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Original Article

LncRNA FGD5-AS1 reduces cardiomyocyte apoptosis and inflammation by modulating Akt and miR-223-3p expression

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Abstract: Objectives: Long non-coding RNAs (lncRNAs) are known to be involved in heart development and function. In this study, we aimed to explore the effect of the lncRNA FGD5 antisense RNA 1 (FGD5-AS1) on acute myocardial infarction (AMI) by targeting miR-223-3p. Methods: An AMI model was established both in vivo and in vitro. The levels of FGD5-AS1, miR-223-3p and inflammatory factors were detected by real-time quantitative reverse transcription PCR. Cardiomyocyte apoptosis was assessed using TdT-mediated dUTP nick-end labeling assay. The protein levels of cleaved caspase-3, Bcl-2 and Bax were examined using Western blot. Cardiac function was evaluated using hemodynamic analysis and hematoxylin-eosin and Masson's trichrome staining. In addition, an underlying competitive endogenous RNA mechanism was revealed by bioinformatics analysis, dual-luciferase reporter assay and rescue experiments. Results: We found decreased expression of FGD5-AS1 in AMI. Furthermore, FGD5-AS1 expression significantly decreased the infarct size, improved cardiac performance and attenuated cardiac fibrosis by reducing myocardial apoptosis and inflammation. miR-223-3p was a direct target of FGD5-AS1. Moreover, miRNA-223-3p directly downregulated the expression of phosphorylated Akt in primary neonatal rat cardiomyocytes. Further experiments demonstrated that FGD5-AS1 modulated Akt activity to reduce myocardial injury through miR-223-3p. Conclusion: The FGD5-AS1/miR-223-3p/Akt pathway is involved in AMI, suggesting that FGD5-AS1 may act as a potential biomarker and therapeutic target for AMI.

Keywords: lncRNA FGD5-AS1, miR-223-3p, apoptosis, inflammation, ischemic reperfusion injury

Introduction

Cardiovascular disease (CVD) is a growing public health concern owing to its high morbidity and mortality worldwide [1, 2]. Although its prevalence has significantly declined over the last two decades, adverse cardiovascular conditions, such as stroke and coronary heart disease, remain at high level [1, 3]. Acute myocardial infarction (AMI), commonly known as a heart attack, can lead to death in patients with ischemic heart diseases [4] and usually results from an occlusion of the coronary artery. Unlike in Western countries, AMI morbidity and mortality have rapidly increased in China over the past few decades [5], and patients with evolving myocardial infarction often die before receiving effective treatment [6]. Thus, early and accurate diagnosis is important for AMI management.

Long non-coding RNAs (lncRNAs) can influence many biological processes [7], including the development and progression of CVDs, and some lncRNAs may be closely involved in myocardial infarction. The lncRNA NRF has been recently shown to be involved in myocardial injury [8], while the lncRNA MALAT1, is closely associated with cardiomyocyte apoptosis [9]. Among lncRNAs, FGD5 antisense RNA 1 (FGD5-

AS1) is abnormally regulated in oral, colorectal, and lung cancer [10-12]. However, the function of this lncRNA in AMI has not been fully explored.

MicroRNAs (miRNAs) regulate many biological processes including cell growth, survival, differentiation and energy metabolism [13, 14]. Previous findings suggest that miRNAs may serve as biomarkers of CVDs, including AMI. For example, miR-93-5p is correlated with cardiac injury [15]. Wang et al. reported that downregulating miR-200a expression protected cardiomyocytes from apoptosis [16]. Additionally, miR-223-3p is well known as a hematopoietic lineage miRNA and is exclusively expressed in tumor and blood cells [17]. Recently, studies suggest that miR-223-3p is associated with CVDs, and in cardiomyocytes, the absence of miR-223-3p represses apoptosis and oxidative stress [18]. Li et al. reported that miR-223-3p reduced the expression of GAS5, which improved chronic heart failure [19]. There are also studies which suggest that lncRNAs may regulate cardiac diseases by sponging miRNAs. In the present study, we explored how the lncRNA FGD5-AS1 interacted with miR-223-3p in AMI.

The PI3K/Akt signaling pathway has been implicated in multiple biological functions including proliferation, differentiation and apoptosis. Activation of Akt protects cardiomyocytes against apoptosis when treated with high glucose or palmitate [20, 21]. Yang et al. reported that the tumor necrosis factor-like weak inducer of apoptosis alleviated apoptosis in cardiomyocytes via the PI3K/Akt signaling pathway [22]. Additionally, FGD5-AS1 and miR-223-3p have been shown to modulate cancer progression via the Akt signaling pathway [23, 24]. Based on these findings, we hypothesized that FGD5-AS1 might regulate Akt signaling by sponging miR-223-3p and thus participates in the pathogenesis of AMI.

Materials and methods

Experimental animals and treatments

Male adult Sprague-Dawley rats (220-280 g) were purchased and acclimated to our laboratory for 1 week with access to food and water *ad libitum*. Rats were randomly divided into a sham-operated group, an ischemia/reperfusion (I/R) group, an Ad-Con group and an Ad-FGD5-AS1 group (n=5-8). The rat model of AMI was created by ligating the left anterior descending coronary artery. The AMI modeling was considered successful if the anterior descending coronary artery turned white with two or more places displaying the J-point elevation on the lead ECG higher than 0.2 mV. Then, the air in the thoracic cavity was gently removed, and the thoracic cavity was sutured layer by layer. Recombinant adenoviruses for FGD5-AS1 (Ad-FGD5-AS1) overexpression and a negative control (Ad-con) were constructed by Gene Chem. Five days before the establishment of the AMI model, the rats were anesthetized with a peritoneal injection of pentobarbital sodium (60 mg/kg), and their pericardium was removed through a left anterior small thoracotomy. Using a micro syringe, 100 μL of adenovirus solution was intramyocardially injected into five separate sites of the left ventricle. The survival rate of rats injected with the adenoviruses was 93%. Also, 1 of the 8 rats with Ad-con had died. After AMI surgery, all of the sham-operated groups survived, and the survival rate of rats with AMI was 75%, with 6 of the 24 (25%) rats had died, two from I/R group, two from Ad-Con group, and two from Ad-FGD5-AS1 group. Rats were euthanized using carbon dioxide (CO_2) at the end of experiment. This research was approved by the Animal Ethics Committee of Laiyang Central Hospital.

Cell culture and transfection

The ventricular cardiomyocytes of neonatal rats were isolated from the hearts of 1-3-day-old rats. Briefly, after anesthesia, the hearts were sheared and immediately placed in cold phosphate buffered saline (Invitrogen Inc., Carlsbad, CA, USA). The heart muscle was then dispersed via 0.4% type 2 collagenase/0.6% pancreatin (Roche). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco) with 10% fetal bovine serum (Gibco) at 5% CO_2 and 37°C. For normoxia treatment, cardiomyocytes were cultured at 37°C in a normoxia incubator containing 21% O_2 and 5% CO_2. For hypoxia treatment, the cardiomyocytes were grown at 37°C for 24 h in a hypoxia incubator containing 1% O_2 and 5% CO_2.

MiR-223-3p mimic (5'-UGUCAGUUUGUCAAAUACCCCA-3'), miR-223-3p inhibitor (5'-UGGGGUAUUUGACAAACUGACA-3') and miRNA scramble (5'-CGUUUGGCUAGUCAGUGUGGCA-3'), which was used as a negative control, were synthesized by Gene chem (Shanghai, China). Plasmids over-expressing FGD5-AS1 were synthesized by Gene Pharma (Shanghai, China) and

Table 1. RT-qPCR primers

Target gene	Forward primers, 5'-3'	Reverse primers, 5'-3'
FGD5-AS1	CGTGGAGAAGAATTGGGC	CGTGGAGAAGAATTGGGC
miR-223	ACACTCCAGCTGGGTGTCAGTTTGTCAAAT	TGGTGTCGTGGATTCG
TNF-α	TTCGGCCTGTTTCCCCATGT	ACCCGGTTGAACTTGTTGAC
IL-6	ATCCCTTGCCCTCTATAAG	TACCCGGAGGGTGACCGCT
IL-8	AGGCACTTGCTGTTCGCGA	GAACCCGACTTCCGTCTTTG
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT-3
GAPDH	CCAGGTGGTCTCCTCTGACTT	GTTGCTGTAGCCAAATTCGTTGT

ligated into the pAd-Flag vector. The primer used to obtain the sequences of the FGD5-AS1 over-expressing plasmid was 5'-CCTGACCTTTC-GCCAACTGACT-3'. Plasmids without any target sequence were used as negative control (5'-GATTACAAGGACGACGATGACAAG-3'). The miR-223-3p mimic, mimic NC, Ad-FGD5-AS1 and Ad-con were transiently transfected into isolated primary cardiomyocytes using Lipofectamine 3000 (Invitrogen Inc., Carlsbad, CA, USA) following the manufacturer's instructions.

HE staining

Heart samples were fixed in 10% formaldehyde, dehydrated in ethanol, embedded in paraffin and sliced at a thickness of 4 μm. Following deparaffinization, the sections were stained with hematoxylin and eosin. Then, the tissue sections were mounted, and microscopy images were taken.

TdT-mediated dUTP nick-end labeling (TUNEL) staining

To detect apoptosis, TUNEL staining was conducted. Cells were fixed with 4% paraformaldehyde. Apoptotic cells were visualized following the manufacturer's instructions (Yisheng, 40-308ES20, Shanghai, China). The fluorescence density was evaluated using Image J.

Masson's trichrome staining

Rat heart tissue was fixed with 10% formaldehyde at 25°C for 24 h. The tissue was decalcified, dehydrated, permeabilized with xylene, embedded in wax, and finally sliced into 5-μm-thick sections. Wiegert's iron hematoxylin solution (Sigma-Aldrich, USA) was utilized to stain nuclei for 5 min. After rinsing, tissue sections were stained with 0.7% Masson-Ponceau-acid fuchsin staining solution (Sigma-Aldrich) for 10 min. Samples were then blocked in 2% glacial acetic acid and differentiated in phosphomolybdic acid for 4 min. Sections were directly stained with 2% aniline blue dye solution (Sigma-Aldrich). Finally, the xylene solution was removed, and tissue sections were mounted with neutral resins.

Dual-luciferase reporter gene assay

The rat FGD5-AS1 wild-type sequence (FGD5-AS1-WT) and the mutant derivative lacking the miR-223-3p binding site (FGD5-AS1-Mut) were subcloned to the downstream of the coding region of the luciferase gene. The recombinant plasmids FGD5-AS1-WT and FGD5-AS1-Mut (FGD5-AS1-Mut: 5'-cctgaccTTTCGCCTTGA-CTG t-5', Gene Chem) were then used to conduct a bioinformatics analysis. HEK293T cells (BeNa Culture Collection, China) were cultured at 37°C with high-glucose DMEM. After being seeded onto 24-well plates, the plasmids and/or miR-223-3p mimic were transfected into cells. A dual-luciferase reporter assay system (Promega) was used to examine the luciferase activity in each group 48 h after transfection.

Western blotting

Cells were lysed using RIPA buffer. Protein samples were loaded onto a 10% SDS-PAGE gel for electrophoresis and then transferred to PVDF membranes. Protein blots were blocked with the following primary antibodies: anti-cleaved caspase-3 (1:1000, CST), anti-Bcl-2 (1:1000, CST), anti-Actin (1:2000, Abcam, ab6276), anti-Bax (1:1000, Abcam), anti-Akt (1:1000, CST), anti-p-Akt (T308, 1:1000, CST) and anti-p-Akt (S473, 1:1000, CST). Then, the blots were incubated with secondary antibodies (1:5000, ABclonal). Finally, the blots were visualized by enhanced chemiluminescence.

Quantitative real-time PCR

To extract total RNA from tissues and cells, TRIzol reagent (Takara, Otsu, Japan) was used. RNA was reverse-transcribed into single-strand complementary DNA using PrimeScript RT

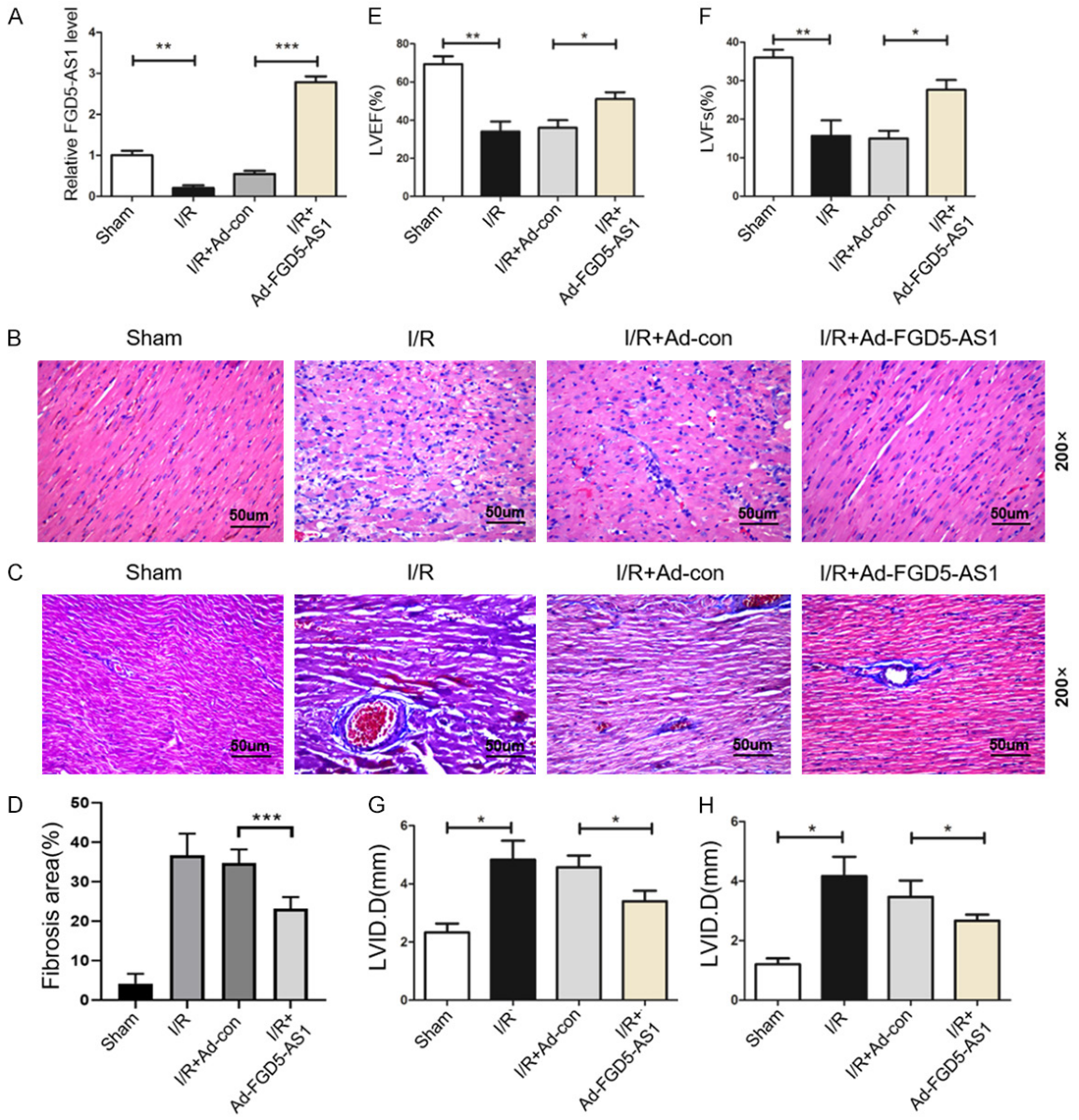

Figure 1. lncRNA FGD5-AS1 overexpression improves cardiac fibrosis and function. A. RT-qPCR results of FGD5-AS1 expression in rats with Ad-con or Ad-FGD5-AS1. B. Histology analysis of the heart of indicated mice by HE staining. C. Masson's trichrome staining in the hearts isolated from four rat heart groups (scale bar =50 μm). D. Bar diagrams represent the area of interstitial fibrosis (%). Values represent the mean ± SEM, n=6 in each group. Compared with Ad-FGD5-AS1-infected AMI rat group, ***p < 0.001. E-H. The LVEF, LVIDd, LVFS, and LVIDs of rats from the four groups. Data represent mean ± SD of three independent measurements. n=6 per group after model establishment. Sham animals (n=5) were used as a control. *p < 0.05; **p < 0.01; ***p < 0.001. AMI: acute myocardial infarction, LVEF: left ventricle ejection fraction, LVFS: left ventricle fractional shortening, LVIDd and LVIDs: left ventricular internal diameter end diastole and end systole.

Reagent Kits (Takara). RNA expression was analyzed via the Step One Plus Real-Time PCR System (Applied Biosystems, Waltham, MA, USA). The relative quantitative expression was determined using the $2^{-\Delta\Delta CT}$ method. The primer sequences are presented in **Table 1**.

Statistical analysis

GraphPad 8.0 was adopted to analyze data. All data are expressed as the mean ± SD. Two-group comparisons were analyzed using the two-tailed Student's t test. Comparisons of

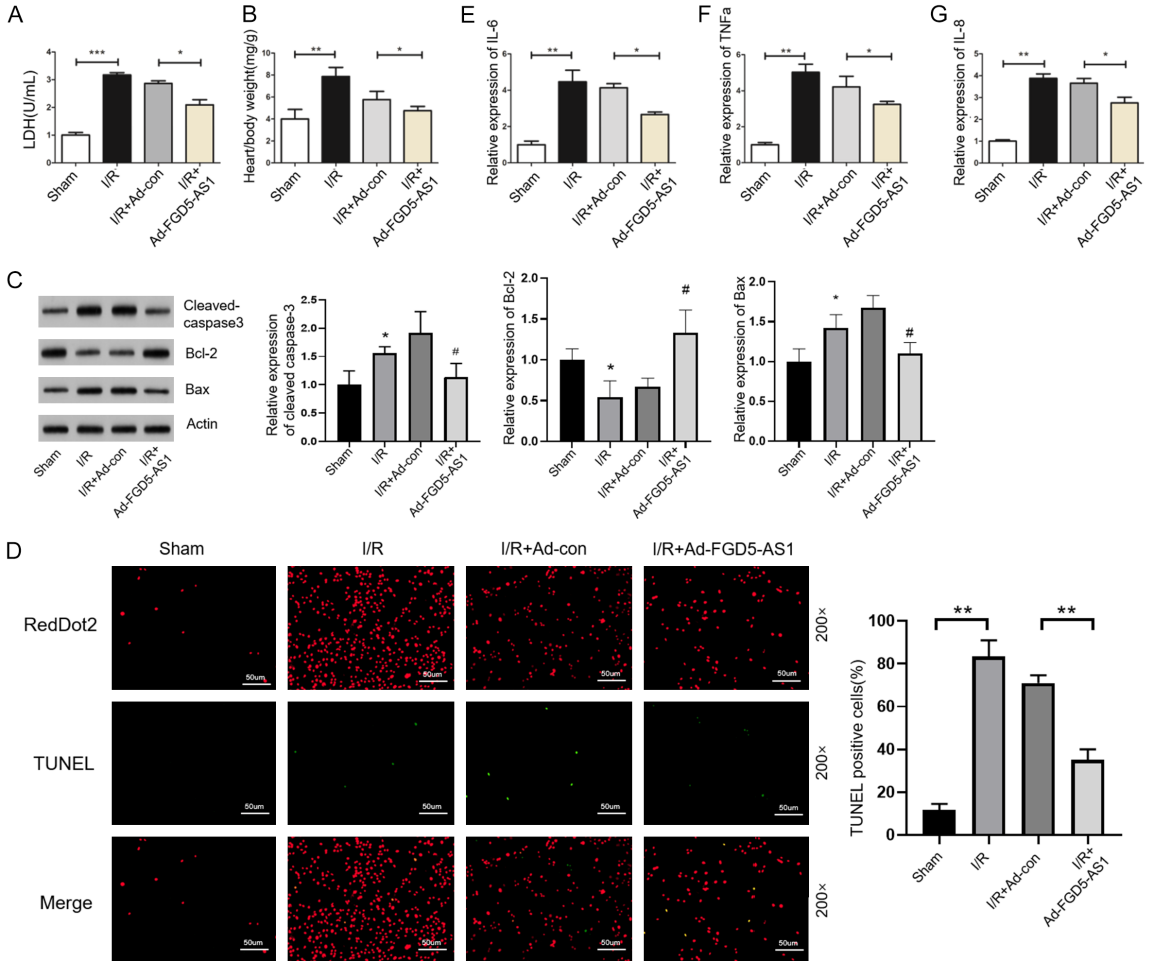

Figure 2. FGD5-AS1 overexpression prevents cardiac apoptosis and inflammation after I/R injury. A. The concentration of serum lactate dehydrogenase was examined in four rat groups. B. The ratio of heart to body weight was measured. C. Western blot was used to examine the protein expression levels of Bcl-2, Bax and cleaved-caspase-3. Results are presented as means ± SE (n=3). Compared with sham group, *P < 0.05; compared with Ad-FGD5-AS1-infected AMI group, #P < 0.05. D. Apoptosis in four rat groups was detected by TUNEL staining (scale bar =50 μm). E-G. PCR analysis was used to determine the mRNA expression of IL-6, TNF-α and IL-8. Data represent the mean ± SD of three independent measurements. *P < 0.05; **P < 0.01; ***P < 0.001. AMI: acute myocardial infarction.

group means were processed by one-way ANOVA, and multiple comparisons among the groups were by LSD and Bonferroni post hoc test. *P < 0.05, **P < 0.01 and ***P < 0.001 were considered statistically significant.

Results

lncRNA FGD5-AS1 overexpression improves cardiac fibrosis and function

To determine whether FGD5-AS is involved in I/R-triggered myocardial injury, rats were intramyocardially injected with Ad-FGD5-AS1 or Ad-Con to stably upregulate FGD5-AS1 expression (**Figure 1A**). HE and Masson's trichrome staining results exhibited a considerable decrease in infarct size in Ad-FGD5-AS1-infected AMI rats (**Figure 1B-D**). Hemodynamic analysis was performed to evaluate cardiac function, including left ventricle ejection fraction (LVEF), left ventricle fractional shortening (LVFS), and left ventricular internal diameter end diastole and end systole (LVIDd and LVIDs). LVEF and LVFS were decreased after I/R treatment, while overexpression of FGD5-AS1 restored LVEF and LVFS (**Figure 1E**, **1F**). LVIDd and LVIDs were significantly increased after I/R treatment, but FGD5-AS1 overexpression significantly decreased LVIDd and LVIDs (**Figure 1G**, **1H**). Therefore, it is indicated that overexpression of FGD5-AS1

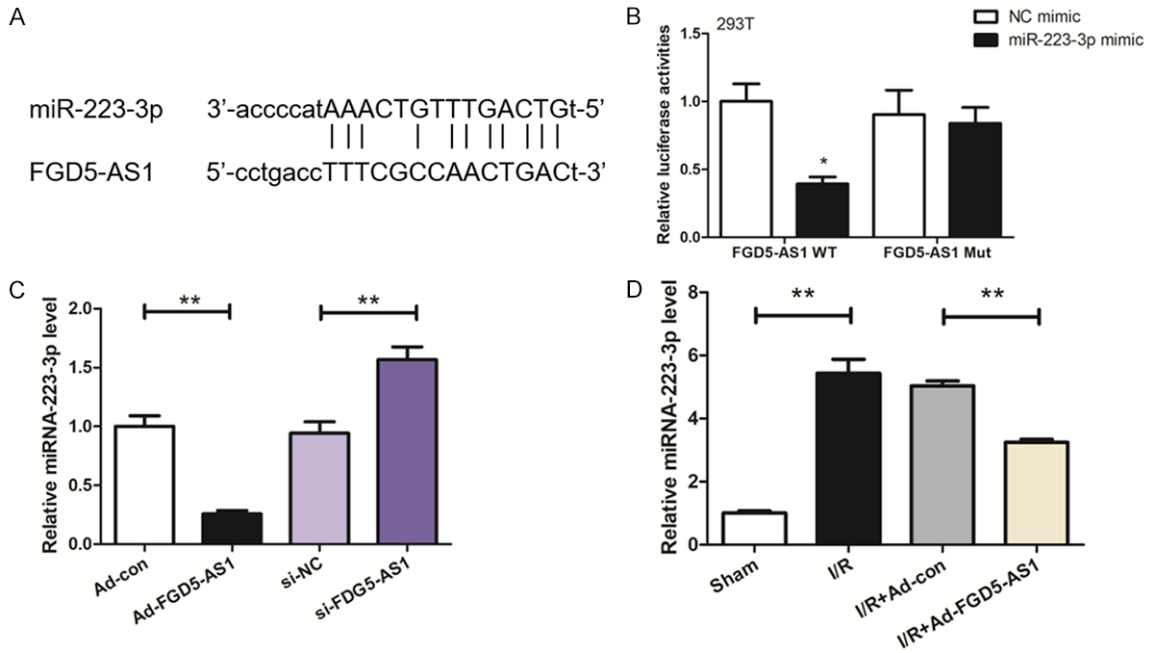

Figure 3. The lncRNA FGD5-AS1 directly targets miR-223-3p. A. The predicted miR-223-3p-binding sequences in FGD5-AS1. B. Luciferase reporter assay was performed to detect the interaction between FGD5-AS1 and miR-223-3p in 293T cells (n=3). C. PCR analysis was performed to determine the mRNA expression of miR-223-3p in different groups of NRCMs (n=3). *P < 0.05; **P < 0.01. D. PCR analysis was performed to determine the mRNA expression of miR-223-3p in the heart of different groups of rats (n=6). *P < 0.05; **P < 0.01. NRCMs: neonatal rat cardiomyocytes.

effectively improves myocardial fibrosis and heart function.

FGD5-AS1 overexpression prevents cardiac apoptosis and inflammation following I/R injury

To further investigate how FGD5-AS1 influences inflammation and apoptosis, the expression levels of apoptosis-related factors were analyzed. The heart to body weight ratio and lactate dehydrogenase (LDH) levels were increased after I/R injury, while FGD5-AS1 overexpression significantly reversed this effect in the I/R injury/Ad-FGD5-AS1 co-treatment group (**Figure 2A**, **2B**). Additionally, the expression levels of cleaved caspase-3 and Bax were markedly decreased in rats transfected with Ad-FGD5-AS1, while Bcl-2 expression was significantly increased at the same time (**Figure 2C**). These results suggest that I/R injury may cause apoptosis in cardiomyocytes, though FGD5-AS1 overexpression significantly inhibited cardiomyocyte apoptosis (**Figure 2D**). Furthermore, FGD5-AS1 overexpression partially reduced inflammation induced by I/R injury (**Figure 2E-G**). It is suggested that FGD5-AS1 overexpression attenuated apoptosis and inflammation in cardiomyocytes.

lncRNA FGD5-AS1 targets miR-223-3p directly

The lncBase Predicted database was adopted to identify putative binding sites between FGD5-AS1 and miR-233-3p (**Figure 3A**). The luciferase constructs of FGD5-AS1 (FGD5-AS1-WT) and a mutated form (FGD5-AS1-Mut) were created. The miR-223-3p mimic suppressed the luciferase activity of FGD5-AS1-WT, yet it did not have any effect on FGD5-AS1-Mut in HEK293T cells (**Figure 3B**). FGD5-AS1 overexpression remarkably decreased miR-223-3p expression, whereas si-FGD5-AS1 increased miR-223-3p expression (**Figure 3C**). miR-223-3p expression was upregulated in the I/R injury group, but FGD5-AS1 overexpression significantly reversed this effect in rats with an I/R injury treated with Ad-FGD5-AS1 (**Figure 3D**). Thus, it is indicated that miR-223-3p can be directly targeted by the lncRNA FGD5-AS1.

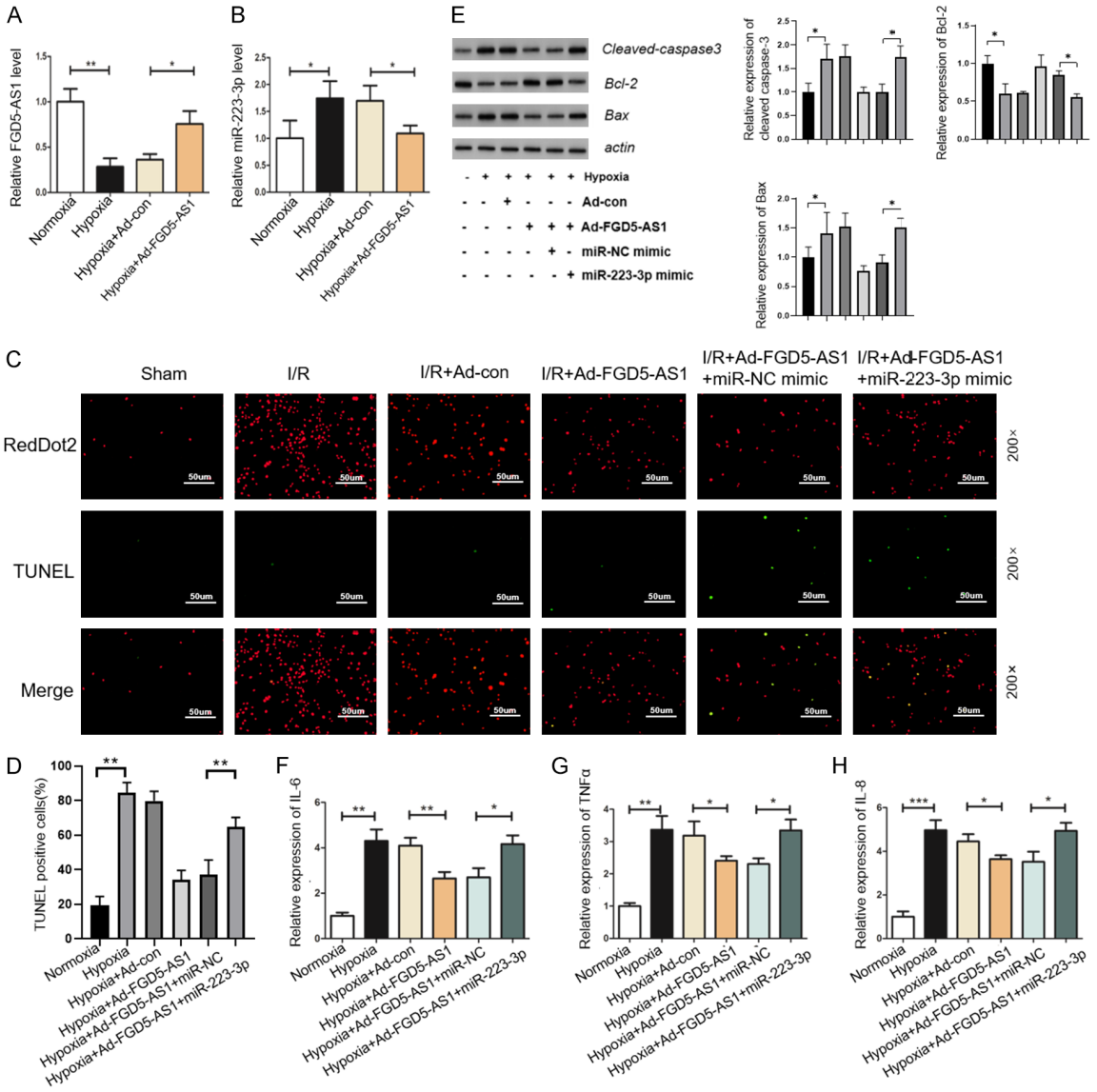

Figure 4. FGD5-AS1 attenuates hypoxia-induced apoptosis and inflammation via miR-223-3p in cultured primary NRCMs. A. PCR analysis was performed to determine the mRNA expression of FGD5-AS1. B. PCR analysis was performed to determine the mRNA expression of miR-223-3p. C, D. Apoptosis in four rat groups was detected by TUNEL staining after overexpression of FGD5-AS1 and/or miR-223-3p (scale bar =50 μm). E. Western blot was performed to examine the protein levels of Bcl-2, Bax and cleaved-caspase-3 after overexpression of FGD5-AS1 and/or miR-223-3p. F-H. PCR analysis was performed to determine the mRNA expressions of IL-6, TNF-α and IL-8. Data represent the mean ± SD of three independent measurements after overexpression of FGD5-AS1 and/or miR-223-3p. n=3, *P < 0.05; **P < 0.01; ***P < 0.001. NRCMs: neonatal rat cardiomyocytes.

FGD5-AS1 attenuates hypoxia-induced apoptosis and inflammation via miR-223-3p in cultured primary neonatal rat cardiomyocytes (NRCMs)

To explore the interaction between FGD5-AS1 and miR-223-3p in hypoxia-treated primary NRCMs, the expression levels of FGD5-AS1 and miR-223-3p were examined. Pretreating cardiomyocytes with Ad-FGD5-AS1 before hypoxia significantly upregulated FGD5-AS1 expression, which was reduced by hypoxia (**Figure 4A**). miR-223-3p expression was increased in NRCMs after hypoxia, but decreased after overexpressing FGD5-AS1 (**Figure 4B**). TUNEL staining was utilized to analyze hypoxia-induced cellular apoptosis, and interestingly, FGD5-AS1 overexpression considerably suppressed cellu-

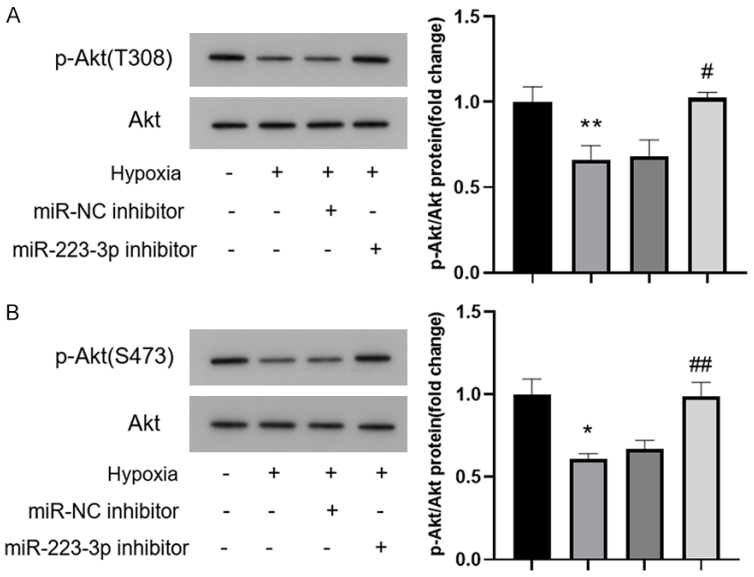

Figure 5. miR-223-3p modulates p-Akt in hypoxia-induced NRCMs. A, B. Western blot was performed to examine the protein levels of P-Akt (T308) and P-Akt (S473) (n=3). NRCMs: neonatal rat cardiomyocytes.

lar apoptosis (**Figure 4C**). However, FGD5-AS1-elicited decline of cell apoptosis was reversed upon the increase of miR-223-3p expression (**Figure 4C**, **4D**). Similar results were observed via Western blot in hypoxia-induced NRCMs (**Figure 4E**). Additionally, in hypoxia-triggered NRCMs, cytokine expression was increased remarkably, but the miR-223-3p mimic restored the suppressive effects of FGD5-AS1 on inflammation (**Figure 4F-H**). Therefore, it is suggested that FGD5-AS1 alleviates hypoxia-induced inflammation and apoptosis in NRCMs through miR-223-3p.

miR-223-3p modulates p-Akt in hypoxia-induced NRCMs

Akt signaling pathway participation in I/R injury is well-characterized. Thus, it was speculated that miR-223-3p might modulate Akt signaling in hypoxia-induced NRCMs. Our results showed that the expression of phosphorylated Akt in NRCMs treated with hypoxia was reduced, but miR-223-3p inhibitor reversed this effect (**Figure 5A**, **5B**). The expression of phosphorylated Akt was reduced in the miR-223-3p inhibitor group compared to that in the miR-NC inhibitor group (**Figure 5A**, **5B**). It is indicated that miR-223-3p regulates p-Akt in hypoxia-induced NRCMs.

FGD5-AS1 suppresses cardiac apoptosis and inflammation through the regulation of Akt signaling in hypoxia-induced NRCMs

We investigated the role of p-Akt signaling on apoptosis and inflammation triggered by hypoxia. LY294002 (MCE, HY-10108, Shanghai, China), a Akt inhibitor which blocks Akt activity, was used in this study. Under hypoxic conditions, the repressive effect of FGD5-AS1 was repressed with a p-Akt inhibitor, as shown by TUNEL staining (**Figure 6A**). In addition, FGD5-AS1 overexpression suppressed hypoxia-induced apoptotic protein expression by inhibiting p-Akt (**Figure 6B**). In cells treated with FGD5-AS1, LY294002 prevented the generation of cytokines induced by hypoxia (**Figure 6C-E**). Thus, under hypoxic conditions, apoptosis and inflammation are inhibited by the overexpression of FGD5-AS1 through p-Akt signaling.

Discussion

The key roles of lncRNAs in the pathogenesis of CVD have previously been reported [25, 26]. Although the lncRNA FGD5-AS1 has been demonstrated to regulate periodontitis as well as a number of cancers [10, 11, 27], little is known about its role in CVDs. Shen et al. found that FGD5-AS1 was a key lncRNA in the development of AMI. Nevertheless, a detailed mechanism of FGD5-AS1 in AMI needs further exploration. In our present study, we showed that FGD5-AS1 alleviated I/R-induced myocardial injury, reduced LVEDD and LVESD, and enhanced LVFS and FS. Moreover, we revealed that FGD5-AS1 was negatively associated with the expression of miR-223-3p. Simultaneously suppressing miR-223-3p and overexpressing FGD5-AS1 showed an additive effect on alleviating I/R-induced myocardial injury and reducing cardiomyocyte apoptosis. Therefore, lncRNAs might serve as competitive endogenous RNAs to modulate miRNAs [11, 28]. Knockdown of the lncRNA MALAT1 improved AMI via miR-320-PTEN signaling [29], while the lncRNA

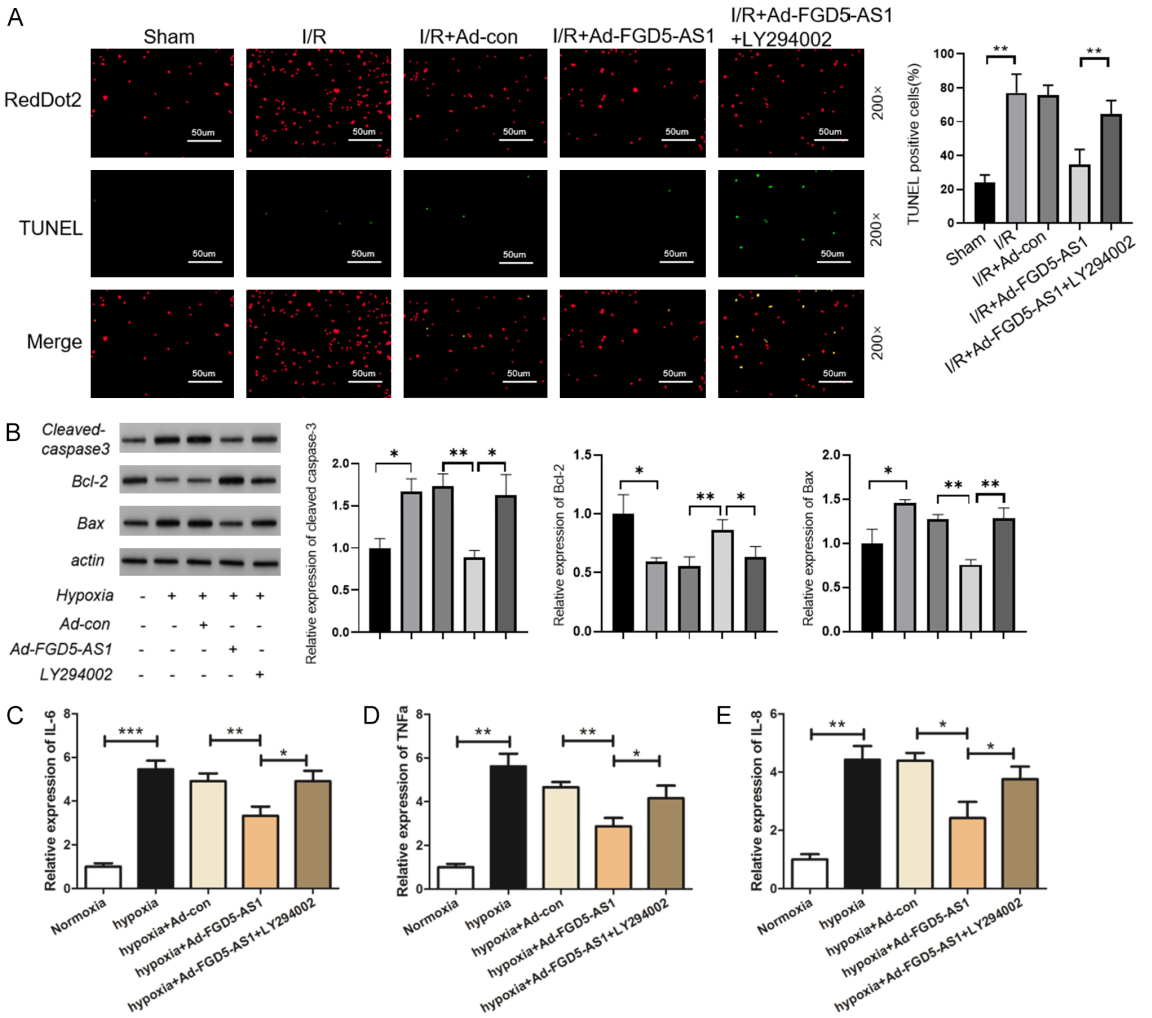

Figure 6. FGD5-AS1 suppresses cardiac apoptosis and inflammation through the regulation of Akt signaling in hypoxia-induced NRCMs. A. Apoptosis in five rat groups was detected by TUNEL staining in the presence of LY294002 (scale bar =50 μm). B. Western blot was performed to examine the protein levels of Bcl-2, Bax and cleaved-caspase-3 in the presence of LY294002 (Akt inhibitor). C-E. PCR analysis was used to determine the mRNA expressions of IL-6, TNF-α and IL-8. Data represent the mean ± SD of three independent measurements in the presence of LY294002. n=3, *P < 0.05; **P < 0.01; ***P < 0.001. NRCMs: neonatal rat cardiomyocytes.

ANRIL protected H9c2 cells from hypoxia-induced injury by targeting the miR-7-5p/SIRT1 axis [30]. Additionally, miR-223-3p could function as a regulator of various kinds of cancers. Yang et al. found that miR-223-3p was involved in gastric carcinogenesis [31]. In addition, miR-223-3p suppressed the metastasis and progression of osteosarcoma [32]. However, miR-223-3p involvement in AMI has been poorly studied. In the present study, miR-223-3p was upregulated in the I/R injury group, and FGD5-AS1 could modulate miR-223-3p directly.

Myocardial infarction is a process of cardiomyocyte apoptosis. The suppression of myocardial apoptosis can prevent heart failure, and cardiac remodeling may be associated with preventing cardiomyocyte apoptosis. Atorvastatin alleviates AMI and hypoxia by reducing cardiomyocyte apoptosis [33]. PEDF improves cardiac function in mice with AMI by blocking vascular permeability and cardiomyocyte apoptosis [34]. Therefore, cardiomyocyte apoptosis is regarded as a pivotal pattern of cell death in I/R injury. Our research revealed that FGD5-AS1 knockdown increased cardiomyocyte apoptosis, whereas FGD5-AS1 overexpression had the opposite effect. Mechanistically, FGD5-AS1 attenuated hypoxia-induced apoptosis by suppressing miR-223-3p expression in NRCMs.

Studies have shown that proinflammatory cytokines are elevated during myocardial injury [35]. Our findings indicate that FGD5-AS1 inhibits the expression of proinflammatory cytokines through miR-223-3p. Previous studies have demonstrated that Akt regulates apoptotic proteins, which in turn mediates cell apoptosis [36, 37]. Moreover, miR-223 modulates cellular proliferation through PI3K/Akt signaling [38, 39]. We therefore surmise that miR-223 may regulate p-Akt in hypoxia-induced NRCMs and showed that miR-223-3p enhanced p-Akt activity in hypoxia-induced NRCMs. Additionally, we demonstrated that the suppressive influence of FGD5-AS1 on hypoxia-triggered apoptosis could be repressed by inhibiting p-Akt. Thus, our results indicate that the Akt signaling pathway is involved in the FGD5-AS1/miR-223-3p interaction during cardiac I/R injury. However, the present study suffers from certain limitations. For example, the mechanism of FGD5-AS1 as a target of myocardial infarction remains to be further explored and verified on patient samples. So far, autophagy has been widely reported to produce an alleviative effect in acute myocardial infarction [40, 41]. MiR-223-3p was reported to involve in isoproterenol-induced myocardial fibrosis by regulating autophagy [42]. There is a lack of exploration on whether the mechanism of FGD5-AS1 inhibiting cardiomyocyte apoptosis is dependent on autophagy.

Overall, we discovered that FGD5-AS1 regulates cardiomyocyte apoptosis in AMI, and miR-223-3p can trigger apoptosis by inhibiting Akt expression. These findings may provide new perspectives for AMI treatment.

Disclosure of conflict of interest

None.

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