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Silencing of long non-coding RNA SOX2-overlapping transcript relieves myocardial ischemia/reperfusion injury through up-regulating miRNA-146a-5p

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Abstract. Long non-coding RNAs (lncRNAs) are involved in the development of myocardial ischemia/reperfusion injury (MIRI). In this study, we aimed to explore the regulatory effect and mechanism of lncRNA SOX2-overlapping transcript (SOX2-OT) in MIRI. The viability of oxygen and glucose deprivation/reperfusion (OGD/R)-treated H9c2 cells was detected by MTT assay. The levels of interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, malondialdehyde (MDA), and superoxide dismutase (SOD) were measured by ELISA. The target relationship between SOX2-OT and miR-146a-5p was predicted by LncBase, and subsequently confirmed by Dual luciferase reporter assay. The effects of SOX2-OT silencing on myocardial apoptosis and function were further validated in MIRI rats. The expression of SOX2-OT was increased in OGD/R-treated H9c2 cells and myocardial tissues of MIRI rats. Silencing of SOX2-OT increased the viability and inhibited the inflammation and oxidative stress of OGD/R-treated H9c2 cells. SOX2-OT negatively regulated its target miR-146a-5p. Silencing of miR-146a-5p reversed the effects of sh-SOX2-OT on OGD/R-treated H9c2 cells. In addition, silencing of SOX2-OT also alleviated myocardial apoptosis and improved myocardial function in MIRI rats. Silencing of SOX2-OT relieved the apoptosis, inflammation, and oxidative stress of myocardial cells *via* up-regulating miR-146a-5p, contributing to the remission of MIRI.

Key words: SOX2-OT — miR-146a-5p — Myocardial ischemia/reperfusion injury — Inflammation

Introduction

Myocardial ischemia-reperfusion injury (MIRI) is a kind of myocardial injury that induced by an initial scarcity of heart blood supply and subsequent perfusion and oxygenation (Zhou et al. 2021). Since MIRI can induce the apoptosis and necrosis of cardiomyocytes, it contributes to many adverse cardiovascular outcomes, such as malignant arrhythmia, cardiac insufficiency, and even heart failure (Hausenloy and Yellon 2013; Vogel et al. 2017). Until now, MIRI is still unavoidable following myocardial ischemia, cardiac surgery, and circulatory arrest, and there are still no efficacious strategies to protect the heart against MIRI (Frank et al. 2012; Mokhtari-Zaer et al. 2018). Therefore, discovering of potential therapeutic targets for MIRI is urgently needed.

Long non-coding RNAs (lncRNAs) are critical regulators involved in the pathogenesis of diverse cardiovascular diseases, including coronary disease, myocardial infarction, and heart failure (Poller et al. 2018). Notably, more than 2000 lncRNAs have been discovered to be dysregulated in MIRI (Ghafouri-Fard et al. 2020). In recent years, studies have determined that some lncRNAs exert protective role against MIRI, such as AK006774 (Nie et al. 2021), Opa interacting protein 5-antisense RNA 1 (Niu X et al. 2020), and prostate androgen-regulated transcript 1 (Guo et al. 2021), and on the contrary, some lncRNAs exert pathogenic role, such as KCNQ1 overlapping transcript 1 (KCNQ1OT1)

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(Rong et al. 2020), Taurine up-regulated 1 (TUG1) (Su et al. 2019), Growth arrest-specific transcript 5 (GAS5) (Han et al. 2020), and Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) (Liu et al. 2022). SOX2-overlapping transcript (SOX2-OT) is a specific lncRNA that functions as an oncogene in cancers (Song et al. 2020; Zhao H et al. 2021; Zhang et al. 2022). Evidence has determined that SOX2-OT also plays an important role in hypoxia-induced myocardial injury (Gu et al. 2020; Yang and Lin 2020). For examples, SOX2-OT exacerbates hypoxia-induced myocardial injury *in vitro* (Yang and Lin 2020). Silencing of SOX2-OT suppresses the apoptosis, oxidative damage, and inflammation in oxygen and glucose deprivation-treated cells, and also improves myocardial dysfunction in a rat model of ischemic heart failure (Gu et al. 2020). However, the action mechanisms of SOX2-OT in MIRI are not fully revealed.

The miRNA-lncRNA interaction network plays a pivotal role in the pathophysiological processes of human diseases (Paraskevopoulou and Hatzigeorgiou 2016). Previous studies have reported that SOX2-OT is involved in cardiovascular diseases through regulating specific miRNAs, such as SOX2-OT-miR-27a-3p in myocardial infarction (Yang and Lin 2020), SOX2-OT-miR-455-3p in ischemic heart failure (He et al. 2020), and SOX2-OT-miR-215-5p in ischemic heart failure (Tu et al. 2021). MiR-146a-5p is known as a tumor suppressor (Iacona and Lutz 2019) that also functions in the protection of ischemia-reperfusion (I/R) injury (Li et al. 2020; Zhenzhen et al. 2022). It has been reported that overexpression of miR-146a-5p inhibits autophagy and attenuates intestinal I/R injury in mice (Zhenzhen et al. 2022). miR-146a-5p protects human renal tubular epithelial cells from hypoxia-reoxygenation injury, contributing to the therapeutic effect of urine-derived stem cells on renal I/R injury (Li et al. 2020). Since miR-146a-5p is predicted as a potential target of SOX2-OT by LncBase, we suspect whether SOX2-OT is involved in MIRI through regulating miR-146a-5p.

In this study, the regulatory effects of SOX2-OT were determined on the viability, inflammation, and oxidative stress of oxygen and glucose deprivation/reperfusion (OGD/R)-treated cells, and on the myocardial apoptosis and function in MIRI rats. In addition, the regulatory relationship between SOX2-OT and miR-146a-5p was further determined *in vitro*. This study aimed to reveal potential therapeutic targets for MIRI.

Materials and Methods

Cell culture and treatment

H9c2 cells, a rat cardiomyocyte cell line (European Collection of Cell Cultures, UK) were cultured in Dulbecco's modified Eagle's medium (DMEM) (Weike Biotechnology, Shanghai, China) containing 100 U/ml penicillin, 100 mg/ml streptomycin, and 10% fetal bovine serum (Gibco, Grand Island, NY, USA) at 37°C with 5% CO_2. OGD/R was induced in H9c2 cells by maintaining in "chemical ischemia" medium (in mmol/l: 140 NaCl, 5 $NaHCO_3$, 3.5 KCl, 1.7 $CaCl_2$, 1.25 $MgSO_4$, 0.43 KH_2PO_4, 20 Hepes; 7.2 to 7.4 pH) for 6 h and then in normal medium for 24 h (Li et al. 2020).

Cell transfection

Lentivirus-packaged short hairpin RNA (shRNA) targeting SOX2-OT (sh-SOX2-OT) and negative control (sh-NC) were constructed in Genechem (Shanghai, China). miR-146a-5p mimic, inhibitor, and negative control (miR-NC) were also purchased from GenePharma. These agents were transfected into H9c2 cells using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) for 24 h according to the instructions.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from cells using a Trizol total RNA extraction kit (Vazyme Biotech, Nanjing, China), and was then reverse transcribed into cDNAs using a PrimeScript RT Reagent kit (Takara, Shanghai, China). qRT-PCR was performed using a SYBR Green Master Mix (Vazyme, Nanjing, China) on ABI7500 system (2016, Applied Biosystems, Foster City, CA, USA). GAPDH was used as an internal control. The primer sequences were shown as follows: SOX2-OT (forward, 5'-GTTCATGGCCTGGACTCTCC-3'; reverse, 5'-ATTGCTAGCCCTCACACCTC-3'); miR-146a-5p (forward, 5'-GTCGATGCAGCAAACCTCAGGGAA-3'; reverse, 5'-GCTCAGAAGCACACAAACAAAACT-3'); GAPDH (forward, 5'-TGTTCGTCATGGGTGTGAAC-3'; reverse, 5'-ATGGCATGGACTGTGGTCAT-3'). Relative expression level of SOX2-OT/miR-146a-5p was calculated by $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

Measurement of cell viability

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed to measure the cell viability. Simply, cells (2×10^5/well) were seeded into 96-well plates, and 10 ml MTT (5 mg/ml) was added into each well. After 4 h of culturing, cells were incubated with 150 μl dimethyl sulfoxide for another 15 min. The optical density (OD) at 570 nm was measured by a Microplate Reader (2016, Multiskan, Thermo Fisher Scientific, Waltham, MA, USA).

Enzyme-linked immunosorbent assay (ELISA)

The contents of interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, malondialdehyde (MDA), superoxide

dismutase (SOD), and lactate dehydrogenase (LDH) were measured using commercial ELISA kits (Jiancheng, Nanjing, China) following the manufacturer's instructions. Simply, the tissue or cell samples were homogenized and the supernatants were collected for specific antibody incubation. The OD at 570 nm was measured by a Microplate Reader (Thermo Fisher Scientific).

Target prediction

The potential target miRNAs of SOX2-OT were predicted by LncBase. A total of 2007 targets were predicted and 357 targets exhibited a score >0.9. miR-146a-5p is a target with a score of 0.973, which plays an important role on the protection of I/R injury (Li et al. 2020; Zhenzhen et al. 2022). Since the regulatory relationship between SOX2-OT and miR-146a-5p is unclear in MIRI, miR-146a-5p was selected as a research target for following assays.

Dual-luciferase reporter (DLR) assay

The fragments of SOX2-OT containing the binding sites of miR-146a-5p were synthesized and cloned into luciferase reporter vector pmirGLO (LMAIBio, Shanghai, China) (SOX2-OT wt). Corresponding SOX2-OT mut was synthesized by cloning the fragments containing the mutated binding sites. H9c2 cells were co-transfected with SOX2-OT wt/mut and miR-146a-5p mimics/miR-NC for 48 h. After the reaction with a DLR kit (Promega, Madison, WI, USA), the fluorescence intensity was measured by a Microplate Reader (Thermo Fisher Scientific). Relative fluorescence was calculated as the ratio of Fireny Luciferase/Renilla Luciferase.

In vivo MIRI with physiological measurements

Animal experiments were approved by the Ethics Committee of Jimo District People's Hospital (JY-2019014) in accordance with the Guide for the Care and Use of Laboratory Animals. Forty Sprague-Dawley rats (male, 180–200 g, 6 weeks) that purchased from Better Biotechnology Co., Ltd. (Nanjing, China) were randomly divided into MIRI ($n = 30$) and Sham groups ($n = 10$). Rats in MIRI group were further randomized into MIRI (MIRI rats without treatment), MIRI+sh-NC, and MIRI+sh-SOX2-OT groups ($n = 10$ each group). Rats in MIRI+sh-NC and MIRI+sh-SOX2-OT groups were intracoronarily injected with 10 μl sh-NC and sh-SOX2-OT (2×10^{11} pfu/ml/rat) at five days before MIRI, respectively. MIRI model was established in rats as previously described (Chen et al. 2019). Simply, rats were anesthetized by intraperitoneal injection of 10% urethane (U2500, Sigma, St. Louis, MO, USA) and were anti-coagulated by intraperitoneal injection of heparin sodium (Suzhou Yacoo Science, Suzhou, China). Left thoracotomy was then performed at the first to third intercostal space, and a 6-0 prolene suture was used for ligating the left anterior descending (LAD) coronary artery. The LAD coronary artery was ligated for 30 min and perfused for 3 h to induce MIRI. Similar surgical procedures, expect the ligation of LAD coronary artery were performed in the Sham group. After the perfusion, the left ventricular end diastolic pressure (LVEDP), left ventricular systolic pressure (LVSP), and left ventricular developed pressure (LVDP) were measured by a multi-lead physiological recorder (2017, Powerlab, ADInstruments Shanghai Trading, Shanghai, China). After measurements, rats were sacrificed by cervical dislocation.

TdT-mediated dUTP nick end labeling (TUNEL) staining

Cell apoptosis in myocardial tissues was detected using a TUNEL kit (Beyotime, Shanghai, China). Simply, the tissue sections were sequentially incubated with DNase-free proteinase K for 20 min, 3% hydrogen peroxide for 10 min, TUNEL reaction mixture for 1 h, and streptavidin-horseradish peroxidase for 30 min. After stained with diaminobenzidine and hematoxylin, the apoptotic cells were observed under a microscope (Olympus, Japan) at five randomly selected fields.

Statistical analysis

Data were presented as mean ± standard deviation and analyzed by SPSS 20.0 (SPSS, Chicago, IL, USA). Student's *t*-test was used for comparing the differences between two groups. One-way ANOVA followed by Tukey's *post hoc* test (pairwise comparison) was used for comparing the differences among multiple groups. A p-value <0.05 was considered as significantly different.

Results

Silencing of SOX2-OT increased the viability of OGD/R-treated H9c2 cells

The expression of SOX2-OT was significantly higher in OGD/R-treated H9c2 cells than that in controls ($p < 0.01$; Fig. 1A). SOX2-OT was then silenced to evaluate the function of SOX2-OT in OGD/R-treated H9c2 cells. As expected, the expression of SOX2-OT in OGD/R-treated H9c2 cells was significantly decreased by the transfection of sh-SOX2-OT ($p < 0.01$; Fig. 1B). MTT assay determined that the viability was lower in OGD/R-treated H9c2 cells than that in controls ($p < 0.01$). Notably, silencing of SOX2-OT (sh-SOX2-OT) significantly increased the viability of OGD/R-treated H9c2 cells ($p < 0.01$; Fig. 1C).

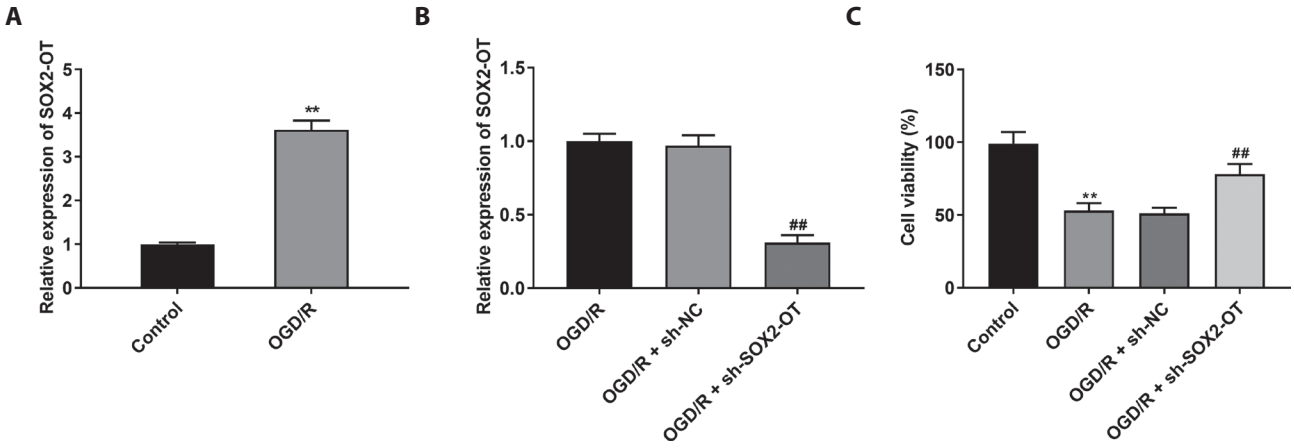

Figure 1. Silencing of SOX2-OT increased the viability of OGD/R-treated H9c2 cells. **A.** The expression of SOX2-OT in OGD/R-treated H9c2 cells and control cells were detected by qRT-PCR. **B.** Knockdown efficiency of sh-SOX2-OT in OGD/R-treated H9c2 cells was verified by qRT-PCR. **C.** Cell viability was examined by MTT assay. ** $p < 0.01$ *vs*. control group, ## $p < 0.01$ *vs* OGD/R+sh-NC group. SOX2-OT, lncRNA SOX2-overlapping transcript; OGD/R, oxygen and glucose deprivation/reperfusion; sh-SOX2-OT, short hairpin RNA targeting SOX2-OT; sh-NC, short hairpin RNA negative control.

Silencing of SOX2-OT inhibited the inflammation and oxidative stress of OGD/R-treated H9c2 cells

The contents of inflammatory factors, including IL-6, IL-1β, and TNF-α were higher in OGD/R group in comparison with those in control group ($p < 0.01$). The transfection of sh-SOX2-OT significantly decreased the contents of IL-6, IL-1β, and TNF-α in OGD/R-treated H9c2 cells ($p < 0.01$; Fig. 2A–C). In addition, the content of MDA (an oxidative stress factor) was higher, and that of SOD (an antioxidant enzyme) was lower in OGD/R-treated H9c2 cells than those in controls ($p < 0.01$). The transfection of sh-SOX2-OT significantly reduced MDA content and increased SOD content in OGD/R-treated H9c2 cells ($p < 0.01$; Fig. 2D,E).

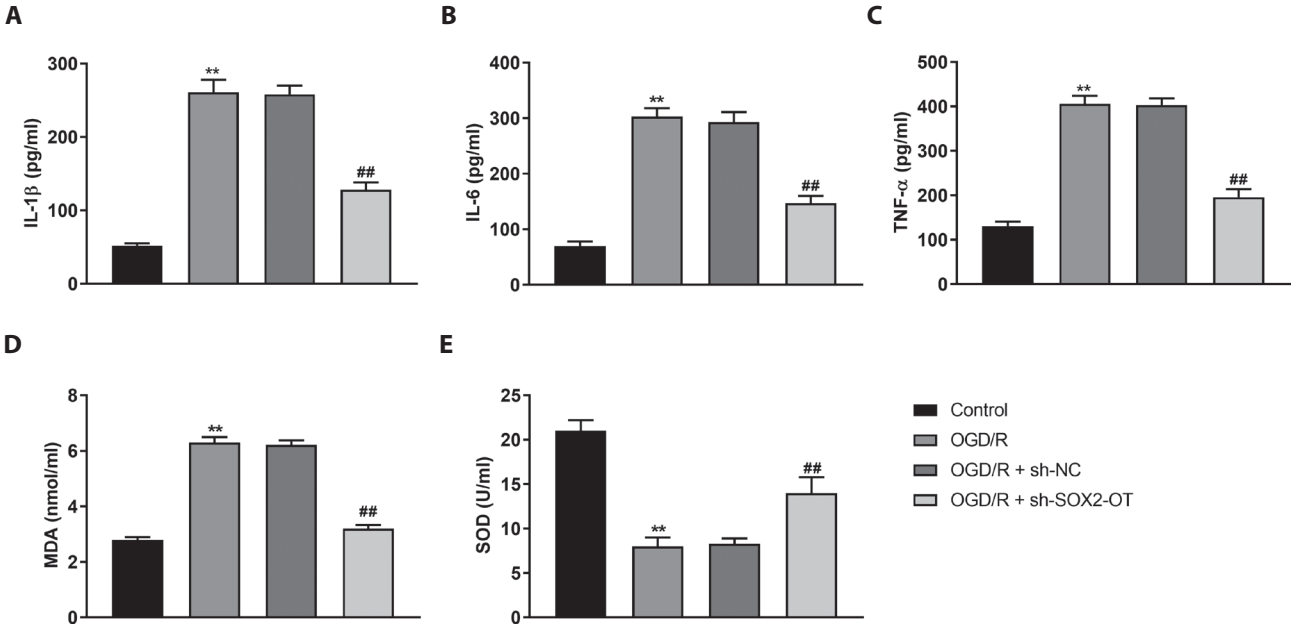

Figure 2. Silencing of SOX2-OT inhibited the inflammation and oxidative stress of OGD/R-treated H9c2 cells. The contents of IL-1β (**A**), IL-6 (**B**), TNF-α (**C**), MDA (**D**), and SOD (**E**) were measured by ELISA. ** $p < 0.01$ *vs*. control group, ## $p < 0.01$ *vs*. OGD/R+sh-NC group. For abbreviations, see Figure 1.

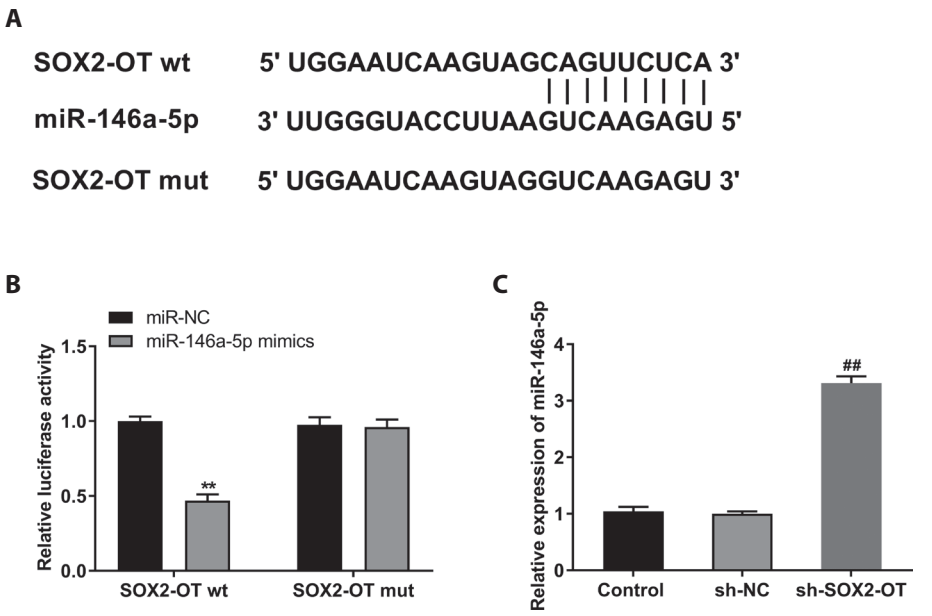

Figure 3. SOX2-OT targeted miR-146a-5p. **A.** Binding region between SOX2-OT and miR-146a-5p was predicted by LncBase. **B.** Relative luciferase activity of H9c2 cells co-transfected with SOX2-OT wild-type/mutant (wt/mut) and miR-146a-5p/miR-NC was measured by DLR assay. **C.** Relative expression of miR-146a-5p in sh-SOX2-OT-transfected H9c2 cells was detected by qRT-PCR. ** $p < 0.01$ *vs.* miR-NC group, ## $p < 0.01$ *vs.* sh-NC group. For abbreviations, see Figure 1.

miR-146a-5p was a target of SOX2-OT

There a binding site between SOX2-OT and miR-146a-5p was predicted by LncBase (Fig. 3A). DLR assay showed that the luciferase activity was significantly decreased in cells co-transfected with SOX2-OT wt and miR-146a-5p mimics compared with cells co-transfected with SOX2-OT wt and miR-NC ($p < 0.01$; Fig. 3B). In addition, silencing of SOX2-OT significantly increased the expression of miR-146a-5p in H9c2 cells ($p < 0.01$; Fig. 3C).

Silencing of SOX2-OT increased the viability of OGD/R-treated H9c2 cells through regulating miR-146a-5p

Whether the regulatory effect of SOX2-OT on cell viability was related to miR-146a-5p was investigated. The expression of miR-146a-5p was significantly lower in OGD/R-treated H9c2 cells than that in controls ($p < 0.01$; Fig. 4A). The transfection of miR-146a-5p inhibitor significantly down-regulated miR-146a-5p in OGD/R-treated H9c2 cells ($p < 0.01$; Fig. 4B). In addition, miR-146a-5p inhibitor also re-

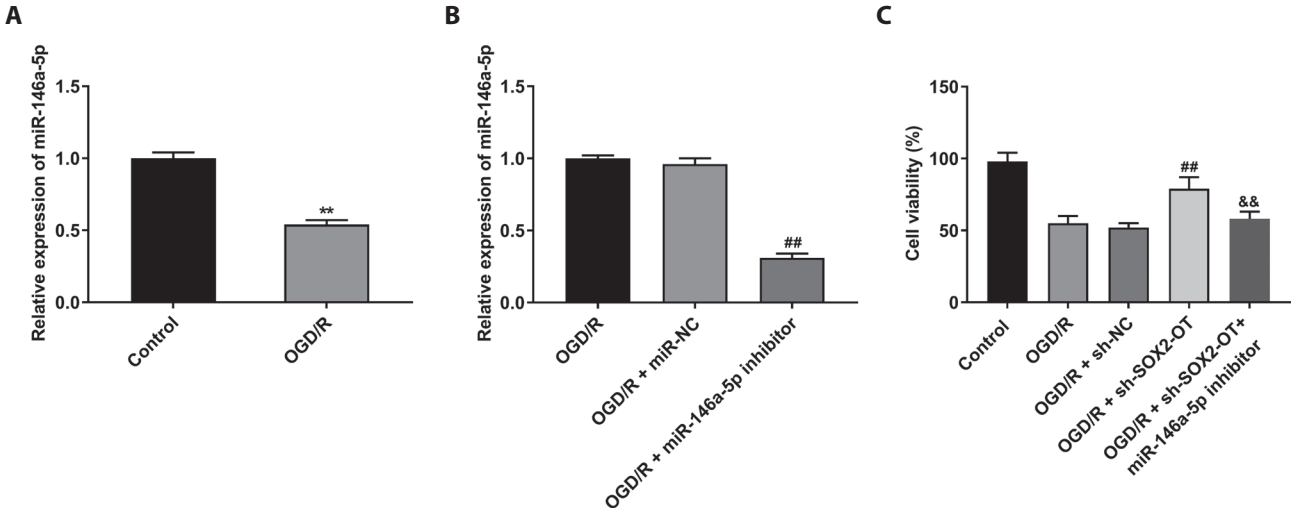

Figure 4. Silencing of SOX2-OT increased the viability of OGD/R-treated H9c2 cells through regulating miR-146a-5p. **A.** Relative expression of miR-146a-5p in OGD/R-treated H9c2 cells and control cells was detected by qRT-PCR. **B.** Knockdown efficiency of miR-146a-5p inhibitor in OGD/R-treated H9c2 cells was verified by qRT-PCR. **C.** Cell viability was detected by MTT assay. ** $p < 0.01$ *vs.* control group, ## $p < 0.01$ *vs.* OGD/R+miR-NC group, && $p < 0.01$ *vs.* OGD/R+sh-SOX2-OT group. For abbreviations, see Figure 1.

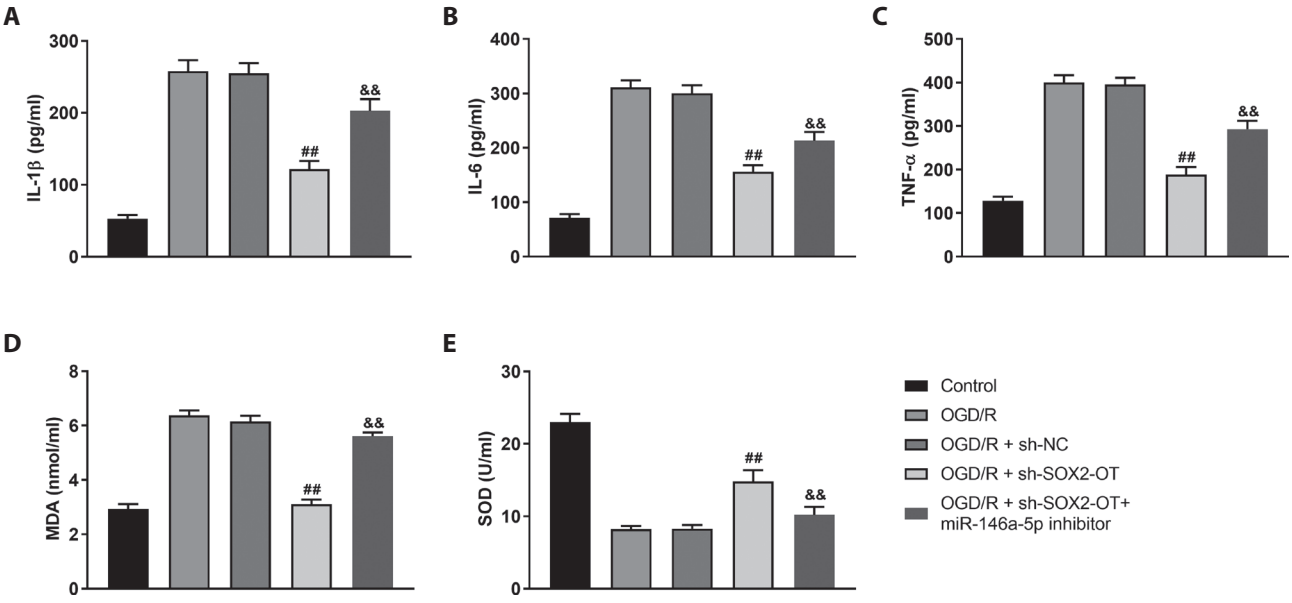

Figure 5. Silencing of SOX2-OT inhibited the inflammation and oxidative stress of OGD/R-treated H9c2 cells through regulating miR-146a-5p. The contents of IL-1β (**A**), IL-6 (**B**), TNF-α (**C**), MDA (**D**), and SOD (**E**) were measured by ELISA. $^{\#\#}p < 0.01$ *vs*. OGD/R+sh-NC group, $^{\&\&}p < 0.01$ *vs*. OGD/R+sh-SOX2-OT group. For abbreviations, see Figure 1.

versed the promoting effect of sh-SOX2-OT on the viability of OGD/R-treated H9c2 cells ($p < 0.01$; Fig. 4C).

Silencing of SOX2-OT inhibited the inflammation and oxidative stress of OGD/R-treated H9c2 cells through regulating miR-146a-5p

The regulatory mechanism of SOX2-OT involving miR-146a-5p was further investigated on cell inflammation and oxidative stress. The contents of IL-6, IL-1β, and TNF-α in OGD/R-treated H9c2 cells were decreased by the transfection of sh-SOX2-OT in comparison with the transfection of sh-NC ($p < 0.01$). miR-146a-5p inhibitor significantly reversed the inhibiting effect of sh-SOX2-OT on the inflammation (IL-6, IL-1β, and TNF-α) of OGD/R-treated H9c2 cells ($p < 0.01$; Fig. 5A–C). In addition, the transfection of sh-SOX2-OT decreased MDA content and increased SOD content in OGD/R-treated H9c2 cells ($p < 0.01$). Silencing of miR-146a-5p significantly weakened the effects of sh-SOX2-OT on decreasing MDA and increasing SOD ($p < 0.01$; Fig. 5D,E).

Silencing of SOX2-OT alleviated MIRI in rats

A rat model of MIRI was established to evaluate the function of SOX2-OT *in vivo*. qRT-PCR showed that the expression of SOX2-OT was increased while the expression of miR-146a-5p was decreased in myocardial tissues of MIRI rats compared with those in sham rats ($p < 0.01$). The injection of sh-SOX2-OT significantly down-regulated SOX2-OT and up-regulated miR-146a-5p in MIRI rats ($p < 0.01$; Fig. 6A,B). When compared with sham rats, there more apoptotic cells in myocardial tissues were observed in MIRI rats by TUNEL staining. The enhanced apoptosis of myocardial cells in MIRI rats was relieved by the injection of sh-SOX2-OT (Fig. 6C). In addition, sh-SOX2-OT also significantly weakened the increasing of LDH and LVEDP, and decreasing of LVSP and LVDP in MIRI rats ($p < 0.01$; Fig. 6D–G).

Discussion

MIRI is a serious myocardial injury following blood flow recovery, contributing to poor cardiovascular outcomes (Frank et al. 2012; Zheng et al. 2021). lncRNAs play important roles in the pathogenesis of MIRI through regulating a variety of physiological processes, such as oxidative stress, inflammatory response, cardiomyocyte apoptosis/autophagy/necrosis, mitochondrial dysfunction, and calcium overload (Zhao Z et al. 2021). Among massive lncRNAs, SOX2-OT has been reported to be up-regulated in hypoxic myocardial cells and in myocardial tissues of ischemic heart failure rats (Gu et al. 2020; Yang and Lin 2020). Consistently, SOX2-OT was also found to be up-regulated in OGD/R-treated H9c2 cells and myocardial tissues of MIRI rats in this study, indicating a possible pathogenic role in MIRI.

There many up-regulated lncRNAs are involved in MIRI progression, such as H19, hypoxia/reoxygenation injury-

related factor in myocytes (HRIM), Gm4419, KCNQ1OT1, TUG1, GAS5, and MALAT1 (Luo et al. 2019; Su et al. 2019; Han et al. 2020; Niu L et al. 2020; Rong et al. 2020; Liu et al. 2022). Previous studies based on *in vitro* experiments have reported that silencing of some specific lncRNAs is a potential strategy for the treatment of MIRI. For examples, silencing of H19 increases cell viability, decreases inflammatory cytokines, and inhibits oxidative stress in OGD/R-treated cardiomyocytes (Luo et al. 2019). Silencing of HRIM inhibits the apoptosis and inflammation of OGD/R-treated cardiomyocytes (Niu L et al. 2020). SOX2-OT is a potential therapeutic target in myocardial infarction and ischemic heart failure (Gu et al. 2020; Yang and Lin 2020). Here, SOX2-OT was silenced to evaluate its function in MIRI. Similarly with previous studies mentioned above, silencing of SOX2-OT increased cell viability and inhibited cell inflammation and oxidative stress in OGD/R-treated H9c2 cells. These *in vitro* findings indicate that SOX2-OT silencing is benefit to the treatment of MIRI. Furthermore, evidence has also determined the protective role of lncRNAs (such as H19, HRIM, and GAS5) silencing against MIRI *in vivo* (Luo et al. 2019; Han et al. 2020; Niu L et al. 2020). Similarly, this study revealed that silencing of SOX2-OT inhibited myocardial apoptosis and improved myocardial

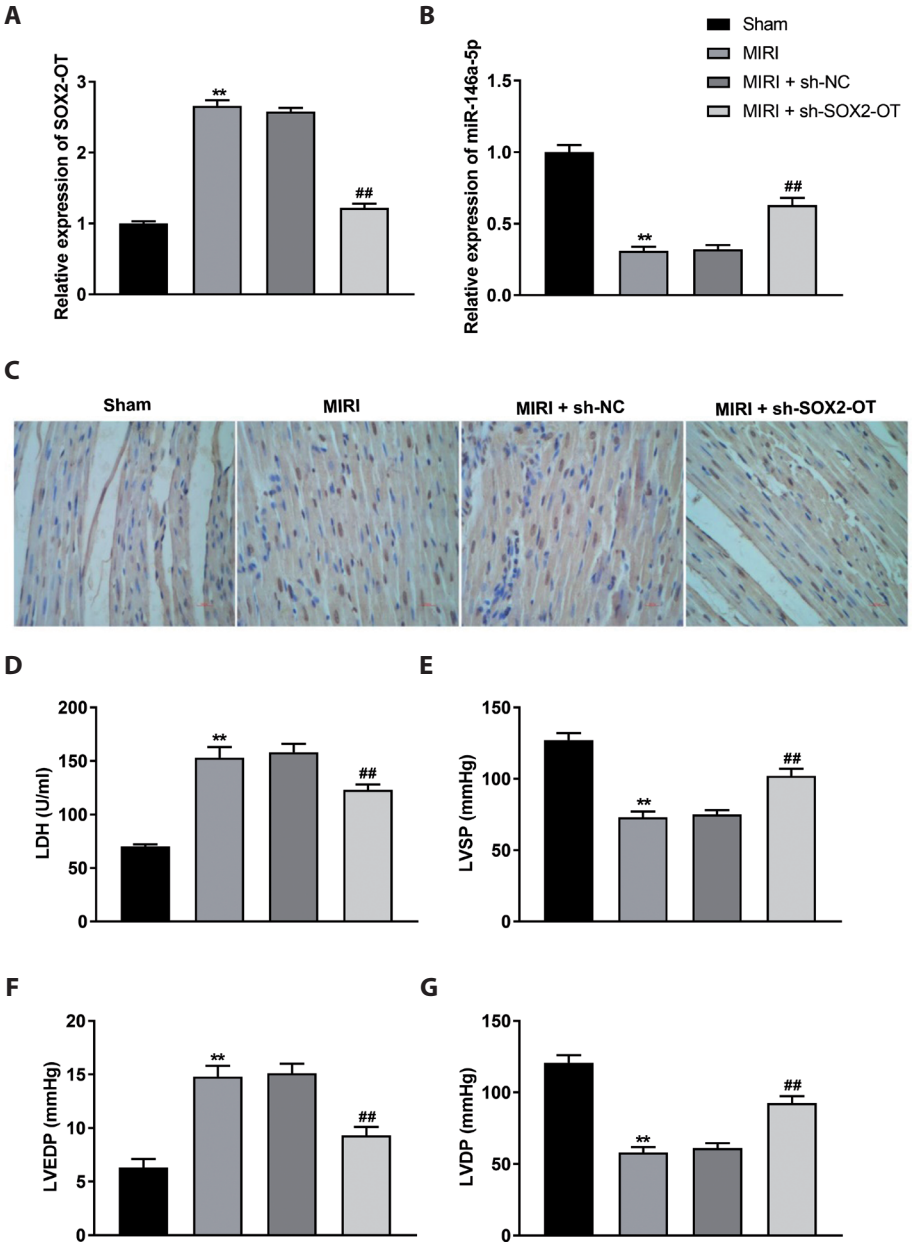

Figure 6. Silencing of SOX2-OT alleviated myocardial ischemia/reperfusion injury (MIRI) in rats. **A.** Relative expression of SOX2-OT in myocardial tissues was detected by qRT-PCR. **B.** Relative expression of miR-146a-5p in myocardial tissues was detected by qRT-PCR. **C.** TUNEL staining of apoptotic cells in myocardial tissues. **D.** Lactate dehydrogenase (LDH) level. **E.** Left ventricular systolic pressure (LVSP) level. **F.** Left ventricular end diastolic pressure (LVEDP) level. **G.** Left ventricular developed pressure (LVDP) level. ** $p < 0.01$ *vs.* sham group, ## $p < 0.01$ *vs.* MIRI+sh-NC group. For more abbreviations, see Figure 1.

function in MIRI rats. Our findings illustrate that SOX2-OT is a potential therapeutic target for MIRI through regulating cell apoptosis, inflammation and oxidative stress. However, the down-stream mechanisms of SOX2-OT involving miR-146a-5p are rarely reported in MIRI.

lncRNAs can regulate relevant target miRNAs *via* competitive binding or sponge effects (Paraskevopoulou and Hatzigeorgiou 2016). There many miRNA targets of SOX2-OT have been determined in diverse cardiovascular diseases, including miR-942-5p in doxorubicin-induced cardiac muscle dysfunction (Wang et al. 2021), miR-27a-3p in myocardial infarction (Yang and Lin 2020), miR-455-3p in ischemic heart failure (Gu et al. 2020), miR-215-5p in ischemic heart failure (Tu et al. 2021), and miR-2355-3p in ventricular arrhythmia associated with heart failure (Liang et al. 2021). In this study, miR-146a-5p was predicted to be a target of SOX2-OT, which was further identified by DLR assay. miR-146a-5p plays an important regulatory role in I/R injury of different tissues. miR-146a-5p inhibits autophagy of OGD/R-treated cells and attenuates intestinal I/R injury in mice (Zhenzhen et al. 2022). Li et al. (2020) have found that urine-derived stem cells protect against renal I/R injury *via* producing exosomal miR-146a-5p. In this study, the down-regulation of miR-146a-5p in MIRI rats was reversed by SOX2-OT silencing. To combine with the protective role of miR-146a-5p in I/R injury, we suspect that SOX2-OT silencing may relieve MIRI through up-regulating miR-146a-5p. This speculation is subsequently confirmed by our following feedback experiments, evidenced by that silencing of miR-146a-5p reversed the effects of sh-SOX2-OT on inhibiting the inflammation and oxidative stress, and on promoting the viability of OGD/R-treated cells.

In conclusion, silencing of SOX2-OT relieves MIRI through inhibiting the inflammation, oxidative stress, and apoptosis of myocardial cells both *in vitro* and *in vivo*. miR-146a-5p is a target of SOX2-OT, which contributes to the protection of myocardial cells against MIRI. Both SOX2-OT and miR-146a-5p may be potential therapeutic targets for MIRI. However, the down-stream mechanisms of SOX2-OT-miR-146a-5p axis in MIRI are not fully discovered. Further researches on the detailed action mechanisms of SOX2-OT in MIRI are still needed.

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Conflict of interests. The authors declare that they have no competing interests.

Ethics approval. Animal experiments were approved by the Ethics Committee of Jimo District People's Hospital (JY-2019014) in accordance with the Guide for the Care and Use of Laboratory Animals.

Authors' contributions. ZL: conceptualization, writing original draft, formal analysis, funding acquisition, project administration; GL: data curation, investigation, methodology, validation, writing – review & editing; HH: resources, software, supervision, writing – review & editing.

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