Long noncoding RNA FTX ameliorates hydrogen peroxide-induced cardiomyocyte injury by regulating the miR-150/KLF13 axis

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Abstract
Background – Myocardial reperfusion is an effective therapy for acute myocardial infarction (AMI). However, ischemia/reperfusion (I/R) injury following myocardial reperfusion is a significant limitation for AMI treatment. Five prime to Xist (FTX) was recognized as a biomarker of multiple diseases, including heart disease. However, the molecular mechanism of FTX in I/R injury is unclear.

Methods – Cell viability was evaluated by using cell counting kit-8 (CCK-8) assay. Apoptosis was analyzed by using a caspase-3 activity detection kit and flow cytometry. The expression of FTX, microRNA (miR)-150, and Kruppel-like factor 13 (KLF13) was measured by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). The interaction of miR-150 and FTX or KLF13 was confirmed by a dual-luciferase reporter and RNA immunoprecipitation (RIP) assays. Protein expression of KLF13 was examined by Western blot. The role of FTX was detected in I/R-injured heart tissues in vivo.

Results – Hydrogen peroxide (H2O2) induced cardiomyocyte injury by decreasing cell viability and expediting cell apoptosis. However, FTX alleviated cardiomyocyte injury by promoting cell proliferation and restricting cell apoptosis of H9C2 cells that were treated with H2O2. In addition, we discovered that FTX directly interacted with miR-150, while KLF13 was a target of miR-150. Rescue experiments showed that miR-150 neutralized the FTX-mediated promotion of cell progression and restriction of cell apoptosis in H9C2 cells treated with H2O2. KLF13 knockdown restored the effect of miR-150 on increased proliferation and decrease in apoptosis in H2O2-treated cardiomyocytes. Furthermore, FTX enhanced the expression of KLF13 protein through interaction with miR-150. Upregulation of FTX repressed apoptosis in I/R-injured heart tissues in vivo.

Conclusion – FTX relieves H2O2-induced cardiomyocyte injury by increasing KLF13 expression via depletion of miR-150, thus providing a novel therapeutic target for the alleviation of I/R injury.

Keywords: I/R injury, Inc FTX, miR-150, KLF13, progression

1 Introduction

Acute myocardial infarction (AMI) is a serious cardiovascular disease accompanied with quadriplegia or paraplegia [1]. AMI pathogenesis is complicated and includes factors such as obesity, sedentary lifestyle, diabetes, smoking, and dyslipidemia. Myocardial reperfusion has dramatically improved the therapeutic outcomes of AMI patients [2,3]. Unfortunately, myocardial reperfusion can often lead to ischemia/reperfusion (I/R) injury and initiate oxidative stress, ventricular arrhythmias, cardiomyocyte death, and neuronal apoptosis [4–6]. Therefore, it is imperative to clarify the molecular mechanism of I/R injury-induced cardiomyocyte injury.

Long noncoding RNAs (lncRNAs) are critical modulators of many diseases through their impact on gene expression [7]. LncRNA five prime to Xist (FTX), located at X-chromosome inactivation center, is associated with the pathogenesis of multiple diseases, such as epilepsy, heart disease, and cancers [8,9]. For example, FTX accelerated cirrhotic patients’ inflammatory response by activating macrophages through the depletion of miR-545 and regulation of Tim-3 expression [10]. In addition, dysregulation of FTX has been observed in a variety of cancers. FTX acts as an oncogene to increase cell growth,
colony formation, and invasion by enhancing AEG-1 expression via targeting miR-342-3p in glioma [11]. By contrast, the elimination of FTX reduced cell cycling, proliferation, and colony formation in renal cell carcinoma [12]. However, the function of FTX in cardiomyocyte injury is still poorly understood.

MicroRNAs (miRNAs) are conserved transcripts that participate in many pathological processes, such as cell cycle, metabolism, growth, differentiation, migration, inflammation, and apoptosis [13–15]. Ectopic expression of miRNAs was identified as a major cause of different diseases [16]. For example, miR-150 is a marker of sepsis and has been shown to reduce inflammation and apoptosis of umbilical vein endothelial cells by regulating NF-κB1 [17]. The abundance of miR-150 could target AKT3 and alleviate LPS-induced lung injury by regulating cell viability, inflammation, autophagy, and apoptosis through the JNK/NF-κB pathway [18]. In addition, miR-150 behaved as a tumor suppressor to inhibit cell growth in non-small cell lung cancer or melanoma by interacting with EPG5 or MYB [19,20]. These findings prompted us to consider that miR-150 may play an important role during cardiomyocyte injury.

Kruppel-like factor 13 (KLF13) is a member of the KLF families, which is overexpressed in the heart [21]. Inhibition of KLF13 has been reported to protect the heart from AMI through its interaction with miR-125b-5p [22]. Therefore, KLF13 may also be involved in cardiomyocyte protection after I/R injury.

We attempted to illuminate the regulatory mechanism of FTX in the cardiomyocyte response to I/R injury. Hydrogen peroxide (H$_2$O$_2$) treatment of cardiomyocytes was utilized to mimic I/R injury. The influences of FTX, miR-150, and KLF13 on H$_2$O$_2$-treated cardiomyocytes were evaluated by rescue experiments.

## 2 Materials and methods

### 2.1 Cell culture and treatment

Cardiac-derived H9C2 cells isolated from rats were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in complete Dulbecco’s modified Eagle medium (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (cat. no. 10099; Gibco). Cells were cultured in a constant temperature biochemical incubator (Thermo Scientific, Waltham, MA, USA) at 37°C in 5% CO$_2$. The H9C2 cells were treated with H$_2$O$_2$ (0, 50, 100, and 200 µM) for 24 h. H9C2 cells treated with 100 µM H$_2$O$_2$ were transfected with different vectors for 48 h.

### 2.2 Cell counting kit-8 (CCK-8) assay

H9C2 cells were plated onto 96-well plates overnight. After H$_2$O$_2$ treatment for 24 h and cell transfection for 48 h, 10 µL of the CCK-8 reagent (Beyotime, Shanghai, China) was added to the H9C2 cells and left for 2 h. Finally, an optical density (OD) value of 490 nm was detected by a spectrophotometer (Thermo Scientific).

### 2.3 Caspase-3 activity detection

H9C2 cells were plated onto 24-well plates overnight, treated with H$_2$O$_2$ for 24 h and transfected with vectors for 48 h. After washing three times with phosphate buffer saline, the H9C2 cells were collected and stained with a caspase-3 activity detection kit (Beyotime). Finally, the caspase-3 activity of the H9C2 cells was measured by a spectrophotometer (Thermo Scientific).

### 2.4 Flow cytometry

H9C2 cells grown in 24-well plates were treated with H$_2$O$_2$ and transfected with different vectors. Then the H9C2 cells were collected and stained with fluorescein isothiocyanate-tagged annexin V/propidium iodide detection kit (Invitrogen, Carlsbad, CA, USA). The apoptotic rate was analyzed by a flow cytometer (ACEA NovoCyte; Agilent BIO, Santa Clara, CA, USA).

### 2.5 Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

RNA extraction from H9C2 cells was performed using TRIzol reagent (Invitrogen) following the manufacturer’s instructions. Next the cDNA for FTX and KLF13 was synthesized by All-in-One First-Strand cDNA Synthesis kit (GeneCopoeia, Guangzhou, China), while miR-150 was reverse transcribed with an All-in-One™ miRNA First-Strand cDNA Synthesis kit (GeneCopoeia). qPCR
was performed using a Fast SYBR™ Green Master Mix (Applied Biosystems, Foster City, CA, USA) with a CFX Touch real-time PCR instrument (Bio-Rad, Hercules, CA, USA). Relative expression was calculated using the \(2^{-\Delta\Delta Ct}\) method. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and U6 were employed as internal references. The primers for FTX, miR-150, KLF13, GAPDH, and U6 are listed as follows: FTX (forward, 5'-TATGCCACCTAGGCTTTCTACA-3'; reverse, 5'-TCTCTTCAAAGGCGCATAT-3'); miR-150 (forward, 5'-GGCTTCCAAAACCTTGTAG-3'; reverse, 5'-GTGCGAGGAGCTCGGGAAG-3'); KLF13 (forward, 5'-CGGCAGAGGAAGCACAA-3'; reverse, 5'-CTCTTCTCGCCGTGT-3'); GAPDH (forward, 5'-AGGTCGCTTGAAACGGAAT-3'; reverse, 5'-GGGGTCGTTGATGGCAACA-3'); and U6 (forward, 5'-ACCCGTGAGAATCCCCCTCATA-3'; reverse, 5'-GACGACTGAGCCCTGATG-3').

2.6 Cell transfection

Small interfering RNA (siRNA) targeting FTX (si-FTX: 5'-GCTAAGACATCCCGAAGTCA-3'), siRNA targeting KLF13 (si-KLF13: 5'-GCCGAGGTCCGAGGTCA-3'), control (si-con: 5'-TGCACTGTGCAAGCCTCTTAA-3'), pcDNA, and FTX overexpression vectors (the sequences of FTX were inserted into pcDNA vector termed FTX) were synthesized by GenePharma (Shanghai, China). MiR-150 mimics (miR-150), miR-150 inhibitor (anti-miR-150), control (miR-con), and control inhibitor (anti-miR-con) were obtained from RIBOBIO (Guangzhou, China). The vectors were transfected into H9C2 cells by Lipofectamine 2000 (Invitrogen, Thermo Scientific).

2.7 Dual-luciferase reporter assay

The interaction between miR-150 and FTX or KLF13 was predicted by bioinformatics software (Starbase, http://starbase.sysu.edu.cn/; DIANA TOOL, http://diana.3mg.ox.ac.uk/diana/index.php?r=site/page&view=software) and determined by a dual-luciferase reporter assay. In brief, the wild-type FTX (FTX-WT), KLF13 (KLF13-WT), mutant-type FTX (FTX-MUT), and KLF13 (KLF13-MUT) luciferase vectors were constructed and separately co-transfected in H9C2 cells with miR-150 or miR-con. Followed by analysis with Dual-Luciferase® Reporter Assay System, the relative luciferase activities were measured using a multimode reader (Tecan, Männedorf, Switzerland).

2.8 RNA immunoprecipitation (RIP) assay

RIP was conducted using the Magna RIP™ RNA-Binding Protein Immunoprecipitation kit (Millipore, MA, USA), according to the manufacturer’s instructions. H9C2 cells transfected with miR-150 or miR-con were lysed by RIP buffer. Next the cell lysate was incubated at 4°C overnight with magnetic beads coated with anti-Ago2 or IgG antibody (Millipore). Finally, the enrichment of FTX and KLF13 was detected by qRT-PCR.

2.9 Western blot

Western blot was conducted as previously reported [23]. In brief, total protein was extracted from H9C2 cells treated with 100 µM H2O2 and transfected with the vectors. The protein was then resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (Millipore), which were then blocked with 5% nonfat milk. After that, the membranes were probed with primary antibodies against KLF13 (cat. no. ab190624; 1:1000; Abcam, Cambridge, UK) or β-actin (cat. no. ab8227; 1:3000; Abcam) and detected using HRP-conjugated secondary antibody (cat. no. D110058; 1:20000; Sangon, Shanghai, China). Protein bands were visualized using ECL detection reagent (Vazyme, Nanjing, China).

2.10 In vivo assay

Ten-week-old male C57BL/6 mice were purchased from Hubei Research Center of Laboratory Animal (Wuhan, China) and kept in an specific pathogen-free (SPF) environment. To evaluate the biological role of FTX, pcDNA vs FTX was delivered into the aortic root, as previously described [24]. At least five mice from each group survived in vivo and were used for subsequent analysis. Five days later, the I/R model was established by ligating the left anterior descending artery for 30 min. After 24 h of reperfusion, the heart was excised. And then, the area of infarction and a total area of the transverse section were measured using NIH Image J software, followed by analysis with qRT-PCR and Western blot.

**Ethical approval:** The research related to animal use has been complied with all the relevant national regulations and institutional policies for the care and use of animals and has been approved by the Institutional Animal Care...
and Use Committee of the First Affiliated Hospital of Airforce Military Medical University.

2.11 Statistical analysis

All the data were presented as mean ± standard deviation (SD). Statistical analysis was performed by GraphPad Prism 7 (San Diego, CA, USA). The correlation between miR-150 and FTX or KLF13 was analyzed by Pearson’s correlation coefficient. P values less than 0.05 ($P < 0.05$) were considered statistically significant.

3 Results

3.1 $\text{H}_2\text{O}_2$ inhibited cardiomyocyte cell proliferation and promoted cell apoptosis

It is well acknowledged that $\text{H}_2\text{O}_2$ can induce cell death [25]. To explore the effects of $\text{H}_2\text{O}_2$ on cardiomyocyte growth and apoptosis, H9C2 cells were treated with $\text{H}_2\text{O}_2$ (0, 50, 100, and 200 µM) for 24 h. As illustrated in Figure 1a, $\text{H}_2\text{O}_2$ repressed cardiomyocyte proliferation in a dose-dependent manner. In addition, caspase-3 activity was enhanced by $\text{H}_2\text{O}_2$ treatment (Figure 1b). Meanwhile, we observed that $\text{H}_2\text{O}_2$ induced cardiomyocyte apoptosis in a dose-dependent manner (Figure 1c). To explore the underlying mechanism, FTX expression was evaluated by qRT-PCR. The result showed that FTX expression was reduced by $\text{H}_2\text{O}_2$ treatment (Figure 1d). Collectively, $\text{H}_2\text{O}_2$ may affect cardiomyocyte development by regulating FTX expression.

3.2 FTX abolished $\text{H}_2\text{O}_2$-induced suppression of proliferation and promotion of apoptosis of cardiomyocytes

In order to investigate the effects of FTX on cell viability and apoptosis of $\text{H}_2\text{O}_2$-treated cardiomyocytes, untransfected or transfected H9C2 cells were treated with 100 µM $\text{H}_2\text{O}_2$ for 24 h. As displayed in Figure 2a, FTX expression was inhibited by $\text{H}_2\text{O}_2$, and the inhibition was reversed by

![Figure 1](image-url)
transfection with FTX. The overexpression efficiency of FTX in H9C2 cells is shown in Figure A1. In addition, the overexpression of FTX restored H2O2-mediated inhibition of proliferation (Figure 2b) and promotion of apoptosis (Figure 2d) of H9C2 cells. In addition, the caspase-3 activity of H9C2 cells was examined by a caspase-3 activity detection kit. The result showed that the caspase-3 activity was enhanced by H2O2 and reduced by FTX (Figure 2c). Taken together, FTX produced regulatory effects opposite to those of H2O2 on cell proliferation and apoptosis of cardiomyocytes.

3.3 FTX directly targeted miR-150

As predicted by the bioinformatics analysis tool StarBase, we identified potential binding sites between FTX and miR-150 (Figure 3a). Reduced luciferase activity in H9C2 cells co-transfected with FTX-WT and miR-150 validated the interaction between FTX and miR-150 (Figure 3b). The transfection efficiency of miR-150 mimics in H9C2 cells is presented in Figure A1. Furthermore, the RIP assay result showed an increased enrichment of FTX in H9C2 cells transfected with miR-150 as compared to miR-con (Figure 3c). The influence of H2O2 on miR-150 expression was further investigated by qRT-PCR. H2O2 treatment increased miR-150 expression in a dose-dependent manner (Figure 3d). The knockdown efficiency of FTX in H9C2 cells is shown in Figure A1. Moreover, miR-150 expression was decreased by FTX and increased by FTX knockdown (Figure 3e). Therefore, we considered the possibility that miR-150 is a direct target of FTX.

3.4 FTX regulated H2O2-induced cytotoxicity of cardiomyocytes by targeting miR-150

After demonstrating the interaction between FTX and miR-150, we predicted that FTX is able to regulate H2O2-induced cytotoxicity of cardiomyocytes by interacting with miR-150. To confirm this, H9C2 cells were transfected with pcDNA, FTX, FTX + miR-con, and FTX + miR-150 and then untransfected or transfected H9C2 cells were treated with 100 µM H2O2. As exhibited in Figure 4a, miR-150 transfection restored the repression of FTX on miR-150 expression in H2O2-treated H9C2 cells. More importantly, miR-150 neutralized the promotion of FTX on H2O2-induced cytotoxicity of H9C2 cells (Figure 4b).
Figure 3: FTX directly interacted with miR-150. (a) Sequence complementarity between FTX and miR-150 was predicted by StarBase. (b) Luciferase activity of H9C2 cells co-transfected with FTX-WT or FTX-MUT and miR-150 or miR-con was determined by dual-luciferase reporter assay. (c) The enrichment of FTX in H9C2 cells transfected with miR-150 and miR-con was analyzed by RIP assay. (d) The expression of miR-150 in H9C2 cells treated with H2O2 was evaluated by qRT-PCR. (e) Determination of miR-150 expression in H9C2 cells transfected with pcDNA, FTX, si-con, and si-FTX using qRT-PCR. *P < 0.05, ***P < 0.001, n = 3.

Figure 4: MiR-150 abrogated FTX-mediated promotion on proliferation and suppression on apoptosis of H2O2-treated cardiomyocytes. H9C2 cells were treated with 100 µM H2O2 for 24 h and transfected with pcDNA, FTX, FTX + miR-con and FTX + miR-150. (a) The expression of miR-150 was examined by qRT-PCR. (b) CCK-8 assay was employed to detect cell viability. (c) Analysis of caspase-3 activity using caspase-3 activity detection kit. (d) Examination of cell apoptosis by flow cytometry. **P < 0.01, ***P < 0.001, n = 3.
By contrast, both H2O2 and miR-150 were promoted, whereas FTX blocked caspase-3 activity in cardiomyocytes (Figure 4c). Consistently, miR-150 facilitated, whereas FTX attenuated cell apoptosis of cardiomyocytes with H2O2 treatment (Figure 4d). These findings revealed that FTX may modulate H2O2-induced cytotoxicity of cardiomyocytes by depleting miR-150.

3.5 KLF13 directly interacted with miR-150

Using DIANA TOOL, we discovered that miR-150 contained the binding sites of KLF13 (Figure 5a). To verify this prediction, the luciferase reporter system was constructed by co-transfecting KLF13-WT or KLF13-MUT and miR-150 or miR-con in H9C2 cells. Luciferase activity of H9C2 cells transfected with miR-150 was inhibited by KLF13-WT, thus confirming the interaction between miR-150 and KLF13 (Figure 5b). In addition, the enrichment of KLF13 was elevated by miR-150 in comparison to miR-con, as determined by the RIP assay (Figure 5c). As expected, KLF13 protein expression in H9C2 cells was repressed by H2O2 in a dose-dependent manner (Figure 5d). Transfection efficiency of miR-150 inhibitor in H9C2 cells is shown in Figure A1. Meanwhile, KLF13 protein expression was decreased by miR-150 and increased by miR-150 inhibitor (Figure 5e).

3.6 Elimination of KLF13 counteracted miR-150 inhibitor-mediated effects on proliferation and apoptosis in H2O2-treated cardiomyocytes

We further investigated the regulatory mechanism of the miR-150/KLF13 axis in H2O2-treated cardiomyocytes. H9C2 cells were transfected with anti-miR-con, anti-miR-150 (miR-150 inhibitor), anti-miR-150 + si-con, and anti-miR-150 + si-KLF13, followed by treatment with 100 µM H2O2. Quantitation of KLF13 expression at the mRNA and protein levels in H2O2-treated H9C2 cells revealed an increase mediated by miR-150 inhibitor and reduction by KLF13 knockdown (Figure 6a). Additionally, the depletion of KLF13 alleviated miR-150 inhibitor-
induced enhancement of proliferation (Figure 6b) and suppression of apoptosis (Figure 6d) in H2O2-treated H9C2 cells. As expected, the caspase-3 activity of H2O2-treated H9C2 cells was enhanced by KLF13 silencing and reduced by miR-150 inhibitor (Figure 6c). In short, miR-150 may regulate H2O2-induced cytotoxicity of cardiomyocytes by targeting KLF13.

3.7 FTX regulated KLF13 expression by sponging miR-150 in cardiomyocytes

The specific molecular mechanism of FTX activity in cardiomyocytes was further explored by Western blot. As shown in Figure 7a, the expression of KLF13 protein was increased by FTX and inhibited by miR-150. Meanwhile, treatment with the miR-150 inhibitor reversed the inhibitory effect of FTX silencing on the KLF13 protein generation (Figure 7b). Therefore, FTX could sponge miR-150 and regulate KLF13 expression in cardiomyocytes.

3.8 FTX suppressed apoptosis in I/R-injured heart tissue in vivo

To assess the biological significance of FTX in hypoxia- or ischemia-challenged cardiomyocytes, we established an I/R model in vivo. As shown in Figure 8a, FTX expression was increased in the transfected-FTX I/R group compared with the transfected-pcDNA I/R group. Moreover, we
Figure 7: FTX increased the expression of KLF13 protein by depleting miR-150 in cardiomyocytes. (a) KLF13 protein expression in H9C2 cells transfected with pcDNA, FTX, FTX + miR-con and FTX + miR-150 was determined by Western blot. (b) KLF13 protein expression in H9C2 cells transfected with si-con, si-FTX, si-FTX + anti-miR-con, and si-FTX + anti-miR-150. **P < 0.01, ***P < 0.001, n = 3.

Figure 8: FTX overexpression inhibited infarction and apoptosis in I/R-injured heart tissue in vivo. (a) FTX level was detected in sham group and I/R groups (untransfected, transfected pcDNA, and transfected FTX) after reperfusion was measured by qRT-PCR. (b) Transsections were measured for infarction size. (c) Caspase-3 activity was evaluated by caspase-3 activity detection kit. (d) MiR-150 level was detected by qRT-PCR assay. (e) KLF13 protein level was assessed by Western blot assay. **P < 0.01, ***P < 0.001, n = 3.
found that the increased expression of FTX decreased the infarct size in I/R mouse in vivo (Figure 8b). We also detected the effect of pcDNA-FTX on caspase-3 activity. Our results suggest that FTX overexpression reduced caspase-3 activity in the I/R group in vivo relative to the transfected-pcDNA I/R group (Figure 8c). Also, miR-150 levels were decreased (Figure 8d), and KLF13 levels increased (Figure 8e) in the transfected-FTX I/R group compared with the transfected-pcDNA I/R group. These results suggest that FTX upregulation represses apoptosis in cardiomyocytes by regulating the miR-150/KLF13 axis in an in vivo model of I/R.

4 Discussion

Accumulating research has clarified that FTX is a significant predictor of different diseases, including cancers. Aberrant expression of FTX has been linked to the cell progression of various cancers. For instance, FTX indicated poor prognosis of colorectal cancer patients, and overexpression of FTX expedited the malignancy of colorectal cancer by accelerating cell growth [26]. The abundance of FTX is thought to influence cell viability, invasion, and aerobic glycolysis of hepatocellular carcinoma cells by regulating the PPARγ pathway [27]. Consistently, enhanced expression of FTX contributed to cell proliferation of hepatocellular carcinoma cells by interacting with miR-545 and regulating RIG-I expression [8]. However, FTX has also been shown to serve as a tumor suppressor by reducing cell proliferation in hepatocellular carcinoma by targeting miR-374a [28]. Moreover, FTX could regulate drug resistance of adriamycin against acute myeloid leukemia cells by competitively binding to miR-342 and altering ALG3 expression [29]. The regulatory effects of FTX in cardiomyocytes remain unclear.

Bioinformatics analysis prediction by StarBase indicated that FTX could potentially bind to miR-150. Reduced luciferase activity in H9C2 cells that were co-transfected with FTX-WT and miR-150 confirmed the interaction between FTX and miR-150. Previous studies demonstrated that miR-150 is a predictor of different diseases, such as neuropathic pain, heart failure, lung injury, and cancers [30–32]. For instance, miR-150 was reported to attenuate cell proliferation and accelerate cell apoptosis in Burkitt lymphoma by regulating LMO4 [33]. Similarly, miR-150 acted as a tumor suppressor in nasopharyngeal carcinoma to repress cell viability and G1/S phase transition by regulating CCND1 and CCNE2 [34]. In hepatocellular carcinoma, miR-150 is downregulated, and miR-150 weakened cell viability, colony formation, migration, and invasion by regulating the GAB1/ERK axis [35]. Therefore, we assumed that FTX participates in cell regulation of H2O2-treated cardiomyocyte by interacting with miR-150.

We initially validated the influence of H2O2 treatment on cardiomyocyte progression by CCK-8, flow cytometry, qRT-PCR, and caspase-3 activity detection assay. The results revealed that H2O2 weakened cell viability and stimulated cell apoptosis of cardiomyocytes, thus indicating that H2O2 is capable of inducing cardiomyocyte injury. Interestingly, we showed that FTX could protect cardiomyocytes from H2O2-induced cytotoxicity. The underlying molecular mechanism of FTX in cardiomyocyte protection was further investigated. The interaction between miR-150 and FTX or KLF13 was confirmed by a dual-luciferase reporter and RIP assay. Furthermore, H2O2 treatment enhanced the expression of miR-150 while reducing the expression of FTK or KLF13 in a dose-dependent manner. In addition, miR-150 abolished FTX-mediated improvement in proliferation and repression of apoptosis in H2O2-treated H9C2 cells. Likewise, KLF13 elimination relieved miR-150 inhibitor-mediated regulatory effects on cell progression in H2O2-treated cardiomyocytes. Also, we found that FTX could regulate KLF13 protein expression by absorbing miR-150 in cardiomyocytes. Additionally, FTX overexpression repressed apoptosis in I/R-stressed heart tissue in vivo by regulating the miR-150/KLF13 axis.

In summary, we revealed the molecular mechanism of FTX for cardiomyocyte protection after I/R injury. Our results demonstrated that FTX could alleviate H2O2-induced cardiomyocyte injury by increasing KLF13 expression through interacting with miR-150. This study demonstrated novel biomarkers for the diagnosis of AMI. One limitation of this study is that H9C2 cardiomyocytes may have different pathophysiological characteristics from human cardiomyocytes, thus suggesting further investigation. The emergence of cardiomyocytes derived from human-induced pluripotent stem cells and engineered cell culture platforms may provide more suitable tools for the establishment of cardiac I/R injury models.

Conflict of interest: The authors state no conflict of interest.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.
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Appendix

Figure A1: Transfection efficiency of pcDNA-FTX, si-FTX, miR-150, and anti-miR-150 in H9C2 cells. (a) FTX level was detected in H9C2 cells transfected with pcDNA, FTX, si-con, and si-FTX. (b) MiR-150 level was measured in H9C2 cells transfected with miR-con, miR-150, anti-miR-con, and anti-miR-150. ***P < 0.001, n = 3.