LncRNA LINC00461 exacerbates myocardial ischemia-reperfusion injury via microRNA-185-3p/Myd88

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Research Article

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Abstract

Objective

Long non-coding RNA (lncRNA) play critically in myocardial ischemia-reperfusion (I/R) injury. Thus, it was proposed to investigate the mechanism of LINC00461 in the disease through mediating microRNA-185-3p (miR-185-3p)/myeloid differentiation primary response gene 88 (Myd88) axis.

Methods

Mice with I/R injury were injected with the vectors that altered miR-185-3p and LINC00461 expression. miR-185-3p, LINC00461 and Myd88 expression in mice with I/R injury were measured, as well as cardiac function, hemodynamics, myocardial fibrosis, infarction area, oxidative stress, and cardiomyocyte apoptosis.

Results

Raised LINC00461 and Myd88 and suppressed miR-185-3p levels were measured in I/R mice. Down-regulating LINC00461 or up-regulating miR-185-3p recovered cardiac function, reduced myocardial collagen hyperplasia, fibrosis and infarction, and attenuated oxidative stress and cardiomyocyte apoptosis in mice with I/R.

Conclusion

Down-regulation of LINC00461 attenuates myocardial I/R injury via suppressing miR-185-3p-targeted Myd88 expression.

Introduction

Myocardial ischemia-reperfusion (I/R) injury is a main factor leading to the morbidity and mortality related to coronary artery disease [1]. Thrombolysis, cardiac surgery and primary angioplasty are effective treatment methods to restore ischemic myocardial blood flow, the purpose of which is to restore the blood supply of ischemic myocardium, and these ways are widely utilized in clinical practice [2]. However, sudden recovery of blood flow may lead to additional cardiovascular trauma as reperfusion injury [3]. In addition to promoting myocardial hypoxia and ischemia, myocardial I/R injury transforms conduction system and excitability of cardiac muscles, damages systolic function and cardiac diastolic, and triggers serious myocardial dysfunction and injury [4]. Although extensive studies have been conducted, the molecular mechanisms implicated in the initiation and development of myocardial I/R injury have not been fully understood [5]. The severe situation asks for more exploration of potential molecular mechanisms for myocardial I/R injury treatment.

Long non-coding RNAs (lncRNAs) have over 200 nucleotides and become new modulators in various of biological processes, containing RNA splicing, cancer generation and development as well as epigenetic regulation [6]. A recent study has identified that lncRNA LINC00461 impacts the survival of renal cell carcinoma patients via functioning as a competitive endogenous RNA of microRNA-942 [7]. Another study has reported that LINC00461 facilitates the development of breast cancer and glioma [8]. MicroRNAs (miRNAs or miRs) are single-stranded small non-coding RNAs with a length of 20–22 nucleotides, and participate in many biological processes [9]. Moreover, it was revealed that endogenous inhibition of miR-185 facilitates the recovery of cardiac function in mice after myocardial infarction [10]. There is a study reporting that circulating miR-185 may be a new biomarker for the clinical prognosis of patients with dilated cardiomyopathy [11]. Myeloid differentiation primary response gene 88 (Myd88) is a key participant in the innate immune system [12]. Moreover, it was presented that bone marrow Myd88 signaling regulates ischemic myocardial injury and neutrophil function [13]. Moreover, it has been proven that inhibition of Myd88 is involved in the protection against myocardial injury induced by I/R in rats [14]. Therefore, we aim to discuss the effect of LINC00461/miR-185-3p/Myd88 axis on myocardial I/R injury in this article.

Materials And Methods

Ethics statement

All animal experiments were in tally with the Guide for the Care and Use of Laboratory Animal by International Committees. The protocol was approved by the Institutional Animal Care Use Committee of The First Affiliated Hospital of Anhui Medical University.

Experimental animals

Male healthy C57BL/6 mice (n = 90), aged 10–12 week and weighed 20–30 g were bought from Beijing Vital River Laboratory Animal Technology Co., Ltd (Beijing, China). The mice were fed adaptively for 1 w in a clean animal house with 22–25°C, water and food available ad libitum and normal circadian rhythm. Twenty mice were divided into the sham group and I/R group with 10 mice in each group to perform the establishment of I/R model and the identification experiment. And then the rest 70 mice were treated according to the successful modeling method.

Modeling of I/R mice

Fasted for 8 h and anesthetized with 1% pentobarbital sodium solution (60 mg/kg) (Sigma, Santa Clara, CA, USA), mice were placed in a supine position with the skin of the regiones colli anterior cut off after disinfection. The tissues and muscles were separated and trachea was exposed; trachea cannula was inserted from the mouth and connected with a respirator. The mice were placed in the right lateral position. The left side of the epidermis of the fifth costal space was made a 2-cm longitudinal incision. The skin was cut open, then the ectopectoralis and entopectoralis were separated, and the fourth costal space was exposed. The fourth costal space was impaled by mosquito forceps, the mediastinum to the

left was penetrated, and then the heart was extruded by squeezing the right chest with hand. At the 0.5 cm of lower edge of left atrial appendage, the left coronary artery was ligated with a 6 − 0 suture, with a slipknot and timing. Left ventricular anterior wall whitening or ST segment elevation in the electrocardiogram (ECG) monitoring indicated successful ligation. After ischemia of 30 min, the slipknot was loosened and then re-clocked. Meanwhile, the ST-segment raised in the ECG (ST segment recovery or a change significantly different from that of the previous waveform) within 5 min was noted as a reperfusion success. The heart was then returned to the chest and the contralateral thoracic cavity was pressed with hand to remove the pneumothorax. The skin was sutured. After the operation, the mice were put back into the animal cage and the vital signs of the mice were observed. The same thoracotomy was used in the sham group, but the left coronary artery was not ligated. Cardiac ultrasound detection and hemodynamic monitoring were used to determine whether the model was successfully established.

Grouping and treatment

Seventy mice were distributed into 7 groups (10 mice/group) and injected with normal saline, si-LINC00461 vector, si-LINC00461 vector negative control (NC), miR-185-3p mimics, miR-185-3p mimics NC, miR-185-3p mimics and overexpression (OE)-LINC00461 vector, or miR-185-3p mimics NC and OE-LINC00461 vector through the tail vein 24 h before modeling. The left coronary artery was ligated, and the ligature line was loosened after 30 min, and re-perfused for 2 h. si-LINC00461, si-NC, miR-185-3p mimics, mimics NC and OE-LINC00461 were bought from GenePharma Co., Ltd. (Pudong District, Shanghai, China).

Cardiac ultrasound detection

The tissues and skins in the chest of mice were sutured layer by layer at the end of reperfusion. Left ventricular end diastolic volume (LVEDV) and left ventricular end systolic volume (LVESV) were gauged; the left ventricular ejection fractions (LVEF) and left ventricular fractional shortening (LVFS) were computed. LVEF (%) = (LVEDV - LVESV)/LVEDV × 100%.

Hemodynamic detection

The abdominal cavity of mice were injected into 1% pentobarbital sodium solution. The neck tissues were bluntly separated to expose the carotid artery, the distal end was ligated and the proximal part was pulled by silk thread, 1.4F Millar catheter (Millar Instruments, Inc. Houston, TX, USA) was inserted into the carotid artery of mice. When the Millar catheter entered to the left ventricle, the maximum rate of rise of left ventricular pressure increase/decrease (± dp/dt max) were recorded. According to the blood pressure waveform, whether the catheter into the left ventricular chamber was determined.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Trizol (Invitrogen, Carlsbad, CA, USA) method was utilized for extracting total RNA in myocardial tissues. The concentration and quality of RNA were determined by NanoDrop2000 (Thermo Fisher Scientific, Massachusetts, USA). Reverse transcription reaction was in accordance with the ReverTra Ace qPCR RT

Kit (TOYOBO, Osaka, Japan) with 500 ng loading sample. RT-PCR reaction system was prepared based on the Sybr green reagent kit (Takara Co., Ltd., Dalian, Liaoning, China). The primers were composed by BGI Co. (Shenzhen, Guangdong, China) (Table 1). U6 was the endogenous control of miR-185-3p while glyceraldehyde phosphate dehydrogenase (GAPDH), the endogenous control of LINC00461 and Myd88. The product was verified by agarose gel electrophoresis. Data were reckoned by $2^{-\Delta\Delta Ct}$ method.

Table 1
Primer sequence

Gene	Sequence (5'→3')
LINC00461	F: 5'-GACATTTACGCCACAACCCACG-3'
	R: 5'-AGACAGACCCTCAGATTCCCCA-3'
miR-185-3p	F: 5'-GGATCCAAAGAACATCAGATCCATGG-3'
	R: 5'-AAGCTTAGCAGACATAGCCAGCCAGA-3'
Myd88	F: 5'– CAACCAGCAGAAACAGGAGTCT-3'
	R: 5'-ATTGGGGCAGTAGCAGATGAAG- 3'
U6	F: 5'-ATTGGAACGATACAGAGAAGATT-3'
	R: 5'-GGAACGCTTCACGAATTTG-3'
GAPDH	F: 5'-ACGGCAAGTTCAACGGCACAG-3'
	R: 5'-GACGCCAGTAGACTCCACGACA-3'

Note: F, forward; R, reverse; LINC00461, long non-coding RNA LINC00461; miR-185-3p, microRNA-185-3p; Myd88, myeloid differentiation primary response gene 88; GAPDH, glyceraldehyde phosphate dehydrogenase.

Western blot analysis

The total protein in myocardial tissues of mice were abstracted. The tissues were cleaned twice or three times with phosphate buffered saline (PBS), cut into small pieces and placed in the homogenizer. The obtained homogenate was moved into a centrifuge tube, followed by an oscillation and ice bath, during which the tissues were triturated repeatedly to ensure the cells were lysed. The liquid supernatant (total protein solution) was amassed by centrifugation at 12,000 rpm for 5 min. The protein concentration was determined by bicinchoninic acid method (Boster Biological Technology Co. Ltd., Wuhan, Hubei, China). The protein was appended with loading buffer and boiled at 95°C for 10 min, with 30 μg loaded in each well. The protein was separated with 10% polyacrylamide gel electrophoresis for 45–70 min, and transferred to a polyvinylidene fluoride membrane and sealed with 5% bovine serum albumin. The membrane was hatched with primary antibody Myd88 (1:1000), Bcl-2 (1:2000), Bax (1:2000) and GAPDH (1:3000) (all from Abcam, Cambridge, MA, USA) and with corresponding secondary antibody (Miaotong Biotechnology Co., Ltd., Xuhui District, Shanghai, China). The image was developed by

chemiluminescence reagent and Bio-rad Gel Doc EZ imager (Bio-rad, California, USA). The target band was analyzed by ImageJ software to performing gray value analysis.

RNA pull-down assay

Three different biotin-labeled miRNA sequences were designed: wild type (WT) miR-185-3p (Bio-miR-185-3p-WT), mutant type (MUT) miR-185-3p (Bio-miR-185-3p-MUT, the sequence complementary to the LINC00461 was mutated), and a random miRNA (Bio-NC) complementary to LINC00461 as a NC. When reached 80–90 confluence, the above mentioned miRNA (Bio-miR-185-3p-WT, Bio-miR-185-3p-MUT and Bio-NC) was transfected. The cells were lysed at 48 h post transfection to obtain a protein lysate. The lysate was hatched with M-280 streptavidin-coated magnetic beads (Sigma). The magnetic beads were washed twice with cold buffer, three times with hypotonic buffer and once with hyperosmotic buffer in turn. Finally, the protein-nucleic acid complex adsorbed by the magnetic bead was eluted. Trizol was utilized to lyse the protein-nucleic acid complex, the RNA was extracted and the expression of LINC00461 was detected by RT-qPCR.

Dual luciferase reporter gene assay

The targeting relationship between miR-185-3p and Myd88 and the binding site between miR-185-3p and Myd88 3'untranslated region (UTR) were predicted by bioinformatics software https://cm.jefferson.edu/rna22/Precomputed. The Myd88 3'UTR sequence containing miR-185-3p binding site was synthesized and the Myd88 3'UTR WT plasmid (Myd88 3'UTR-WT) was constructed. On the basis of the plasmid, the Myd88 3'UTR MUT plasmid (Myd88 3'UTR-MUT) was constructed. The logarithmic growth 293T cells were cultured into 70% confluence on 96-well plates. Myd88 3'UTR WT plasmid and miR-185-3p mimics plasmid or its NC/Myd88 3'UTR MUT plasmid and miR-185-3p mimics plasmid or its NC were transfected into 293T cells according to Lipofectamine 2000 (Invitrogen) specification. The luciferase activity was detected by Dual-Luciferase@ Reporter Assay System kit (Promega Corporation, Madison, WI, USA) 48 h later.

Hematoxylin-eosin (HE) staining

Preparation of myocardial tissue sections: after 2 h of cardiac I/R in mice, 4 mice were selected for thoracotomy again. The myocardial tissues were flushed in 4°C pre-cooled PBS and fastened with paraformaldehyde for 24 h. The tissues were embedded with paraffin and sliced to 5 μm using a paraffin slicer (Leica, Stockholm, Sweden), and the slice was baked in an oven at 60°C for 20 min. The tissue wax block was sliced with a paraffin slicing machine (Leica), and the tissues were baked at 60°C in an oven for another 20 min.

The slices were dyed with coaggulating reagent TO□ and TO□ (Guangzhou Jetway Biotech Co., Ltd, Guangzhou, China) for 10 min, respectively. The tissues were dehydrated in 95%, 80% and 75% ethanol for 2 min in turn. Then, the tissues were cleaned with running water, dyed with hematoxylin, returned to blue by tap water and differentiated with hydrochloric acid alcohol. The slices were rinsed under the running water for 5 min when the color was turned red from blue, dyed with eosin for 1 min, hydrated with

inverse concentration gradient ethanol, and placed into TO for 10 min and finally blocked with neutral gum. The tissues were examined with a light microscope for histopathology and photographed.

Picric acid-Sirius red staining

The myocardial tissue sections of mice were dewaxed in turpentine I, solution for 15 min, respectively, then step-by-step hydrated in 100% ethanol I, , 95%, 90%, 80% and 75% ethanol and distilled water for 5 min, respectively, placed in hematoxylin solution for 2–3 min, rinsed with clear water for 30 min, dehydrated with 75%, 80%, 95% and 100% for 3 min, respectively. The sections were cleared with xylene and sealed with neutral gum. The degree of myocardial fibrosis was observed under a light microscope.

Terminal deoxynu-cleotidyl transerase-mediated dUTP-biotin nick end-labeling (TUNEL) staining

Preparation of the frozen section of the mice heart: the remaining 3 mice were euthanized at the end of the experiment, and the heart was perfused with physiological saline to remove the residual blood from the heart, and 10% muriate was injected. The mouse heart was immediately buried in the optimal cutting temperature (OCT) (the bottom of the heart was down, the tip of the heart was up). The embedded tissue can be stored in a -4℃ refrigerator. A continuous frozen section was prepared into 4 μm and the tissue sections were placed on a polylysine coated slide after exposure of the papillary muscle.

The frozen sections were fastened with 4% paraformaldehyde, cleaned with PBS, added with 50 μL DNA labeling solution avoiding light for 1 h, reacted with 100 μL antibody solution for 0.5 h avoiding light and dyed with 100 μL 7-AAD/RNase solution for 0.5 h. Nucleus was stained with 4'-6-diamidino-2-phenylindole solution. The sections were sealed and observed by a microscope.

Serum index detection

All the mice at the end of the reperfusion were taken 4 mL blood by a disposable venous blood sample collection container separation glue or coagulant promoter from the abdominal aorta. The blood was centrifuged at 3000 r/min for 10 min to take the supernatant. According to the instructions of the lactate dehydrogenase (LDH) kit, the creatine kinase-MB (CK-MB) kit, the cardiac troponinI (cTn-I) kit and the nitric oxide (NO) kit (all from NanJing JianCheng Bioengineering Institute, Nanjing, China), corresponding sample and reagent was added for detection. With the optical density (OD) values determined by a microplate reader, the standard curve was drawn and the activity or content of each index in the sample was calculated.

Detection of myocardial tissue index in I/R mice

At the end of reperfusion, all the apical tissues of the mouse heart were removed, shredded and put into the 10 mL homogenate tube. The tissues were homogenized in the precooled 0.9 NaCl solution (1 mL/100 mg) at the weight to volume ratio of 1 : 9, and then the homogenate was placed in precooled homogenate medium (0.01M Tris-HCl, 0.0001M ethylene diamine tetraacetic acid-2Na, 0.01M sucrose, 0.8% NaCl solution, pH 7.4) to prepare 10% homogenate. Reactive oxygen species (ROS) and

malondialdeyde (MDA) content, as well as superoxide dismutase (SOD) activity were determined according to ROS, MDA and SOD kits (JianCheng Bioengineering Institute).

Statistical analysis

All date were processed by SPSS 21.0 software (IBM Corp. Armonk, NY, USA). Measurement data were interpreted as mean ± standard deviation. Comparisons between two groups were conducted by *t*-test in data in normal distribution. Comparisons among multiple groups were assessed by one-way analysis of variance (ANOVA) and the pairwise comparisons after ANOVA were analyzed by Fisher's least significant difference t test (LSD-t). *P* value < 0.05 was indicative of statistically significant difference.

Results

Up-regulating miR-185-3p and down-regulating LINC00461 enhance heart function, LVEF, LVFS, ±dp/dt max and decrease LVEDP of I/R mice

It was reported that the heart function, LVFS, LVEF, ±dp/dt max decreased and LVEDP raised in I/R mice. Up-regulating miR-185-3p or down-regulating LINC00461 improved heart function, LVFS, LVEF, ±dp/dt max and declined LVEDP. miR-185-3p overexpression followed by LINC00461 up-regulation promoted heart function, LVFS, LVEF, ±dp/dt max and reduced LVEDP (Fig. 1A-E).

LINC00461 binds to miR-185-3p; LINC00461 is up-regulated while miR-185-3p is down-regulated in I/R mice

The specific binding region of LINC00461 and miR-185-3p was predicted by the bioinformatics software (Fig. 2A). Dual luciferase reporter gene assay showed that miR-185-3p mimics impaired the luciferase activity of WT LINC00461, while imposed no effect on that of MUT LINC00461 (Fig. 2B).

RNA pull-down assay revealed that compared to the Bio-NC group, LINC00461 expression raised in the Bio-miR-185-3p-WT group ($P < 0.05$), and there was no distinct difference of LINC00461 expression in the Bio-miR-185-3p-MUT group ($P > 0.05$) (Fig. 2C).

RT-qPCR was utilized to detect LINC00461 and miR-185-3p expression in the myocardial tissues. The results demonstrated that LINC00461 expression was enhanced and miR-185-3p expression was down-regulated in I/R mice. LINC00461 down-regulation decreased LINC00461 expression while heightened miR-185-3p expression. miR-185-3p restoration had no effect on LINC00461 expression but elevated miR-185-3p expression. miR-185-3p enhancement followed by LINC00461 overexpression imposed no effect on LINC00461 expression while raised miR-185-3p expression (Fig. 2D).

MiR-185-3p targets Myd88 and Myd88 expression increases in I/R mice

The binding site of Myd88 and miR-185-3p was predicted by the bioinformatics software (Fig. 3A). Myd88 was the target gene of miR-185-3p, which was verified by dual luciferase reporter gene assay. The results displayed that miR-185-3p mimics destroyed the luciferase activity of Wt-Myd88, indicating that miR-185-3p could specifically bind to Myd88 (Fig. 3B).

Western blot analysis and RT-qPCR detected Myd88 expression in the myocardial tissues. It was presented that Myd88 expression was elevated in I/R mice. miR-185-3p restoration or LINC00461 knockdown degraded Myd88 expression. miR-185-3p up-regulation followed by LINC00461 overexpression caused a reduction in Myd88 expression (Fig. 3C-E).

Up-regulating miR-183-3p and down-regulating LINC00461 alleviate the pathological injury of I/R mice

HE staining revealed that in normal mice, myocardial fiber cells were arranged neatly and tightly, the cytoplasm and nucleus staining were uniform, with only a small amount of bleeding, mild edema and without necrosis, apoptosis, or inflammatory cell infiltration. In I/R mice, and I/R mice injected with si-NC, mimics NC or OE-LINC00461 + miR-185-3p mimics, broken myocardial fibers were loosely arranged with obvious intervals, serious bleeding and edema, obvious cell necrosis and serious infiltration of inflammatory cells. Mice injected with down-regulated LINC00461 or elevated miR-185-3p showed with neatly and tightly arranged myocardial fiber cells with less bleeding, mild edema, a small number of cell necrosis and inflammatory cell infiltration, and the overall condition was better than I/R mice. In mice injected with OE-LINC00461 and mimics NC in order, aggravated myocardial fiber rupture, loosely arranged cells with obvious intervals, aggravated bleeding and edema, and severe cell necrosis and inflammatory cell infiltration were observed; the overall condition was severer than I/R mice (Fig. 4).

Restoration of miR-185-3p and depletion of LINC00461 reduce the degree of myocardial fibrosis in I/R mice

Picric acid-Sirius red staining reported that the myocardial collagen was obviously proliferated and thickened, and the cardiomyocytes were divided into a mesh-like shape, the area of myocardial tissue fibrosis increased in I/R mice. Silencing LINC00461 or restoring miR-185-3p alleviated myocardial collagen hyperplasia and reduced area of myocardial tissue fibrosis. miR-185-3p enhancement followed by LINC00461 overexpression alleviated myocardial collagen hyperplasia and narrowed the area of myocardial tissue fibrosis (Fig. 5A,B).

Restored miR-185-3p and depleted LINC00461 suppress apoptosis of cardiomyocytes in I/R mice

Western blot analysis tested Bcl-2 and Bax protein expression while TUNEL staining tested cell apoptosis. The results displayed that Bcl-2 expression reduced while Bax expression and cell apoptosis rate increased in I/R mice. Bcl-2 expression increased while Bax and cell apoptosis rate degraded by down-regulating LINC00461 or enhancing miR-185-3p. miR-185-3p enhancement followed by LINC00461

overexpression heightened Bcl-2 expression whereas reduced Bax expression and cell apoptosis rate (Fig. 6A-D).

Up-regulating miR-185-3p and down-regulating LINC00461 decline LDH, CK-MB and cTnI as well as raise NO in I/R mice

The blood samples were collected from the abdominal aorta of mice in each group, and the indices were detected. The results presented that LDH, CK-MB and cTnI contents enhanced and NO content decreased in I/R mice. LDH, CK-MB and cTnI contents depressed and NO content elevated by silencing LINC00461 or restoring miR-185-3p. miR-185-3p enhancement followed by LINC00461 overexpression decreased LDH, CK-MB and cTnI contents and elevated NO content (Fig. 7A-D).

Restoring miR-185-3p and silencing LINC00461 decline ROS and MDA while enhance SOD activity in I/R mice

The oxidative stress level of myocardial tissue in mice was measured. The results reported that ROS and MDA contents elevated and SOD activity impaired in I/R mice. Restoring miR-185-3p or silencing LINC00461 degraded ROS and MDA contents and enhanced SOD activity. miR-185-3p enhancement followed by LINC00461 overexpression reduced ROS and MDA contents and reinforced SOD activity (Fig. 8A-C).

Discussion

Myocardial I/R injury is a complicated pathophysiological processes [15]. A previous study has discussed that lowly expressed lncRNA KCNQ1OT1 protects against myocardial I/R injury after acute myocardial infarction [16]. Another study has reported that miR-497 accelerates proliferation and suppresses cardiomyocyte apoptosis in myocardial I/R injury [17]. Furthermore, it was revealed a critical role for MyD88-dependent signaling pathway during myocardial I/R injury while the regulation of the IL-1R/MyD88 interaction may be a strategy for declining myocardial ischemic injury [18]. As the related mechanisms of LINC00461 and miR-185-3p in myocardial I/R injury still remained enigmatic, our study was to inquire LINC00461/miR-185-3p/Myd88 axis on myocardial I/R injury and their inner mechanisms.

Our study has provided evidence that LINC00461 and Myd88 increased while miR-185-3p decreased in I/R mice. Recently, a study has promoted that LINC00461 expression was heightened in hepatocellular carcinoma (HCC) tissues and also positively related to advanced stage and metastasis as well as its up-regulation in HCC patients forecasts unfavorable outcome [8]. Another study has presented that LINC00461 expression was dramatically elevated in breast cancer tissues and cell lines, and related to differentiation and TNM stage [19]. It is reported that miR-185 expresssion was notably declined in myocardial cells in the process of cardiac hypertrophy caused by transverse aortic constriction [20]. Similarly, a previous study has pointed out that miR-185-5p expression was markedly reduced in hearts of mice with myocardial infarction [10]. It has been demonstrated that MyD88 expression was dramatically enhanced in cardiomyocytes treated with hypoxia/reoxygenation [21]. Another study has proven that

MyD88 expression was markedly raised in the I/R group by comparison with the sham group [22]. Furthermore, our study has proven that LINC00461 binds to miR-185-3p and Myd88 is targeted by miR-185-3p. It was showed that LINC00461 functions as a competing endogenous RNA of miR-942 [7]. And it was also presented that LINC00461 act as a sponge for miR-15a/16 [23]. It was revealed that miR-940 modulates the inflammatory response of chondrocytes via targeting MyD88 in osteoarthritis [24]. Another study has reported that miR-128 mediates negative modulation in staphylococcus aureus caused inflammation via targeting MyD88 [25]. While the relationship between LINC00461 and miR-185-3p as well as Myd88 and miR-185-3p has not been elucidated.

In addition, it was revealed in our study that depleted LINC00461 and restored miR-185-3p decreased LVEDP, LDH, CK-MB, cTnI, ROS and MDA levels, and enhanced LVEF, LVFS, ±dp/dt max, NO and SOD levels, as well as attenuated cardiomyocyte apoptosis in I/R mice. It has been suggested previously that LINC00461 down-regulation notably inhibited proliferation and promoted apoptosis of multiple myeloma cells [23]. Another study has verified that miR-185 up-regulation suppresses endoplasmic reticulum stress-caused apoptosis in the heart [26]. LDH is an critical enzyme that produces energy in hypoxia through anaerobic glycolysis [27]. The excessive production of ROS is usually associated with inflammation or cancer as well as may cause tissue damage [28]. Creatine kinase, which located in mitochondria and plasma of tissues including brain tissue, skeletal muscle, and cardiac muscle, is a key kinase correlated with ATP regeneration, intracellular energy transportation and muscle contraction and is widely utilized as a biomarker of myocardial injury [29]. Additionally, an experiment presented that LVEF and LVFS in mice with myocardial I/R injury was improved by treatment [30]. A study revealed that myocardial I/R insult declined LVEF and + dp/dtmax, increased LVEDP, serum CK, LDH, and cTnT levels, also reduced SOD activity and raised MDA level in the myocardial tissue [31]. In addition, it was displayed that LDH, ROS and MDA was decreased and SOD was increased by treatment in myocardial tissues [32]. It has been documented that myocardial I/R resulted in a distinct ascend in CK-MB, MDA and LDH activities, markedly descend in SOD and NO levels, while necrosis, myocardial and interstitial edema, infiltration of neutrophil granulocytes was appeared in tissues of the I/R group [33].

Conclusion

In summary, this investigation reveals that depleted LINC00461 and restored miR-185-3p alleviate myocardial I/R injury via suppressing Myd88 expression. Thus, LINC00461/miR-185-3p may serve as a potential target for the treatment of myocardial I/R injury. However, a conclusion about the effects of LINC00461 and miR-185-3p cannot be made clearly due to limited known researches on this. It needs to be monitored rigorously and reported appropriately in the future clinical trials.

Abbreviations

lncRNA : long non-coding RNA

I/R: ischemia-reperfusion

miR-185-3p: microRNA-185-3p

Myd88: myeloid differentiation primary response gene 88

ECG : electrocardiogram

NC: negative control

OE : overexpression

LVEDV: Left ventricular end diastolic volume

LVESV: left ventricular end systolic volume

LVEF: left ventricular ejection fractions

LVFS: left ventricular fractional shortening

RT-qPCR : Reverse transcription quantitative polymerase chain reaction

GAPDH : glyceraldehyde phosphate dehydrogenase

PBS: phosphate buffered saline

PVDF: polyvinylidene fluoride

WT : wild type

MUT : mutant type

UTR: untranslated region

HE : Hematoxylin-eosin

TEM : Transmission electron microscope

TTC : triphenyltetrazolium chloride

LAD: left anterior descending coronary artery

TUNEL : transerase-mediated dUTP-biotin nick end-labeling

OCT : optimal cutting temperature

LDH: lactate dehydrogenase

CK-MB: creatine kinase-MB

cTn-I : cardiac troponinI

NO: nitric oxide

OD : optical density

EDTA: ethylene diamine tetraacetic acid

ROS: Reactive oxygen species

MDA: malondialdeyde

ANOVA: analysis of variance

LSD-t: least significant difference t test

Declarations

Conflict of interest

The authors declare that they have no conflicts of interest.

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Ethical statement

This study was approved and supervised by the animal ethics committee of The First Affiliated Hospital of Anhui Medical University. The treatment of animals in all experiments conforms to the ethical standards of experimental animals.

Consent for publication

Not applicable

Availability of data and material

Not applicable

Authors' contributions

Xianhe Lin, Banglong Xu finished study design, Feng Gao, Tingting Fan, Zhidan Luo, Mengqin Ma finished experimental studies, Xiaochen Wang, Guangquan Hu, Yue Li, Yi Liang finished data analysis, Feng Gao, Xiaochen Wang finished manuscript editing. All authors read and approved the final manuscript

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Figures

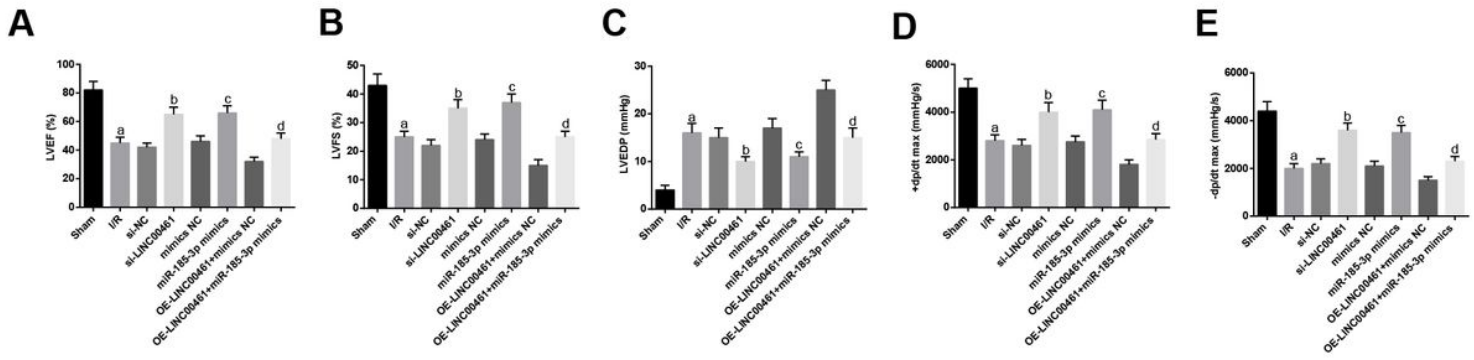

Figure 1

Up-regulating miR-185-3p and down-regulating LINC00461 enhance heart function, LVEF, LVFS, ±dp/dt max as well as decline LVEDP of I/R mice. A, Comparison of LVEF in each group of mice. B, Comparison of LVFS in each group of mice. C, Comparison of LVEDP in each group of mice. D, Comparison of +dp/dt max in each group of mice. E, Comparison of -dp/dt max in each group of mice. a $P < 0.05$ vs. the sham group. b $P < 0.05$ vs. the si-NC group. c $P < 0.05$ vs. the mimics-NC group. d $P < 0.05$ vs. the OE-LINC00461 + mimics NC group.

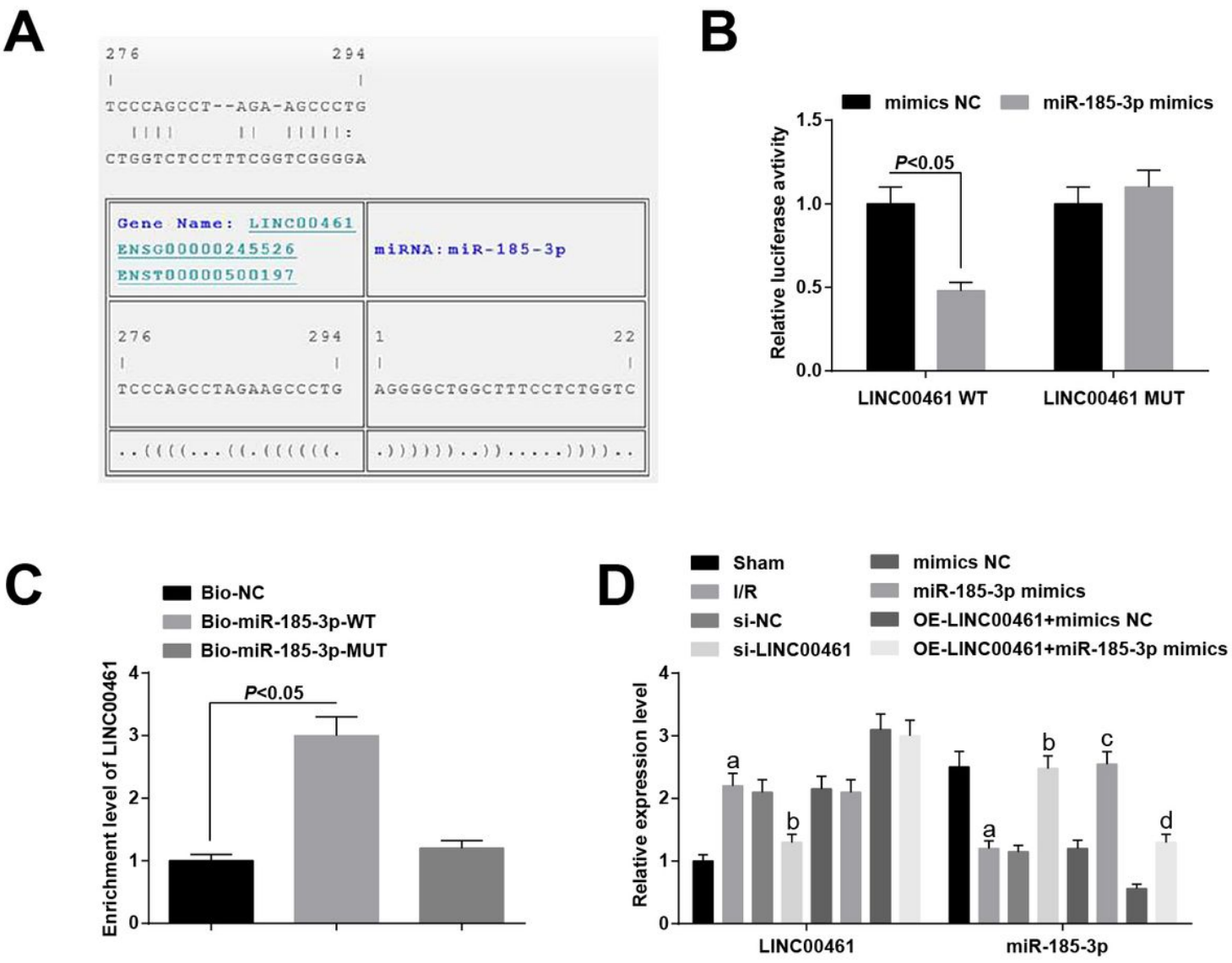

Figure 2

LINC00461 binds to miR-185-3p; LINC00461 is up-regulated while miR-185-3p is down-regulated in I/R mice. A, Binding sites of LINC00461 to miR-185-3p. B, Dual luciferase reporter gene assay verified the regulatory relationship between LINC00461 and miR-185-3p. C, The relationship between LINC00461 and miR-185-3p in myocardial tissue by RNA-pull down assay. D, Expression of LINC00461 and miR-185-3p in myocardial tissue of mice in each group. a $P < 0.05$ vs. the sham group. b $P < 0.05$ vs. the si-NC group. c $P < 0.05$ vs. the mimics-NC group. d $P < 0.05$ vs. the OE-LINC00461 + mimics NC group.

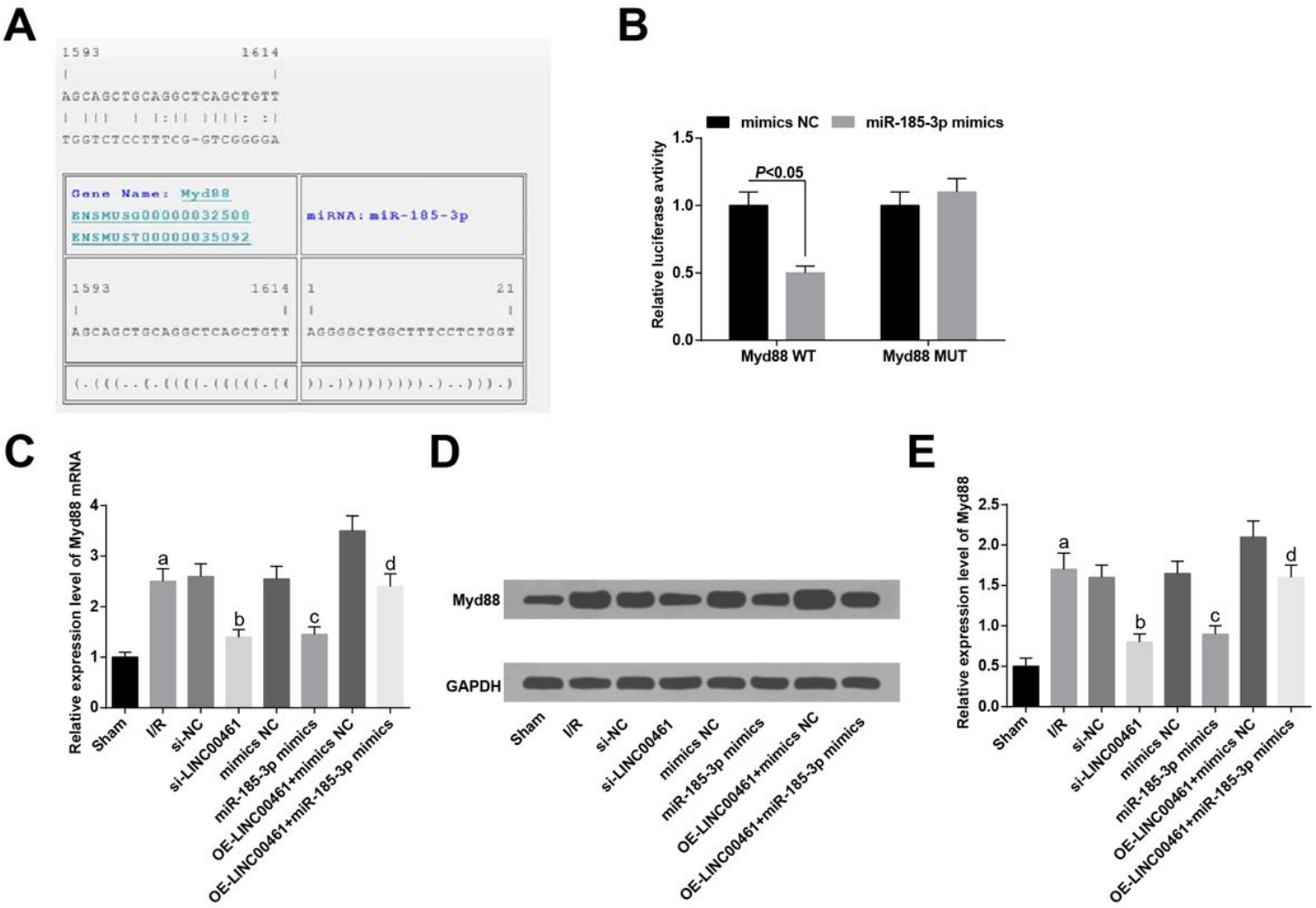

Figure 3

MiR-185-3p targets Myd88 and Myd88 expression increases in I/R mice. A, The binding sites between miR-185-3p and Myd88. B, The targeting relationship between Myd88 and miR-185-3p was verified by dual luciferase reporter gene assay. C, Comparison of Myd88 mRNA expression in myocardial tissue of mice. D, Protein bands of Myd88 of mice in each group. E, Comparison of Myd88 protein expression in the myocardial tissue of each group. a $P < 0.05$ vs. the sham group. b $P < 0.05$ vs. the si-NC group. c $P < 0.05$ vs. the mimics-NC group. d $P < 0.05$ vs. the OE-LINC00461 + mimics NC group.

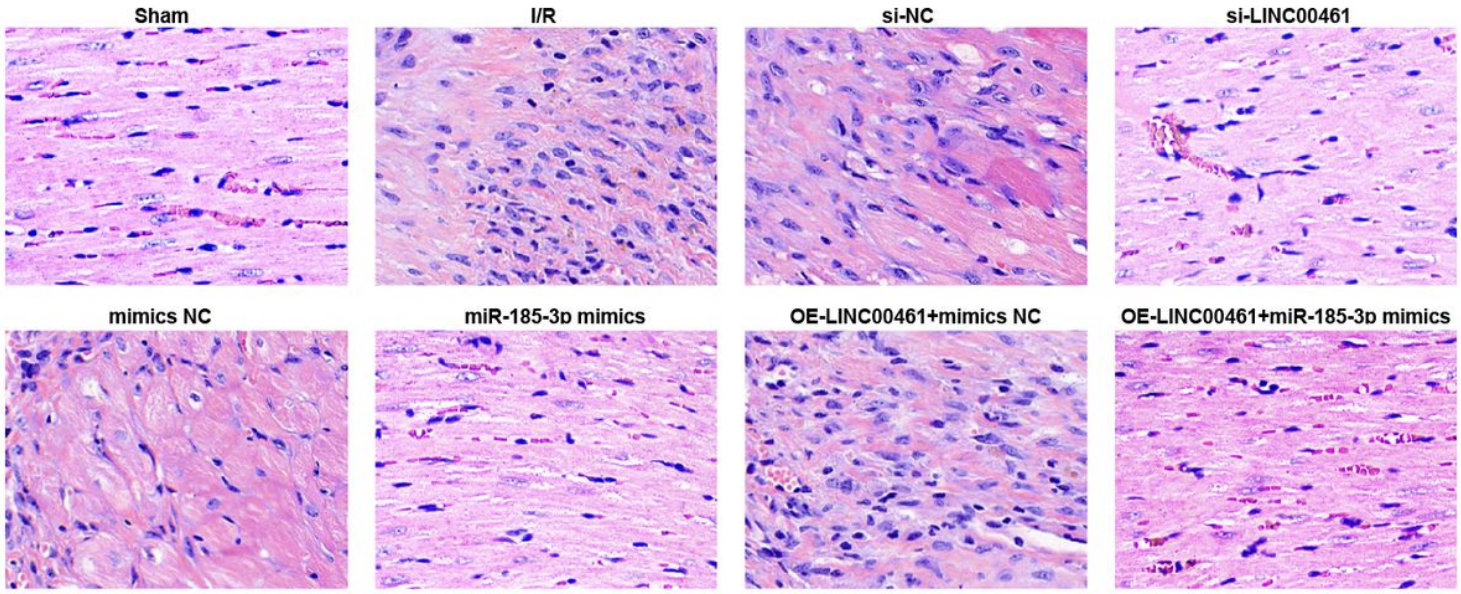

Figure 4

Up-regulating miR-183-3p and down-regulating LINC00461 alleviate the pathological injury of I/R mice. The changes of myocardial tissue structure in each group of mice by HE staining.

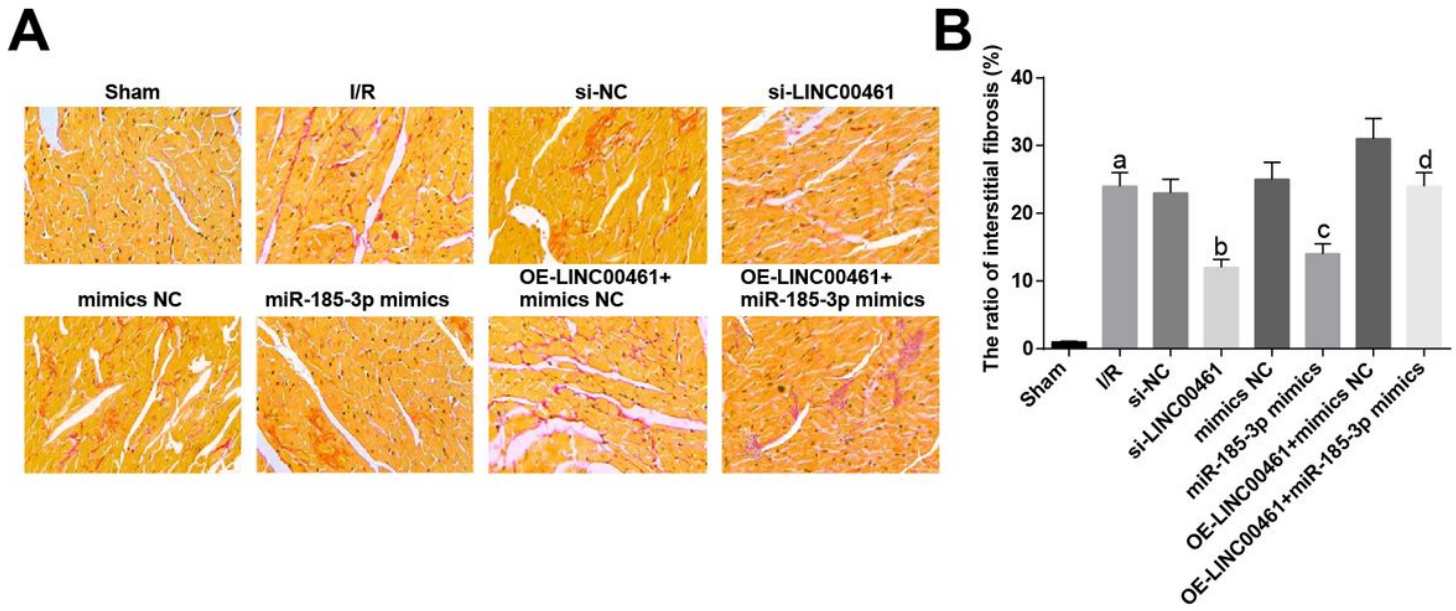

Figure 5

Restoration of miR-185-3p and depletion of LINC00461 reduce myocardial collagen hyperplasia and the area of myocardial tissue fibrosis in I/R mice. A, Degree of myocardial fibrosis in each group of mice. B, Comparison of myocardial fibrosis area in each group of mice. a $P < 0.05$ vs. the sham group. b $P < 0.05$ vs. the si-NC group. c $P < 0.05$ vs. the mimics-NC group. d $P < 0.05$ vs. the OE-LINC00461 + mimics NC group.

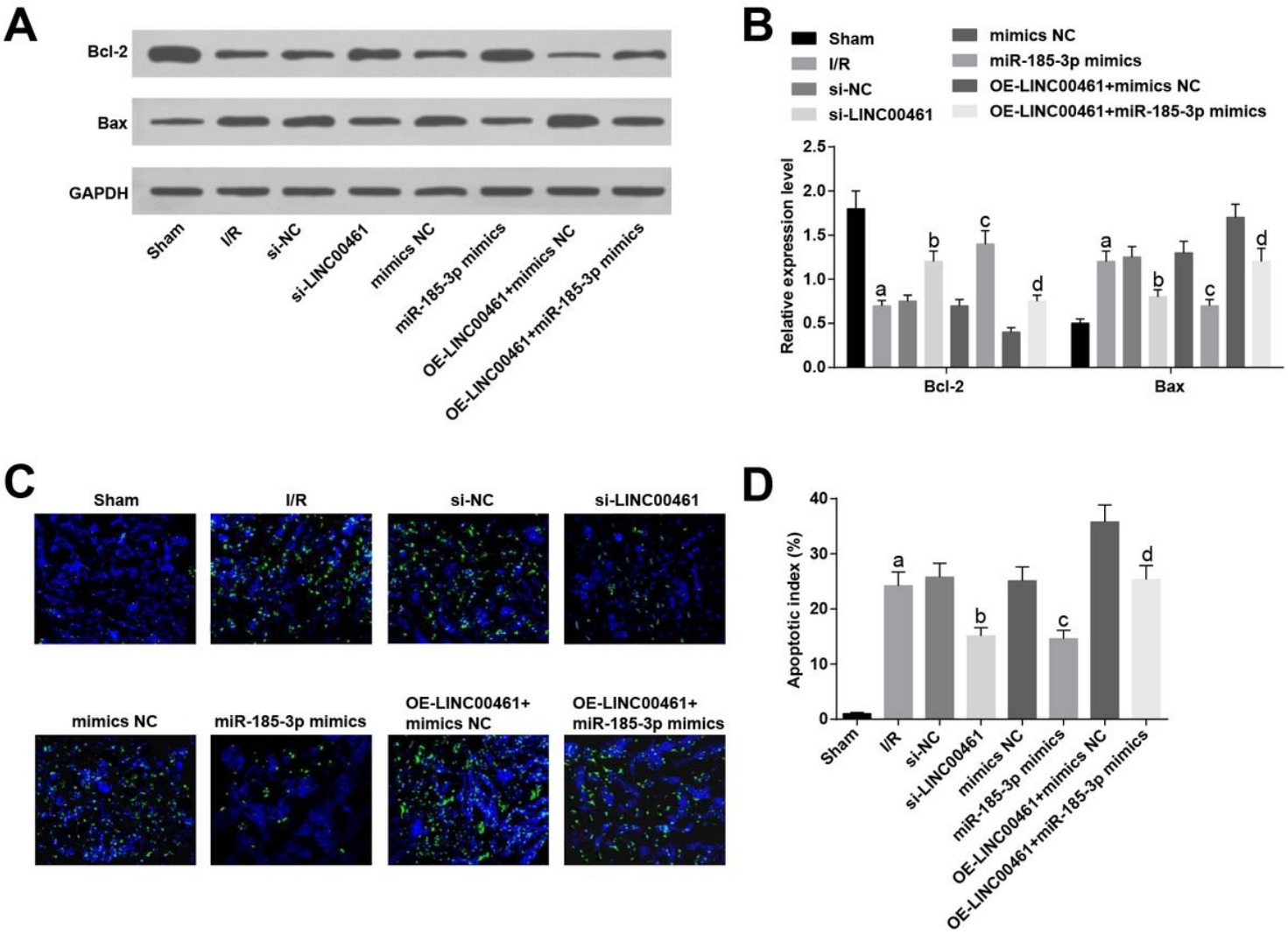

Figure 6

Restored miR-185-3p and depleted LINC00461 suppress apoptosis of cardiomyocytes in I/R mice. A, Protein bands of Bcl-2 and Bax in myocardial tissues of mice. B, Comparison of the protein expression of Bcl-2 and Bax in the myocardial tissue of mice. C, Cell apoptosis tested by TUNEL staining. D, Comparison of the apoptosis index of cardiomyocytes of mice. a $P < 0.05$ vs. the sham group. b $P < 0.05$ vs. the si-NC group. c $P < 0.05$ vs. the mimics-NC group. d $P < 0.05$ vs. the OE-LINC00461 + mimics NC group.

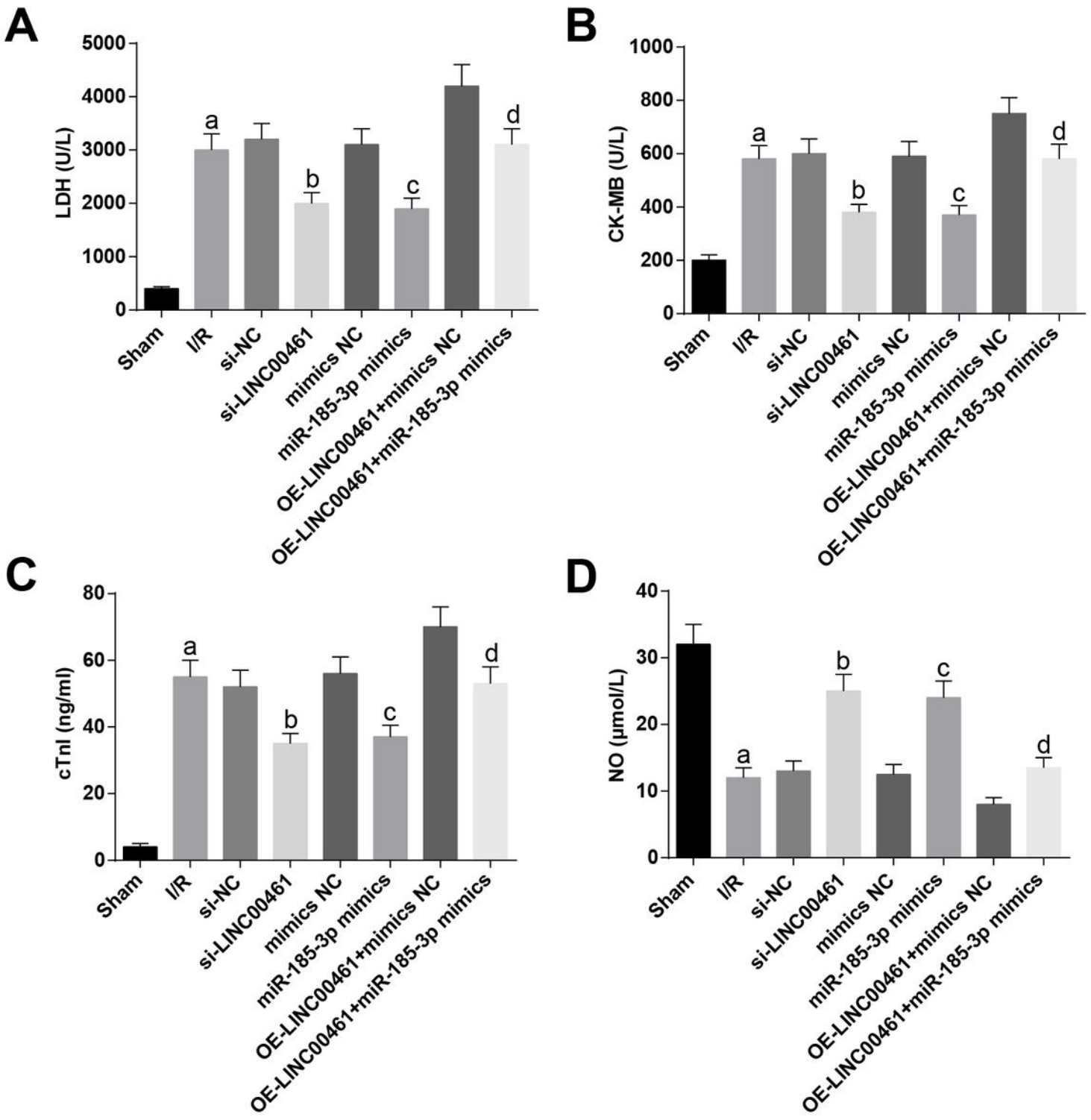

Figure 7

Up-regulating miR-185-3p and down-regulating LINC00461 decrease LDH, CK-MB and cTnI contents as well as increase NO content in I/R mice. A, Content of LDH in serum of abdominal aorta of mice in each group. B, Content of CK-MB in serum of abdominal aorta of mice in each group. C, Content of cTnI in serum of abdominal aorta of mice in each group. D, Content of NO in serum of abdominal aorta of mice

in each group. a P < 0.05 vs. the sham group. b P < 0.05 vs. the si-NC group. c P < 0.05 vs. the mimics-NC group. d P < 0.05 vs. the OE-LINC00461 + mimics NC group.

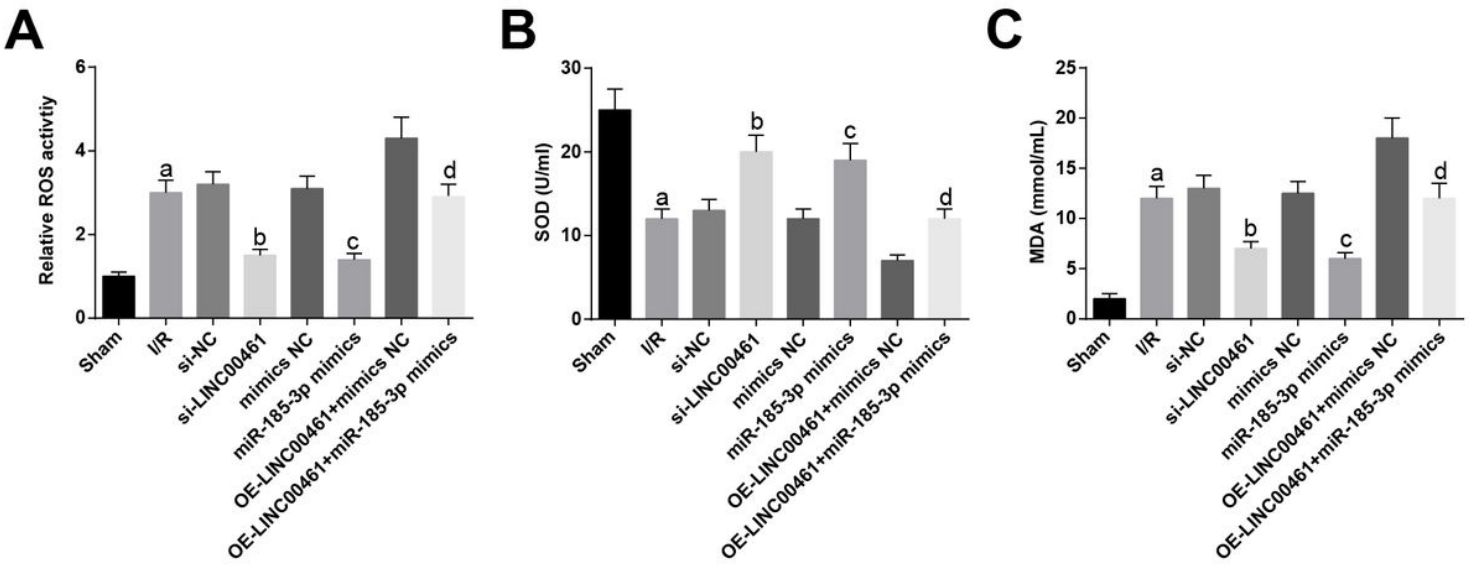

Figure 8

Restoring miR-185-3p and silencing LINC00461 decrease ROS and MDA contents while enhance SOD activity in I/R mice. A, Comparison of ROS levels in myocardial tissue of mice. B, Comparison of SOD levels in myocardial tissue of mice. C, Comparison of MDA levels in myocardial tissue of mice. a P < 0.05 vs. the sham group. b P < 0.05 vs. the si-NC group. c P < 0.05 vs. the mimics-NC group. d P < 0.05 vs. the OE-LINC00461 + mimics NC group.