MicroRNA-155-5p suppresses cardiomyocytes apoptosis induced by hypoxia/reoxygenation via targeting fos-related antigen 2

Ge Jin[1], Xue Qiang Guan[1], Jia Li[1] and Jun Ma[1]

[1]The Department of Cardiology, 2nd Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China

Correspondence to: *Jun Ma,* ***email:*** *ainannan@phpwangpan.cn*

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ABSTRACT

Myocardial ischemia/reperfusion (I/R) injury results in cardiomyocytes apoptosis and cardiac fibrosis, which accompanied with fibroblasts trans-differentiate to myofibroblasts and the deposition of collagen. Substantive researches have demonstrated that microRNAs are involved in myocardial I/R injury. Nevertheless, the behind mechanisms remain not well investigation. In this study, cardiomyocyte (M6200 cells) were exposed to hypoxia/reoxygenation (H/R). Our results implied that H/R induced M6200 cells apoptosis and increased the expression of fibrosis-associated proteins, including collagen I, collagen II, collagen III and fibronectin, as well as decreased the level of miR-155-5p. Over-expression of miR-155-5p inhibited H/R-induced apoptosis and the fibrosis of cardiomyocyte M6200 cells. Both the bioinformatics analysis and luciferase reporter assay demonstrated fos-related antigen 2 (FRA2) is one of the direct targets of miR-155-5p, and miR-155-5p negatively regulated the expression of FRA2 in M6200 cells. Moreover, knocked-down of FRA2 accelerated cell growth whereas suppressed the apoptosis and fibrosis in M6200 cells induced by H/R. Altogether, our results demonstrated that miR-155-5p restrain H/R-induced both cellular apoptosis and fibrosis of cardiomyocytes, partly via directly inhibit FRA2.

INTRODUCTION

Ischemia/reperfusion (I/R) injury is one of the mainly causes in cardiomyocyte apoptosis and cardiac fibrosis [1]. Suppression of myocardial cell apoptosis and myocardial fibrosis could effectively reduce the degree of myocardial injury caused by I/R as well as improve the survival in patients with heart failure [2]. Thus, reveal the basic molecular mechanisms are urgent for the development effective strategies of myocardial ischemia/reperfusion injury [3].

MicroRNAs is a class of non-coding RNAs, which directly bind to the 3′-UTR region of target genes and induce the degradation of target genes or inhibit the translation of target protein [4]. Substantial studies have demonstrated that miRNAs play core roles in the regulation of a variety of cellular processes, including cells growth, cellular apoptosis and fibrosis [5–7]. Previously research has suggested miR-155 to be closely associated with cardiovascular heart diseases [8, 9]. Overexpression of miR-155 in endothelial cell inhibits cells proliferation and re-endothelialization and thus increases the permeability of vascular endothelial [10, 11]. In addition, miR-155 regulates cardiac fibrosis through TGF-β1-Smad2 signaling pathway, which suggests miR-155 may be a potential therapeutic target for preventing cardiac fibrosis [12, 13]. Pharmacological inhibition of miR-155-5p by anti-miRs (antisense oligonucleotides) successfully inhibits cardiac infiltration by monocyte/macrophages, improves cardiac function and attenuates myocardial damage during viral myocarditis (VM) [14]. Altogether, these results imply that therapeutic targeting miR-155 may benefit myocarditis patients. However, the roles miR-155 in H/R-induced cardiomyocytes apoptosis and the cardiac fibrosis have not been entirely evaluated.

Fos-related antigen 2 (FRA2) is a member of Fos family, which contains various immediate-early serum-inducible genes [15]. FRA2 forms stabilized heterodimer

complexes with Jun family members, including c-Jun, Jun-B and Jun-D. Once formed, these complexes bind to activator protein 1 (AP-1) sites [16]. The phosphorylation of FRA2 increases itself DNA binding activity. Consistent with FRA1, the FRA2 lacks C-terminal trans-activating domain [17]. Previous study indicates that FRA-2 mediates oxygen-sensitive induction of transforming growth factor-β (TGF-β) in cardiac fibroblasts [18]. In addition, the level of FRA2 is up-regulation in the infarcted myocardial tissue and implicates in accelerating TGF-β transcription. Owing to these well-known transcriptional targets of FRA2 are proved to be up-regulated in the tissue fibrosis, FRA2 might function as a crucial driver of myocardial fibrosis. However, the precise molecular mechanism of FRA2 involved into myocardial I/R injury remains not well explored. The present study reveals the cellular and molecular mechanisms of miR-155-5p/FRA2 axis involved in H/R-mediated the cardiomyocytes apoptosis and myocardial fibrosis.

RESULTS

H/R induces the apoptosis of cardiomyocytes

The mouse cardiomyocyte M6200 was cultured under hypoxia condition for 24 h, followed by re-oxygenation for 1 hour. MTT proliferation analysis was performed to assess M6200 cells viability. As shown in Figure 1A, hypoxia/re-oxygenation (H/R) resulted in remarkably reduction of M6200 cells viability. Then, we hypothesized that H/R inhibited the proliferation of M6200 cells might be caused by cellular apoptosis. Thus, flow cytometry assays were subjected to analysis the apoptosis of M6200 cells after H/R treatment. As shown in Figure 1B, the apoptotic rate of M6200 cells was markedly elevated upon cells exposed to H/R. To ascertain these observations, immunoblotting and qRT-CPR assay was conducted to evaluate the level of apoptosis-associated genes. As shown in Figure 1C, the expression of Bcl-2, which is a core inhibitor of

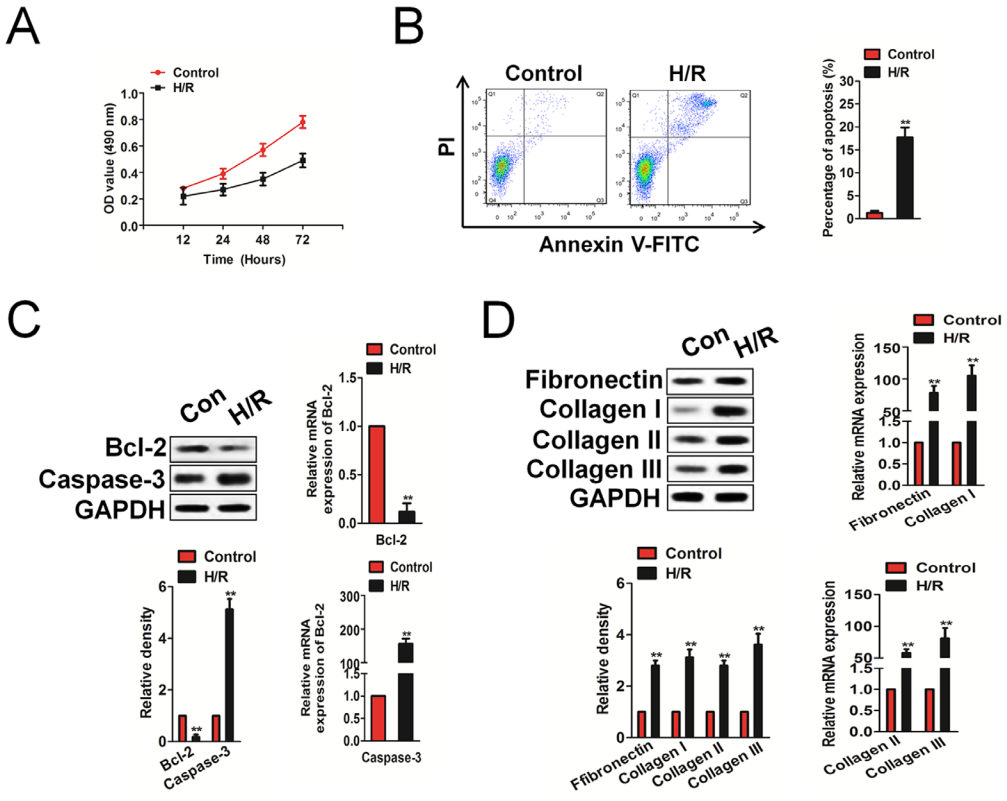

Figure 1: H/R induces the apoptosis of M6200 cells and inhibits miR-155-5p. (A) MTT analysis was conducted to assess the growth of M6200 cells treated with hypoxia/reoxygenation (H/R). **(B)** Flow cytometry analysis was performed to assess the apoptosis rate of M6200 cells exposed to H/R. **(C)** M6200 cells were exposed to with H/R. Immunoblotting and qRT-PCR analysis were subjected to evaluate the level of apoptosis-related proteins. **(D)** qRT-PCR and immunoblotting analysis of the expression of fibronectin, collagen I, collagen II and collagen III in M6200 cells that treated with H/R.

apoptosis, was significantly down-regulated, whereas caspase-3 (an inducer of cells apoptosis) was markedly up-regulated in M6200 cells that exposed to H/R. Owing to myocardial I/R damage accelerates cardiac fibrosis, we analysis the level of fibrotic-associated genes by qRT-PCR and immunoblotting assays. As shown in Figure 1D, the expression of fibronectin, collagen I, collagen II and collagen III were remarkably up-regulated in M6200 cells after exposed to H/R. Altogether, H/R induced the apoptosis and fibrosis of cardiomyocytes M6200 cells.

Over-expression of miR-155-5p inhibits H/R-induced M6200 cells apoptosis and fibrosis

Subsequently, we investigated the expression of miR-155-5p in H/R treated M6200 cells. As shown in Figure 2A, the mRNA level of miR-155-5p was markedly down-regulated after H/R treatment, as compared to control M6200 cells, which implied miR-155-5p play vital roles in myocardial I/R injury. In order to identify the potential role of miR-155-5p in H/R-treated M6200 cells, the model of miR-155-5p gain-of-function in M6200 cells was then performed. MiR-155-5p expression was markedly elevated in after M6200 cells transfected with miR-155-5p as compared to the control cells (Figure 2B). We found that the proliferation of miR-155-5p over-expressing M6200 cells that exposed to H/R treatment was higher than that of the control cells (Figure 2C). In addition, the apoptosis rate of miR-155-5p over-expressing M6200 cells that exposed to H/R was lower (Figure 2D), and accompanied with an increasing the level of Bcl-2, and the decreasing of caspase-3 (Figure 2E). Finally, the up-regulation of miR-155-5p effectively inhibited the expression of fibrosis-related genes (Figure 2F). Thus, over-expression of miR-155-5p inhibits H/R-induced M6200 cells apoptosis and fibrosis.

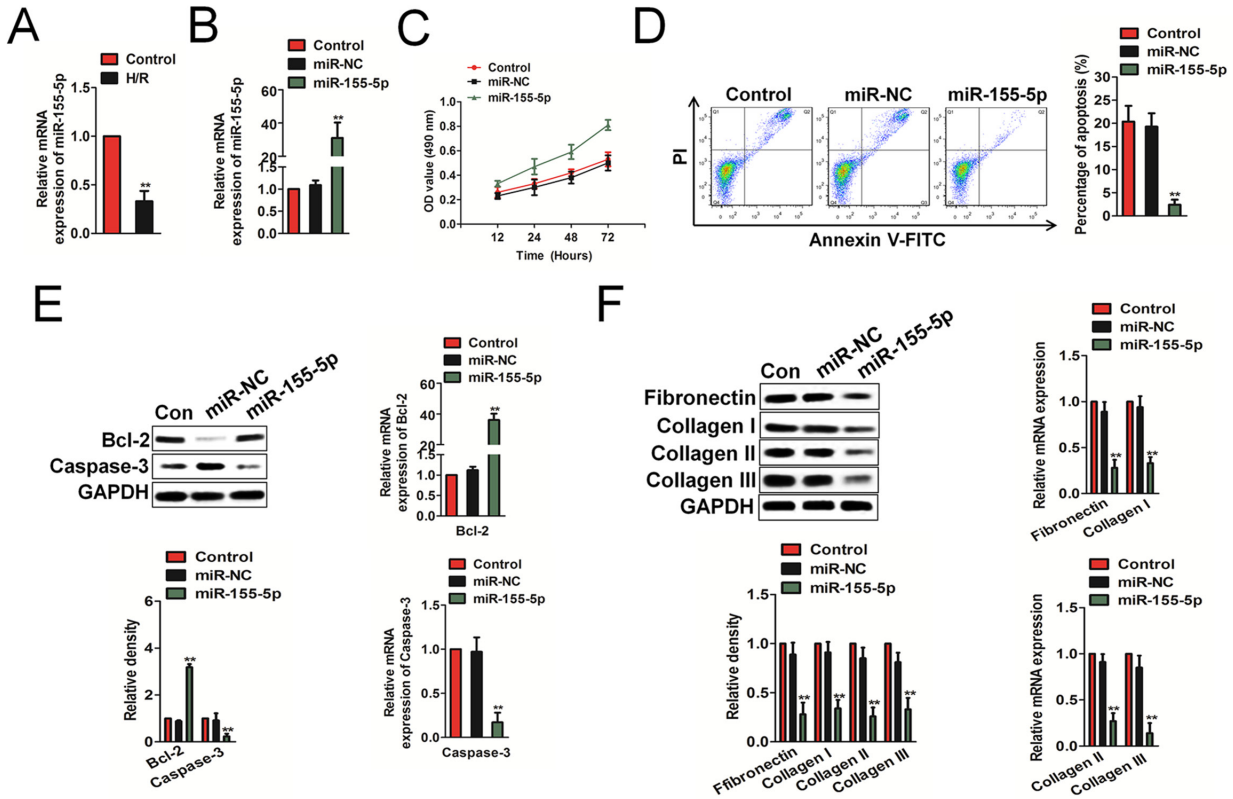

Figure 2: Overexpression of miR-155-5p rescues proliferation of M6200 cells. **(A)** M6200 cells were exposed to H/R and the mRNA level of miR-155-5p was determined by qRT-PCR. **(B)** miR-NC or miR-155-5p mimic was transfected into M6200 cells, respectively and the mRNA level of miR-155-5p was assessed by qRT-PCR. ** $p < 0.01$ compared to control. **(C)** MTT analysis was conducted to determine the growth of H/R-treated miR-155-5p over-expressing M6200 cells. **(D)** Flow cytometry analysis was performed to evaluate the apoptosis in H/R-treated miR-155-5p over-expressing M6200 cells. **(E)** qRT-PCR and western blot analysis was used to assess the expression of Bcl-2 and caspase-3. **(F)** qRT-PCR and immunoblotting analysis of the expression of fibronectin and collagen I, collagen II and collagen III in H/R-treated M6200 cells that previously transfected with miR-155-5p or miR-NC.

FRA2 is a target of miR-155-5p

Three prediction websites (Targetscan, miRDB, and PicTar) were used to identify the targets of miR-155-5p. Notably, 9 genes (ZBTB18, NFIB, RAP1B, BTF3L4, ARHGEF26, FRA2, GOLGA8A, ZFPM2, and WNT10A) were present in the databases (Figure 3A). Among these candidates, we focused on FRA2 because its expression was most significantly inhibited in cells transfected with miR-155-5p (Figure 3B). To confirm FRA2 was one of the functionally targets of miR-155-5p, the WT or MT of FRA2 3′-UTR was inserted into psiCHECK-2 vector that contained a Renilla luciferase gene (Figure 3C). The luciferase reporter assay was performed in M6200 cells that co-transfected with the FRA2-3′-UTR-psiCHECK-2 and miR-155-5p or miR-NC. As shown in Figure 3D, the luciferase activity was remarkably decreased in M6200 cells that transfected with the WT 3′-UTR of FRA2 and miR-155-5p. Nevertheless, the luciferase activity was almost unchanged in M6200 cells transfected with MT 3-′UTR of FRA2-3 and miR-155-5p. In addition, we demonstrated that miR-155-5p over-expression inhibited the FRA2 level whereas M6200 cells transfection with miR-155-5p inhibitor exhibited down-expression of FRA2 (Figure 3E). These results suggested that FRA2 was negatively mediated by miR-155-5p in M6200 cells.

Knock-down of FRA2 inhibits H/R-induced M6200 cells apoptosis and fibrosis

To elucidate whether FRA2 was involved in miR-155-5p-regulated the apoptosis and fibrosis of M6200

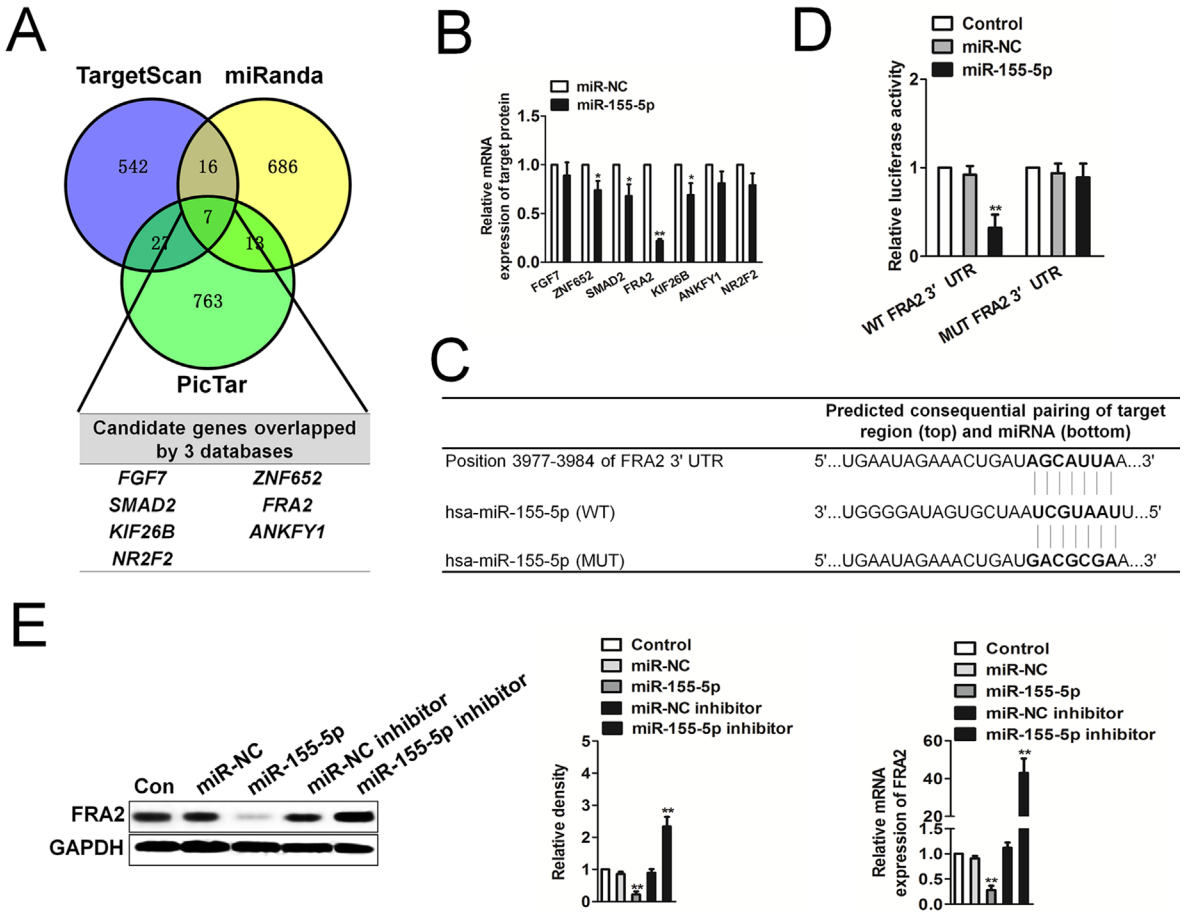

Figure 3: FRA2 is the target gene of miR-155-5p. **(A)** MiR-155-5p target genes were predicted by silico analyses. The Venn graph represented the number of candidate genes determined by three prediction algorithms. **(B)** miR-NC or miR-155-5p-3p was transfected into M6200 cells and the levels of FGF7, ZNF652, SMAD2, KIF26B, NR2F2, ANKFY1 and FRA2 were determined by qRT-PCR. Data are expressed as mean ± SD. **(C)** Schematic diagram of WT or MT of FRA2 3′-UTR inserted into psiCHECK-2 vector. **(D)** The relative luciferase activity in M6200 cells that co-transfected with miR-155b-5p and WT or MUT of FRA2-3′-UTR was analyzed by luciferase reporter assay. **(E)** miR-155-5p or miR-155-5p inhibitor was transfected into M6200 cells. The levels of FRA2 were evaluated by qRT-PCR and western blot assay. Data are expressed as mean ± SD. ** p <0.01, compared with control.

cells, M6200 cells were transfected with shRNA targeting FRA2. After transfection, the expression of FRA2 was significantly decreased as compared to control cells (Figure 4A-4B). In addition, after H/R treatment, the viability of FRA2 knock-down M6200 cells was increased, accompanied with a decrease in apoptosis rate (Figure 4C-4D). In addition, down-regulation of FRA2 inhibited the level of fibrotic-related proteins (Figure 4E). Thus, these results suggested that knock-down of FRA2 mimics the up-regulation of miR-155-5p, which inhibited H/R-induced M6200 cells apoptosis and fibrosis.

Confirmation of the role of FRA2 in H/R-induced M6200 cells apoptosis and fibrosis

To investigate whether miR-155-5p regulates the H/R-mediated apoptosis and fibrosis by targeting FRA2, we transfected miR-155-5p over-expressing M6200 cells with FRA2. Both qRT-PCR and western blot analysis showed that ectopic expression of FRA2 rescued its levels in M6200 cells that were inhibited by the miR-155-5p over-expression (Figure 5A). Both the MTT and flow cytometry analysis (Figure 5B-5C) showed that overexpression of FRA2 inhibited M6200 cells proliferation and induced cells apoptosis when compared to cells that transfected with the miR-155-5p alone. qRT-PCR and western blotting analysis showed that overexpression of FRA2 restored the expression of fibrosis-related genes in miR-155-5p over-expressing M6200 cells, as compared to that in cells transfected with miR19b-3p alone (Figure 5D-5E). In summary, these results demonstrated the important role of FRA2 in H/R-induced over-expressing M6200 cells apoptosis and fibrosis.

DISCUSSION

Myocardial I/R injury accelerate both cardiomyocyte apoptosis and cardiac fibrosis, which finally trigger heart failure [19]. Consequently, intensive investigation the potential molecular mechanisms are crucial for development effective treatment option for myocardial

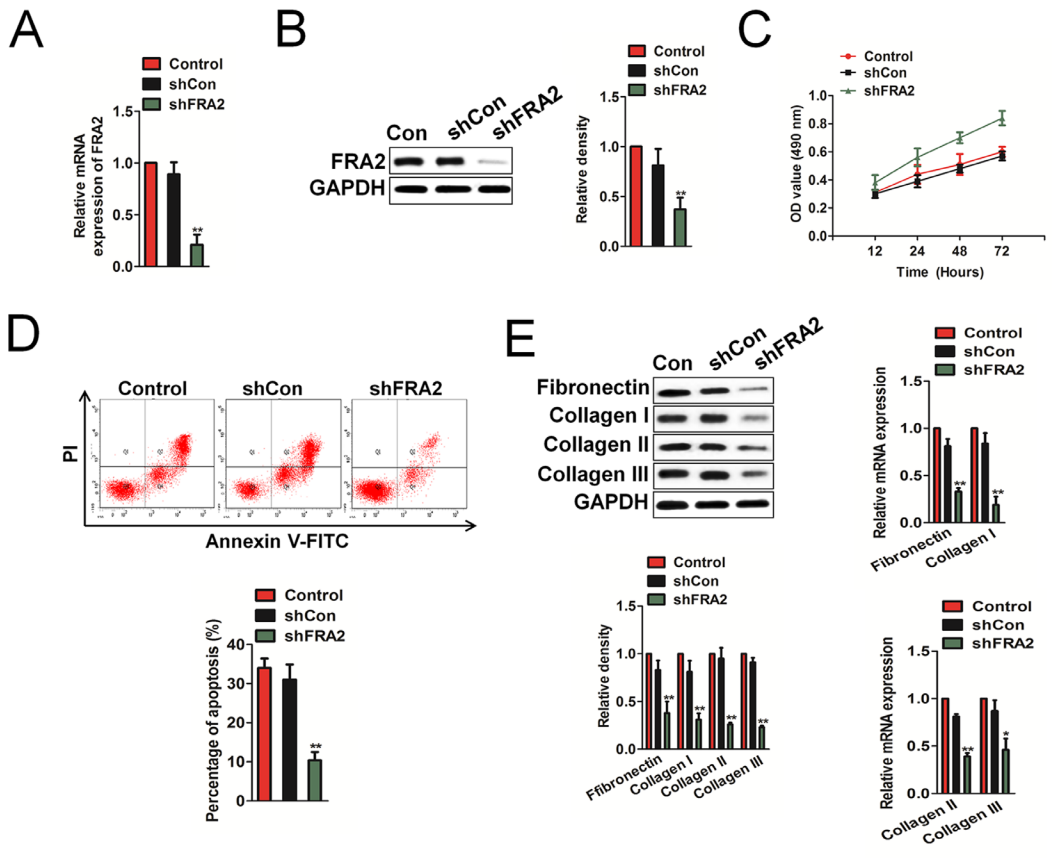

Figure 4: Down-regulation of FRA2 inhibits H/R-mediated M6200 cells apoptosis and fibrosis. (A-B) qRT-PCR and immunoblotting analysis of the expression of FRA2 in M6200 cells transfected with FRA2 shRNA (shFRA2) or negative control shRNA (shCon). **(C)** MTT assay was subjected to determine the proliferation of FRA2 knock-down M6200 cells after H/R treatment. **(D)** Flow cytometry analysis was subjected to examine the apoptosis rate of FRA2 knock-down M6200 cells after H/R treatment. **(E)** qRT-PCR and western blot assay were performed to analysis the levels of fibrosis-related proteins in FRA2 knock-down M6200 cells.

I/R injury. In the present study, we revealed the effect of miR-155-5p on H/R-mediated cardiomyocyte M6200 cells fibrosis and apoptosis. Our results suggested that cardiomyocyte M6200 cells exposed to H/R suppressed the M6200 cells growth and increased apoptosis. Meanwhile, the level of miR-155-5p was suppressed in M6200 cells treated with H/R whereas the expression of fibrosis-associated genes (fibronectin, collagen I, collagen II and collagen III) was accompanied up-regulation. Mechanically investigation revealed that miR-155-5p inhibited H/R-induced M6200 cells apoptosis and fibrosis through directly inhibiting FRA2 expression.

Substantial reporters demonstrate alter expression of miR-155 participate into tumorigenesis and tumor development [20, 21]. The level of miR-155-5p was remarkably increased in patients with clear cell renal cell carcinoma (ccRCC) and miR-155 functions as a tumor promoter by directly targeting E2F transcription factor 2 (E2F2) in ccRCC [22]. In addition, miR-155 is overexpressed in breast cancer cells and contributes to growth of cancer cells through down-regulating p53-inducible nuclear protein 1 (p53INP1) [23]. Recently, miR-155 has also been implicated in cardiovascular diseases. The level of miR-155 was elevated in the serum and atherosclerotic lesions of patients with atherosclerosis (AS), which suggest miR-155 might be a diagnostic biomarker and therapeutic target in AS. Meanwhile, circulating miR-155 was a powerful marker in detecting coronary artery disease (CAD). Noteworthy, miR-155 was also up-regulated in the plasma of patients with septic cardiac dysfunction compared

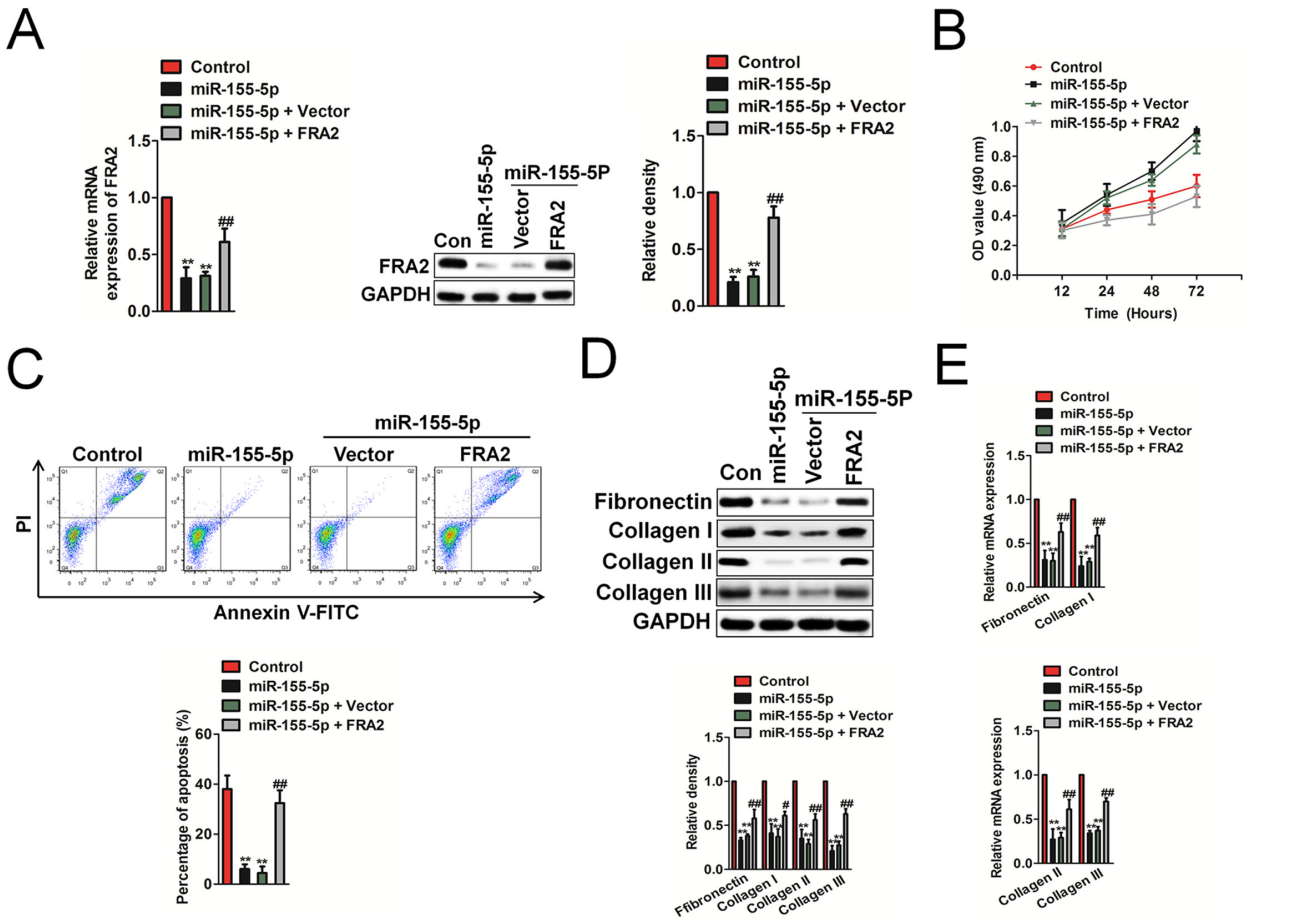

Figure 5: The role of FRA2 in the apoptosis and fibrosis of miR-155-5p over-expressing M6200 cells. (A) miR-155-5p over-expression M6200 cells were transfected FRA2. The levels of FRA2 were determined by qRT-PCR and western blot analysis. Data are expressed as mean ± SD. ** p <0.01, compared with control. $^{\#\#}$ p <0.01, compared with miR-155-5p. **(B)** miR-155-5p over-expression M6200 cells were transfected FRA2. Cell proliferation was measured by MTT assay as described. **(C)** Flow cytometry analysis was conducted to evaluate the apoptosis rate of H/R-treated M6200 cells. **(D-E)** qRT-PCR and immunoblotting analysis of the expression of fibronectin and collagen I, collagen II and collagen III in H/R-treated M6200 cells transfection with miR-155-5p alone or miR-155-5p combine with FRA2.

to the corresponding sepsis of patients without cardiac dysfunction, which indicating the potential clinical relevance of miR-155. Herein, we revealed that H/R treatment significantly decreased the level of miR-155-5p in M6200 cells and induced cells apoptosis and fibrosis. We assumed that down-regulation of miR-155-5p play a functional role in H/R-mediated M6200 cells apoptosis and fibrosis. Therefore, M6200 cells that transfected with miR-155-5p was performed to several experiments. Our data indicated that the over-expression of miR-155-5p significantly inhibited the H/R-caused M6200 cells apoptosis and fibrosis.

On account of miRNA function via negatively regulating its target genes, we investigated the potential targets of miR-155-5p utilized both bioinformatics and luciferase assay. We confirmed that FRA2 was a direct target of miR-155-5p and demonstrated FRA2 was negatively regulated by miR-155-5p. FRA2, which is a component of AP-1 transcription factor, potentiates the process of cardiomyocyte differentiation in zebra fish. Meanwhile, FRA2-containing AP-1 activity is necessary for cardiomyocyte differentiation of P19 embryonal carcinoma cells. The transcriptional targets of FRA2 positively regulate cardiomyocyte differentiation and several transcriptional targets have been demonstrated to be the crucial regulators of cardiomyocyte differentiation. Previous research has identified that FRA2 function as an O2-sensitive transcriptional regulator of inducible TGF-β expression, hypothetically positions FRA2 as an important player in reoxygenation-induced fibrosis.

In conclusion, our results demonstrate that miR-155-5p inhibits H/R-induced cardiomyocytes apoptosis and fibrosis by direct inhibiting FRA2. Over-expression of miR-155-5p protects cells from H/R induced damage and attenuates H/R induced cardiomyocytes injury. Down-regulation of FRA2 decreases in H/R-regluated myocardial apoptosis and ectopic expression of FRA2 abolishes the protection of miR-155-5p over-expressing M6200 cells that exposed to H/R. These findings provide a rationale for the development of miRNA-based strategies for the attenuation of H/R induced cardiomyocytes apoptosis and fibrosis.

MATERIALS AND METHODS

M6200 cells culture

Mouse cardiomyocyte M6200 cells were bought from CHI Scientific Inc (Jiangyin, Jinagsu, China). M6200 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplement with 10% Fetal Bovine Serum (FBS) in an incubator containing 5% CO_2 at 37°C. In H/R treatment, M6200 were exposed to hypoxic condition in a Hypoxia Modular Incubator Chamber (MIC-101, Billups Rothenberg, Inc., Del Mar, CA, USA) with 94% N2, 1% O2 and 5% CO2 at 37°C, followed by re-oxygenation for 1 hour [24].

Reverse transcription-quantitative polymerase chain reaction (qRT-PCR)

Total RNA was extracted using TRIzol reagent (Beyotime, Nnanjing, Jiangsu, China). cDNA was synthesized using 1 μg RNA with the PrimeScript RT reagent kit (TakaraBio, Tokyo, Japan). qRT-PCR was conducted with IQ™ SYBR Green supermix and iQ5 real-time detection system (Bio-Rad Laboratories, Hercules, CA). The mRNA level of target genes was calculated by $2^{(-\Delta\Delta Ct)}$ method, $^{\Delta}Ct$ = Ct (target gene) - Ct (GAPDH) and Ct value is the threshold cycle. The primers used for PCR were as follows: FRA2 sense, 5′-CCAGCGAGTACACCTACCG and antisense, 5′-TCCTTGTCCTCATAGGAGCAG-3′; Fibronectin sense, 5′-TCTGTGCCTCCTATCTATGTGC-3′ and antisense, 5′- GAGGGACCACGACAACTCTTC-3′; Bcl-2 sense, 5′-GTCTTCGCTGCGGAGATCAT-3′ and antisense, 5′-CATTCCGATATACGCTGGGAC-3′; Caspase-3 sense, 5′-GTCTTCGCTGCGGAGATCAT-3′ and antisense, 5′-CATTCCGATATACGCTGGGAC-3′; Collagen I sense, 5′-CTCTGCCTCCGACTCAACG-3′ and antisense, 5′-ACTGCATCCCTAATCCCTTGC-3′; Collagen II sense, 5′-CTTCCTACGGGGAATCTGTGT-3′ and antisense, 5′-CAATGGCGTTTTGGGTGTTC-3′; Collagen III sense, 5′-GGAGGAGTGTGACGACGGTA-3′ and antisense, 5′-CTCGCATGTCAGGTAGCCAAA-3′; and antisense, 5′-TCCTTGAGTGGAGCTTCCATT-3′; and GAPDH sense, 5′-AATGGATTTGGACGCATTGGT-3′ and antisense, 5′-TTTGCACTGGTACGTGTTGAT-3′.

Western blot analysis

Total protein from M6200 cells was prepared in RIPA lysis. 30 μg protein was separated with 10% sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto PVDF membrane (Pierce Chemical Co., Rockford, IL, USA). Then, the membranes were incubated with 5% milk at 4°C for 2 hours. Membranes were incubated with anti-caspase-3 (1:1000; Santa Cruz Biotechnology, Dallas, Texas, USA), ant-Bcl-2 (1: 1000; Santa Cruz Biotechnology, Dallas, Texas, USA), anti-fibronectin (1:1000; Santa Cruz Biotechnology, Dallas, Texas, USA), anti-collagen I (1:1000; Santa Cruz Biotechnology, Dallas, Texas, USA), anti-collagen II (1: 1000; Santa Cruz Biotechnology, Dallas, Texas, USA), anti-collagen III (1:1000; Santa Cruz Biotechnology, Dallas, Texas, USA), anti-collagen IV (1: 1000; Santa Cruz Biotechnology, Dallas, Texas, USA), anti-FRA2 (1: 1000; Santa Cruz Biotechnology, Dallas, Texas, USA) and anti-GAPDH (1: 1000; Santa Cruz Biotechnology, Dallas, Texas, USA) monoclonal antibodies (all from Abcam, Cambridge, MA, USA) at 4°C for 12 hours. Finally, PVDF membranes were incubated with goat anti-rabbit monoclonal IgG antibody (1: 10,000; Santa Cruz

Biotechnology, Dallas, Texas, USA) for 1.5 h. The PVDF was visualized by with ECL kit (Pierce).

3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide (MTT) assay

Control M6200 cells or H/R treated with M6200 cells were seeded in 96 well plates, and cultured for 12 h, 24 h, 48 h or 72 h, respectively. Then, 10 μl MTT was added into each well. After 96 well plate incubation for 4 h, 200 μl DMSO was added. After incubation for 15 min at room temperature, the optical density (OD) was assessed at 490 nm [25].

Cell apoptosis analysis

Annexin V-FITC/PI apoptosis kit (Beyotime, Nnanjing, Jiangsu, China) was used for cells apoptosis analysis. Following exposed to H/R, M6200 cells were re-suspended with 400 μl binding buffer. Then, cells were incubated with 5 μl Annexin V and 5 μl propidium iodide (PI). After 15 min, the apoptosis of M6200 cells was analyzed using a BD C6 flow cytometer (BD Biosciences, San Jose, CA, USA).

miRNA mimic and shFRA2 transfection

MiR-155-5p mimics, miR-NC, miR-NC inhibitor and miR-155-5p inhibitor (synthesized by Gene Pharma, Shanghai, China) were used for the transient gain of functional studies. Small interfering RNA duplex (shRNA) for FRA2 (named shhFRA2) (Sequence: CCGGGCGCTC TGTCATCAAGCCCATCTCGAGATGGGCTTGATGAC AGAGCGCTTTTTG) was used for FRA2 knock-down. Stable overexpression of FRA2 was carried out using the lentiviral expression system (Gene Pharma, Shanghai, China). Lipofectamine 2000 reagent (Invitrogen, USA) was used for transient transfection [26].

Bioinformatics and luciferase reporter analysis

The miRDB, Targetscan and PicTar software were selected to identify the targets of miR-155-5p. In the luciferase reporter assays, the wild type (WT) or mutant type (MT) of FRA2 3′-UTR was inserted into psiCHECK-2 vector (Promega, Madison, WI, USA) downstream of the Renilla luciferase gene (Amspring, Changsha, China). FRA2 3′-UTR-psiCHECK-2 combination with miR-155-5p or miR-NC was co-transfected into M6200 cells. Following transfection for 48 h, the luciferase activity was assessed using the luciferase reporter assay system (Promega, Madison, WI, USA) [27].

Statistical analysis

The data were presented as means ± SD. Statistical analysis was conducted with GraphPad Prism. Comparisons between groups were performed by one-way analysis. P value < 0.05 was considered as statistically difference.

Abbreviations

I/R: ischemia/reperfusion; H/R: hypoxia/reoxygenation; FRA2: fos-related antigen 2; TGF-β: transforming growth factor-β; AP-1: activator protein 1; qRT-PCR: reverse transcription-quantitative polymerase chain reaction; SDS-PAGE: sodium dodecylsulphate polyacrylamide gel electrophoresis; DMEM: Dulbecco's Modified Eagle Medium; FBS: supplement with 10% Fetal Bovine Serum.

Author contributions

Ge Jin and Xue Qiang Guan write and revise the paper. Jia Li and Jun Ma design the experimental program.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the publication of this paper.

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