAAAA Reverse: GGCTGTTGTCATACTTCTCATGG

Note: NEAT1, nuclear enriched abundant transcript 1; miR-140, microRNA-140; GAPDH, glyceraldehyde-3-phosphate dehydrogenase

Figures

Figure 1

Sev acts as a cardio-protector in mice with injury caused by myocardial I/R. A, LVEF and LVFS in mice with I/R injury and Sev-treated model evaluated by ECG; B, myocardial infarct area in mice tested by TTC staining; C, the apoptosis rate of I/R and Sev treated model assessed by TUNEL staining. * $p < 0.05$ according to the one-way ANOVA. Results are expressed as mean $\pm$ SD ($n = 3$ per group). Experiment was repeated 3 times.

Figure 2

NEAT1 is downregulated after Sev treatment in mice with myocardial I/R. A, differentially expressed lncRNAs between mice subjected to I/R injury with or without Sev treatment screened out by microarray analysis; B, NEAT1 expression in mice under different treatments evaluated by RT-qPCR; C, NEAT1 expression in Sev-injected mice administrated with NEAT1-OE or NEAT1-NC. In panel B, $*p < 0.05$ according to the one-way ANOVA; In panel C, $*p < 0.05$ according to unpaired t test. Results are expressed as mean $\pm$ SD ($n = 3$ per group). Experiment was repeated 3 times.

Figure 3

NEAT1 upregulation weakens the cardioprotective role of Sev on mice with myocardial I/R injury. A, LVEF and LVFS in mice injected with NEAT1-OE or NEAT1-NC evaluated by ECG; B, myocardial infarct area in mice injected with NEAT1-OE or NEAT1-NC tested by TTC staining; C, the apoptosis rate of injected with NEAT1-OE or NEAT1-NC-treated model assessed by TUNEL staining. * $p < 0.05$ according to the unpaired t test. Results are expressed as mean $\pm$ SD ($n = 3$ per group). Experiment was repeated 3 times.

Figure 4

miR-140, restored after Sev treatment, is involved in the modulation of Sev-alleviated myocardial I/R injury. A, differentially expressed miRNA between mice subjected to I/R injury with or without Sev treatment screened out by microarray analysis; B, the intersected miRNA in microarray and StarBase prediction results; C, the binding relationship between miR-140 and NEAT1 predicted by StarBase and validated by a dual luciferase reporter gene assay; D, miR-140 expression in mice under different treatments evaluated by RT-qPCR; E, miR-140 expression in Sev-injected mice administrated with NEAT1-OE or NEAT1-NC; F, miR-140 expression in Sev-injected mice administrated with miR-140 inhibitor or miR-140 control; G, LVEF and LVFS in mice injected with miR-140 inhibitor or miR-140 control evaluated by ECG; H, myocardial infarct area in mice injected with miR-140 inhibitor or miR-140 control tested by TTC

staining; I, the apoptosis rate of injected with miR-140 inhibitor or miR-140 control-treated model assessed by TUNEL staining. In panel C, * $p < 0.05$ according to the two-way ANOVA; In panel D-I, * $p < 0.05$ according to unpaired t test. Results are expressed as mean $\pm$ SD ($n = 3$ per group). Experiment was repeated 3 times.

Figure 5

RhoA, a target of miR-140, is involved in the modulation of miR-140-dependent myocardial I/R injury. A, the binding relationship between miR-140 and RhoA predicted by StarBase and validated by a dual luciferase reporter gene assay; B, the protein expression of RhoA in nude mice injected with NEAT1-NC, NEAT1-OE, miR-140 control and miR-140 inhibitor determined by western blot analysis; C, the protein expression of RhoA in nude mice injected with miR-140 inhibitor + C3 or miR-140 inhibitor + DMSO determined by western blot analysis; D, LVEF and LVFS in mice injected with miR-140 inhibitor + C3 or miR-140 inhibitor + DMSO evaluated by ECG; E, myocardial infarct area in mice injected with miR-140 inhibitor + C3 or miR-140 inhibitor + DMSO tested by TTC staining; F, the apoptosis rate of injected with miR-140 inhibitor + C3 or miR-140 inhibitor + DMSO-treated model assessed by TUNEL staining. In panel A-C, * $p < 0.05$ according to the two-way ANOVA; In panel D-F, * $p < 0.05$ according to unpaired t test. Results are expressed as mean $\pm$ SD ($n = 3$ per group). Experiment was repeated 3 times.

Figure 6

A diagram illustrating Sev diminishes injury caused by myocardial I/R via the NEAT1/miR-140/RhoA axis. NEAT1 sponged miR-140 to mediate RhoA expression. Sev treatment mitigated I/R injured mice by downregulating NEAT1 and RhoA, while upregulating miR-140 expression.

Supplementary Files

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