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Original Article

Lncrna FGD5-AS1 Aggravates Myocardial Ischemia-Reperfusion Injury by Sponging Mir-129-5p

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Abstract

Background: LncRNA FGD5-AS1 regulates the pathogenesis of many human diseases. We aimed to elucidate the function of lncRNA FGD5-AS1 and the regulatory mechanism of lncRNA FGD5-AS1/miR-129-5p in myocardial ischemia-reperfusion (I/R) injury.

Methods: Myocardial I/R injury mice model and H/R treated H9c2 cells were established. RT-qPCR and Western blot analysis were used to detect the mRNA and protein expression. Cell viability was detected by MTT assay. Dual luciferase reporter assay was applied to confirm the relationship between lncRNA FGD5-AS1 and miR-129-5p.

Results: LncRNA FGD5-AS1 was upregulated in myocardial I/R injury mice models and H/R treated H9c2 cells. Functionally, knockdown of lncRNA FGD5-AS1 promoted cell viability and inhibited apoptosis in H/R treated H9c2 cells. In addition, lncRNA FGD5-AS1 directly targets miR-129-5p. Upregulation of lncRNA FGD5-AS1 weakened the protective effect of miR-129-5p on myocardial I/R injury.

Conclusion: LncRNA FGD5-AS1 aggravates myocardial I/R injury by downregulating miR-129-5p.

Keywords: Myocardial ischemia; Reperfusion injury; Cardiology

Introduction

Myocardial ischemia refers to a pathological state in which the blood perfusion of the heart is reduced. It can lead to a decrease in oxygen supply to the heart and abnormal myocardial energy metabolism (1). With the improvement of people's living standards, the incidence of myocardial ischemia in China is increasing (2). Myocardial ischemia-reperfusion (M-I/R) injury indicates a cardiovascular dysfunction after myocardial ischemia (3). Currently, effective treatment is limited to restoring coronary blood flow to prevent myocardial infarction. Therefore, there is an urgent need for new therapeutic strategies to prevent M-I/R injury.

Long non-coding RNA (lncRNA) is a type of RNA molecule with a transcript length of more than 200 nt. LncRNAs do not encode proteins, but regulate gene expression at multiple levels (epigenetic, transcription, post-transcriptional regulation) (4). Moreover, lncRNAs play im-

portant roles in the pathogenesis of M-I/R injury. For example, knockdown of lncRNA TTTY15 alleviated M-I/R injury through the miR-374a-5p/FOXO1 axis (5). In addition, lncRNA A2M-AS1 has been reported to lessen the injury of cardiomyocytes caused by hypoxia and reoxygenation via regulating IL1R2 (6). Here, the role of lncRNA FGD5-AS1 was investigated in the pathogenesis of M-I/R injury. lncRNA FGD5-AS1 was involved in the development of human cancers. For instance, silencing of lncRNA FGD5-AS1 inhibited the progression of non-small cell lung cancer by regulating the miR-493-5p/DDX5 axis (7). lncRNA FGD5-AS1 promoted tumor growth by regulating MCL1 via sponging miR-153-3p in oral cancer (8). However, the function and regulatory mechanism of lncRNA FGD5-AS1 remains unclear in the pathogenesis of M-I/R injury.

lncRNAs have "sponge-like effects" on numerous miRNAs, including lncRNA FGD5-AS1. In this study, miR-129-5p was found to have a binding site with lncRNA FGD5-AS1. Overexpression of miR-129-5p mitigate sepsis-induced acute lung injury by targeting High Mobility Group Box 1 (9). More importantly, miR-129-5p protects H9c2 cardiac myoblasts from hypoxia/reoxygenation injury by targeting TRPM7 and inhibiting NLRP3 inflammasome activation (10). In addition, lncRNA NEAT1 promoted myocardiocyte apoptosis and suppressed proliferation through regulation of miR-129-5p (11). However, little is known about the interaction between lncRNA FGD5-AS1 and miR-129-5p in myocardial I/R injury.

In the present study, the expression level of lncRNA FGD5-AS1 was detected in mouse I/R models. Then, the biological effect of lncRNA FGD5-AS1 in H9c2 cell H/R model was investigated. In addition, the regulatory mechanism of lncRNA FGD5-AS1/miR-129-5p in M-I/R injury was discovered.

Materials and Methods

Experimental animals

Male c57BL6/J mice (20-24 g, Guangdong Medical Laboratory Animal Center) were feed in a standard pathogen-free environment (25°C, 60% humidity). Food and water were freely provided. All procedures were performed in accordance with the Care and Use of Laboratory Animals issued by the Chinese Association for Laboratory Animal Care and approved by our Hospital.

M-I/R injury mice model

The myocardial I/R injury model was established by ligating the left anterior descending coronary artery. C57BL/6 mice were anesthetized with 3% pentobarbital sodium and a longitudinal incision. The mouse's thoracic cavity was open by left thoracotomy. Ligation was performed at approximately 3 mm from the source of the descending left anterior coronary artery with line 6–0. After 30 min induction of ischemia, the ligature was untied. Then, the mice were reperfused at various time points (6h, 12h, 24h).

Cell culture and H/R treatment

The cardiomyocytes cell line H9c2 (Chinese Academy of Sciences, China) were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) in a humid incubator with 5% CO_2 at 37 °C.

H/R treatment was used to establish an I/R injury model in H9c2 cells. H9c2 cells were exposed for 24 h under hypoxia (5% CO_2, 95% N_2) and then re-oxygenated (5% CO_2, 95% O_2) for 12 h at 37°C. Control cells were incubated under normoxic conditions (NC).

Cell transfection

FGD5-AS1 siRNA and vector or miR-129-5p mimics and inhibitor were designed and synthesized by GenePharma (Shanghai, China). They were transfected into H9c2 cells using Lipofectamine® 2000 transfection reagent, respectively.

Detection of lactate dehydrogenase (LDH) and creatine kinase-muscle/brain (CK-MB)

When the M-I/R injury mice model was established, the levels of CK-MB and LDH were measured by using commercial assay kits (Invi-

trogen, Carlsbad, CA) in accordance with the manufacturer's protocol.

RT-qPCR

Total RNA was extracted using TRIzol reagent (Thermo Fisher Scientific, MA, USA). Then, RT was conducted using a RevertAid First Strand cDNA Synthesis kit (K1622; Thermo Fermentas, USA). PCR was performed on an ABI Prism 7900 detection system (Thermo Fisher Scientific, Inc.) using iQ™ SYBR®-Green SuperMix (Bio-Rad Laboratories, Inc., Hercules, CA). GAPDH was applied as internal reference. MiRNA and mRNA expression levels were quantified using the $2^{-\Delta\Delta Cq}$ method.

MTT assay

H9c2 cells (2×10^3cells/well) were seeded into 96-well plates and cultured for 12 h in 5% CO_2 at 37°C. Then, cells were subjected to H/R exposure. Next, the cells were incubated with 15 μL/well MTT solution (5 mg/mL, Sigma) at 37 °C for 4 h. The absorbance value was determined at a wavelength of 490 nm by using a Bio-Rad 680 microplate reader (Bio-Rad Laboratories, Inc.).

Western blot analysis

H9c2 cells were collected and lysed by RIPA Lysis Buffer (Beyotime, Shanghai, China). Protein concentration was measured using Enhanced BCA Protein Assay kit (Beyotime, Shanghai, China). Next, protein samples (40 μg) were separated by 10% SDS-PAGE and transferred to PVDF membranes. The membranes were blocked with 5% skimmed milk for 2 h at room temperature and incubated with Bax, Bcl-2 and GAPDH primary antibodies (Abcam, Shanghai, China) overnight at 4°C. After washing, protein samples were incubated with horseradish peroxidase-conjugated secondary antibodies (Abcam, USA) for 2 h. Finally, the blots were detected using an enhanced chemiluminescence (ECL) reagent and analyzed with ImageJ software.

Dual luciferase reporter assay

Wild-type and mutant FGD5-AS1 containing miR-129-5p binding site were amplified and inserted into the pGL3 vector (Promega) to construct recombinant reporter plasmids WT-FGD5-AS1 and MUT-FGD5-AS1. The reporter plasmids and miR-129-5p mimics or miR-NC was co-transfected into H9c2 cells. After 48 h, dual luciferase assay system (Promega, USA) was used to detect luciferase activities.

Statistical analysis

Data were analyzed SPSS 19.0 (IBM Corp., Armonk, NY, USA) and expressed as mean ± SD. Graphs are made by Graphpad Prism 6. Student *t*-test was adopted to compare the difference between two groups, and multiple comparison was performed by one-way analysis of variance followed by Tukey's post hoc test. $P < 0.05$ indicates statistically significant difference.

Results

LncRNA FGD5-AS1 is upregulated in myocardial I/R tissues

To explore the expression level of lncRNA FGD5-AS1 in myocardial I/R injury, myocardial I/R injury mice models were established. To assess whether the myocardial I/R injury mice mouse model is successfully established, the serum levels of LDH and CK-MB were detected. LDH and CK-MB serum levels were significantly increased in myocardial I/R injury group (Fig. 1A, 1B). Especially, the highest serum levels of LDH and CK-MB were occurred at 12 h after reperfusion (Fig. 1A, 1B). The myocardial I/R injury mice mouse model was successfully established. Next, RT-qPCR showed that lncRNA FGD5-AS1 expression was apparently increased at 6h and 12 h after reperfusion in myocardial I/R injury group compared with Normoxia group (Fig. 1C).

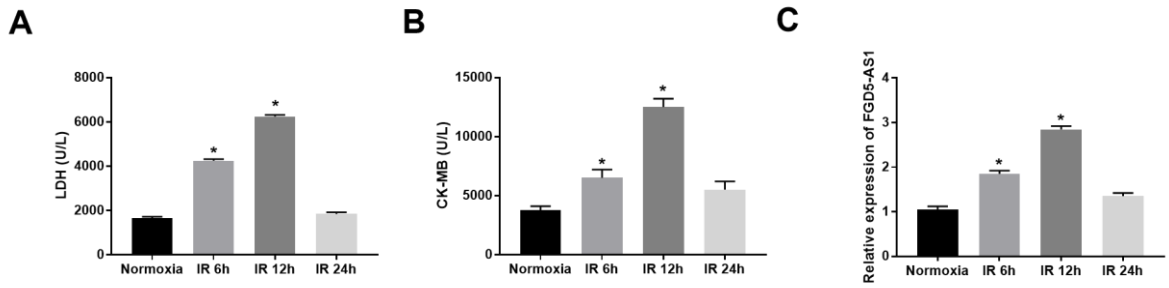

Fig. 1: LncRNA FGD5-AS1 is upregulated in myocardial I/R tissues. (A, B) Detection of LDH and CK-MB levels in serum of the myocardial I/R injury mice (C) The lncRNA FGD5-AS1 expression was detected in the myocardial I/R injury mice tissues. *$P < 0.05$

Knockdown of lncRNA FGD5-AS1 ameliorates H/R-induced cell injury

To explore the role of lncRNA FGD5-AS1 in myocardial I/R injury, H9c2 cell H/R model was established. RT-qPCR showed that lncRNA FGD5-AS1 expression was upregulated in H9c2 cells treated with H/R compared with Normoxia group (Fig. 2A).

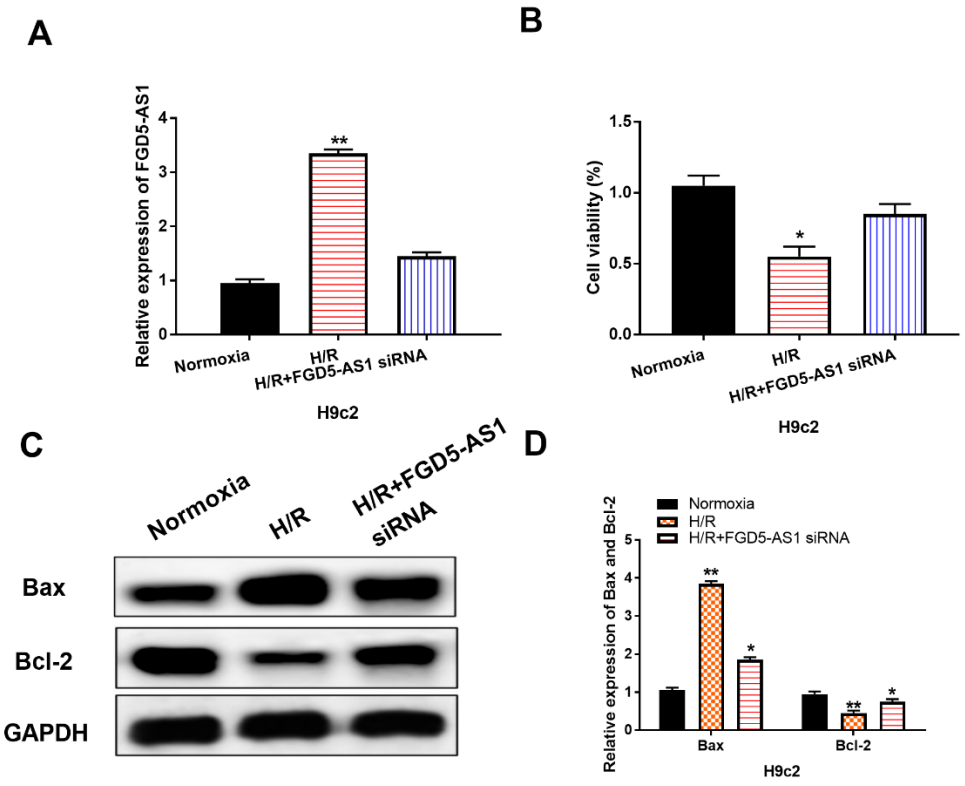

Fig. 2: Knockdown of lncRNA FGD5-AS1 ameliorates H/R-induced cell injury. (A) LncRNA FGD5-AS1 expression was detected in Normoxia group, H/R group and H/R+ FGD5-AS1 siRNA group. (B) H9c2 cell proliferation was measured in Normoxia group, H/R group and H/R+ FGD5-AS1 siRNA group. (C, D) The apoptosis-related proteins (Bcl-2 and Bax) were detected in Normoxia group, H/R group and H/R+ FGD5-AS1 siRNA group. *$P < 0.05$, ** $P < 0.01$

After transfection of FGD5-AS1 siRNA, FGD5-AS1 expression was reduced in H/R treated H9c2 cells, but still higher than that in Normoxia group (Fig. 2A). Functionally, cell proliferation was suppressed in H9c2 cells treated with H/R compared to Normoxia group. Downregulation of FGD5-AS1 promoted cell proliferation in H/R treated H9c2 cells. However, H9c2 cell proliferation in H/R + FGD5-AS1 siRNA group was still inhibited compared to Normoxia group (Fig. 2B). Additionally, the effect of lncRNA FGD5-AS1 on apoptosis-related protein (Bcl-2/Bax) was also detected in H9c2 cells. Compared with the Normoxia group, increased expression of Bax and decreased expression of Bcl-2 were identified in H/R group. Compared with H/R group, knockdown of FGD5-AS1 reduced Bax expression and enhanced Bcl-2 expression. The expression of Bax and Bcl-2 in H/R + FGD5-AS1 siRNA group tended to the levels in Normoxia group but could not reach the levels in Normoxia group (Fig. 2C, 2D). Briefly, lncRNA FGD5-AS1 could aggravate myocardial I/R injury by suppressing cell viability and inducing apoptosis.

LncRNA FGD5-AS1 directly targets miR-129-5p

To explain the regulatory mechanism of lncRNA FGD5-AS1 in myocardial I/R injury, the target of lncRNA FGD5-AS1 was searched in the starBase database (http://starbase.sysu.edu.cn). We found that lncRNA FGD5-AS1 has a binding site with miR-129-5p (Fig. 3A). Dual-luciferase reporter assay showed that miR-129-5p mimics reduced the luciferase activity of wt-FGD5-AS1 (Fig. 3B), indicating that miR-129-5p is a direct target of lncRNA FGD5-AS1. Next, RT-qPCR showed thta miR-129-5p expression was reduced by FGD5-AS1 vector and promoted by FGD5-AS1 siRNA in H9c2 cells (Fig. 3C). At the same time, lncRNA FGD5-AS1 was upregulated in H9c2 cells with miR-129-5p mimics and downregulated in H9c2 cells with miR-129-5p inhibitor (Fig. 3D).

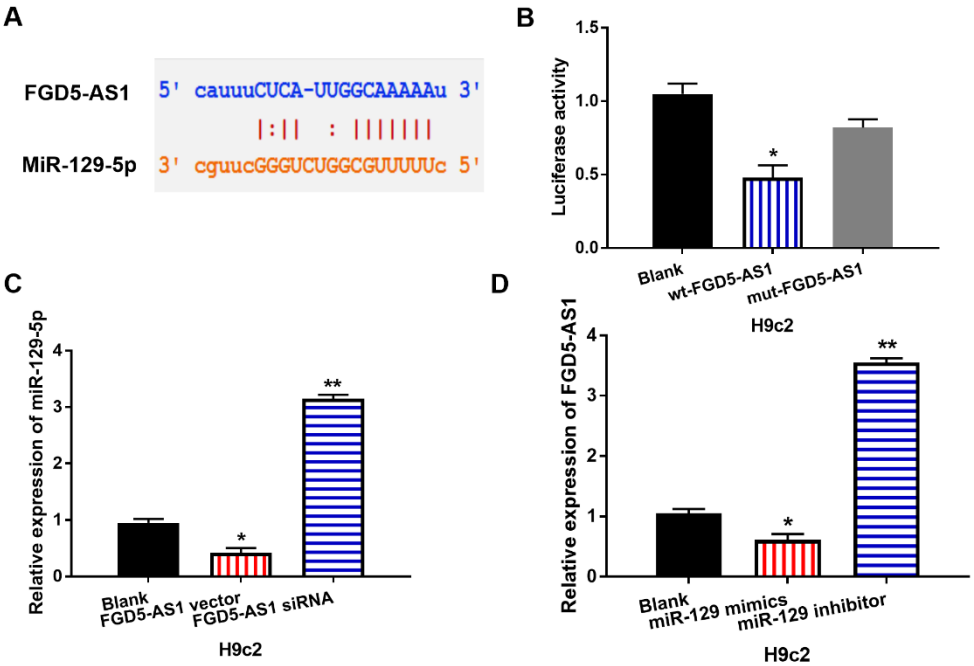

Fig. 3: LncRNA FGD5-AS1 directly targets miR-129-5p. (A) The binding sites between lncRNA FGD5-AS1 and miR-129-5p. (B) Luciferase reporter assay (C) MiR-129-5p expression was detected in H9c2 cells with FGD5-AS1 vector or siRNA (D) LncRNA FGD5-AS1 expression was measured in H9c2 cells with miR-129-5p mimics or inhibitor. *$P < 0.05$, ** $P < 0.01$

LncRNA FGD5-AS1 regulates myocardial I/R injury by sponging miR-129-5p.

To investigate the interaction between lncRNA FGD5-AS1 and miR-129-5p in myocardial I/R injury, miR-129-5p mimics and miR-129-5p mimics+FGD5-AS1 vector were transfected into H/R treated H9c2 cells. Compared with the Normoxia group, miR-129-5p was downregulated in H/R group. Upregulation of FGD5-AS1 reduced the increased expression of miR-129-5p induced by miR-129-5p mimics. However, miR-129-5p expression was still lower than that in Normoxia group (Fig. 4A). MTT assay showed that miR-129-5p overexpression promoted H/R treated H9c2 cell viability compared with H/R group. However, FGD5-AS1 vector weakened the promoting effect of miR-129-5p overexpression on H/R treated H9c2 cell viability (Fig. 4B). Compared to H/R group, overexpression of miR-129-5p reduced Bax expression and promoted Bcl-2 expression in H/R treated H9c2 cells. However, upregulation of FGD5-AS1 increased Bax expression and reduced Bcl-2 expression in H/R treated H9c2 cells compared with H/R+ miR-129-5p mimics group (Fig. 4C, 4D). These results indicate that overexpression of miR-129-5p ameliorates myocardial I/R injury. lncRNA FGD5-AS1 aggravates myocardial I/R injury by downregulating miR-129-5p.

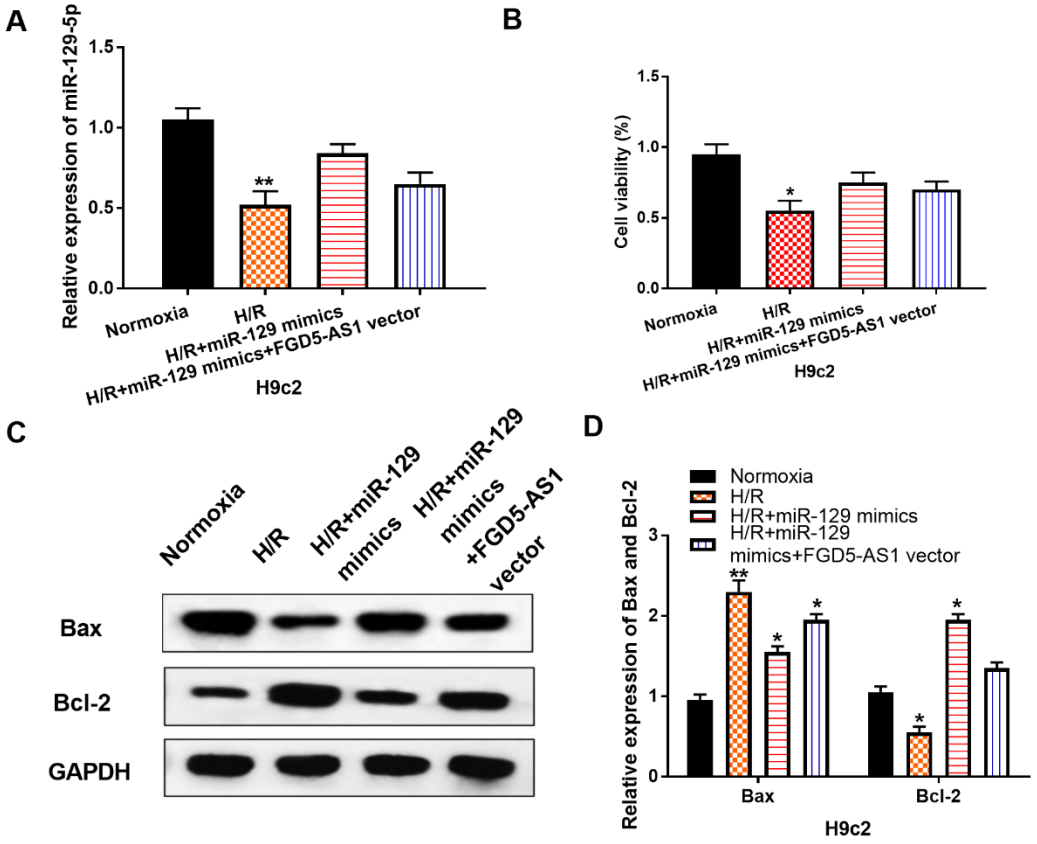

Fig. 4: LncRNA FGD5-AS1 regulates myocardial I/R injury by sponging miR-129-5p. (A) MiR-129-5p expression was detected in Normoxia group, H/R group, H/R+miR-129-5p mimics group and H/R+ miR-129-5p mimics+FGD5-AS1 vector group. (B) H9c2 cell proliferation was measured in Normoxia group, H/R group, H/R+miR-129-5p mimics group and H/R+ miR-129-5p mimics+FGD5-AS1 vector group (C, D) The apoptosis-related proteins (Bcl-2 and Bax) were detected in Normoxia group, H/R group, H/R+miR-129-5p mimics group and H/R+ miR-129-5p mimics+FGD5-AS1 vector group. *$P < 0.05$, ** $P < 0.01$

Discussion

Recently, many lncRNAs and microRNAs have been reported to participate in the pathogenesis of myocardial injury. For example, Downregulation of lncRNA NEAT1 promoted cell proliferation and inhibited cell apoptosis by targeting miR-193a in myocardial I/R injury (12). In this study, lncRNA FGD5-AS1 was upregulated in myocardial I/R injury mice models and H/R treated H9c2 cells. Functionally, knockdown of lncRNA FGD5-AS1 promoted cell viability and inhibited apoptosis in H/R treated H9c2 cells. In addition, miR-129-5p was confirmed to be a target of lncRNA FGD5-AS1. The protective effect of miR-129-5p on myocardial I/R injury was impaired by upregulation of lncRNA FGD5-AS1. These results demonstrate that lncRNA FGD5-AS1 aggravates myocardial I/R injury by downregulating miR-129-5p.

Consistent with our results, other lncRNAs also have been found to regulate myocardial I/R injury. For example, lncRNA FOXD3-AS1 aggravated I/R injury of cardiomyocytes through promoting autophagy (13). However, the role of lncRNA FGD5-AS1 has not been reported in myocardial I/R injury. Most studies reported that lncRNA FGD5-AS1 play important roles in human cancers. For instance, upregulation and carcinogenesis of lncRNA FGD5-AS1 has been detected in colorectal cancer and glioblastoma (14,15). However, lncRNA FGD5-AS1 expression was found to be decreased in oxygen-glucose deprivation and simulated reperfusion (OGD/R)-induced neurons injury. Up-regulation of FGD5-AS1 could recover proliferation and inhibit apoptosis of OGD/R-injured neurons (16). These results are contrary to our results in this study. This difference may be caused by different experimental materials.

In the present study, lncRNA FGD5-AS1 directly targeted miR-129-5p and had a negative correlation with miR-129-5p expression in cardiomyocytes. More importantly, overexpression of miR-129-5p ameliorated myocardial I/R injury. Consistent with our study, miR-129-5p alleviates myocardial injury after ischemia/reperfusion (17). miR-129-5p ameliorated ischemia-reperfusion injury by targeting HMGB1 in myocardium (18). All these results indicate that miR-129-5p play a positive effect on myocardial I/R injury. In addition, upregulation of lncRNA FGD5-AS1 impaired the protective effect of miR-129-5p on myocardial I/R injury. lncRNA FGD5-AS1 could aggravate myocardial I/R injury.

Interaction between lncRNA FGD5-AS1 and miR-129-5p in myocardial I/R injury has not been found in previous studies.

Conclusion

Upregulation of lncRNA FGD5-AS1 is detected in myocardial I/R tissues and cardiomyocytes. Upregulation of lncRNA FGD5-AS1 reduced cell proliferation and induced apoptosis in H/R treated cardiomyocytes. More importantly, lncRNA FGD5-AS1 aggravates myocardial I/R injury by downregulating miR-129-5p. Our results may provide a novel therapeutic or diagnostic target for myocardial I/R injury.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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