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FABP4 silencing ameliorates hypoxia reoxygenation injury through the attenuation of endoplasmic reticulum stress-mediated apoptosis by activating PI3K/Akt pathway

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ABSTRACT

Endoplasmic reticulum (ER) stress and subsequent apoptosis play a vital role in myocardial ischemia reperfusion (IR) injury. Fatty acid binding protein 4 (FABP4) may induce ER stress. The aim of this study was to investigate the mechanism and effect of FABP4 on IR injury *in vitro*. Rat H9c2 cells were exposed to hypoxia reoxygenation (HR) to create an IR model *in vitro*. FABP4 was overexpressed in HR-injured H9c2 cells. Transfection with FABP4 siRNA increased cell viability and decreased LDH upon HR stimulation. FABP4 cessation also suppressed apoptotic cells and caspase-3 activity after HR. Downregulation of FABP4 significantly inhibited ER stress by decreasing the protein expression of p-PERK, GRP78, and ATF6. FABP4 silencing also restrained the ER stress-mediated apoptotic pathway, as indicated by decreased pro-apoptotic proteins p-JNK, CHOP, Bax, and caspase-12, as well as upregulation of Bcl-2 during HR. Furthermore, FABP4 silencing activated the PI3K/Akt pathway. Blocking this pathway by the specific PI3K inhibitor-LY294002 restored HR-induced ER stress and subsequently reversed the protective effect of FABP4 silencing on HR injury. Taken together, our findings revealed that FABP4 silencing exerts protective effects against HR injury in H9c2 cells through inhibiting ER stress-induced cell apoptosis *via* activation of the PI3K/Akt pathway.

1. Introduction

Ischemic heart disease is a leading cause of death worldwide. Currently, the most effective therapy for ischemic heart disease is thrombolytic therapy, percutaneous coronary intervention, and coronary artery bypass grafting. Myocardial ischemic reperfusion (IR) injury is the most common complication of percutaneous coronary intervention or intravenous thrombolytic therapy that occurs in patients with acute myocardial infarction [6,42]. The restoration of blood supply of the ischemic myocardium and associated damages, such as destruction of myocardial tissue and the aggravated myocardial damage, are life-threatening for patients with myocardial infarction [9,23]. Despite various therapies, there is no effective prevention or treatment of myocardial IR injury. Therefore, it is important to explore its underlying mechanism [16].

Cardiac IR injury is a complicated pathological process, and its mechanism is not fully understood [8]. Previous evidence has proved that myocardial apoptosis plays an important role in the pathophysiology of myocardial IR injury [45]. Recent studies have shown that excessive endoplasmic reticulum (ER) stress can activate apoptotic signal transduction pathway and ultimately induces apoptosis [3]. Other studies have confirmed that apoptosis mediated by ER stress is closely related to the occurrence of myocardial IR injury [37,39]. During IR, ischemia, hypoxia, massive oxygen free radicals, calcium overload, and other factors destroy ER homeostasis and induce ER stress response. Studies have shown that ER stress is an important pathological mechanism of IR; thus, inhibiting ER and subsequent apoptosis can effectively reduce myocardial cell injury [34].

Fatty acid binding proteins (FABPs) are a low-molecular-weight (14–15 K) protein in cytoplasm. FABPs can reversibly bind to free fatty acids and other hydrophobic ligands, such as cholesterol, arachidonic acid, and sulfonic acid, then transport them to various target locations, such as the endoplasmic reticulum, mitochondria, peroxide enzyme proliferation body, and nucleus *etc*. [11]. Fatty acid binding protein-4 (FABP4), a member of the FABP family, is located in the human 8q21 chromosome region. FABP4 encodes 132 amino acids, and it was first

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detected in adipocyte [33] and adipose [18] tissues. It is mainly expressed in macrophages [24] and adipocytes [4]. FABP4 also has been shown to be associated with multiple diseases, such as inflammation [14], obesity [32], diabetes [7], insulin resistance [28], atherosclerosis and cardiovascular diseases [12].

Recently, it also has been reported that FABP4 plays a vital role in multiple injuries. Zhang et al. reported that FABP4 overexpression in cardiomyocytes aggravated cardiac hypertrophy by activating the ERK signaling pathway [44]. FABP4 decreases the contractility of myocardial muscle cells [25]. Ectopic FABP4 expression in the vascular endothelium contributes to neointima formation after vascular injury [13]. FABP4 also regulates fatty infiltration after rotator cuff tear [26]. Moreover, IR significantly induces expression of FABP4 in the liver [17]. However, whether FABP4 plays a role in myocardial IR injury has never been investigated.

Therefore, the present study investigated the effect of FABP4 on cardiomyocyte HR injury by exposing H9c2 cells to hypoxia reoxygenation (HR) to mimic myocardial IR injury.

2. Materials and methods

2.1. Cell culture

Rat H9c2 cardiac cells obtained from Cell Bank of the Chinese Academy of Sciences (Shanghai, China) were cultured in Dulbecco's Modified Eagle Medium (Life Technologies, Carlsbad, CA, USA) containing 10% fetal bovine serum with 5% CO_2 at 37 °C.

2.2. HR induction

To create the HR cell model, H9c2 cells were maintained in an environment according to a previous method [20], H9C2 cells were incubated in a hypoxia chamber with 5% CO_2 and 95% N_2 at 37 °C for 6 h. Then, the cells were reoxygenated under 95% air and 5% CO_2 normoxic conditions at 37 °C for different durations (0 h, 2 h, 4 h, 8 h, and 16 h).

2.3. CCK-8 assay

The CCK-8 assay was conducted to assess cell viability. H9c2 cells were maintained in 96-well plates (2×10^3 cells/well). After HR treatment, CCK-8 reagents (10 μL/well; Beyotime Institute of Biotechnology, Jiangsu, China) were added. The absorbance was measured with a microplate reader (Bio-Rad, Hercules, CA, USA) at 450 nm.

2.4. Cell transfection

When H9c2 cells in 6-well plates grew to about 80%, the medium was replaced by the serum-free medium. The cells were then transfected with FABP4 siRNA-1 (siFABP4-1), FABP4 siRNA-2 (siFABP4-2), or nonspecific siRNA (siNC-1, siNC-2) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

2.5. LDH activity measurement

H9c2 cells were cultured in 6-well plates, and the supernatants were collected. LDH activity in the cultured supernatant was determined by using an LDH assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) with a microplate reader at 450 nm.

2.6. Caspase-3 activity measurement

Caspase-3 activity was measured by using a caspase-3 assay kit (Beyotime Institute of Biotechnology). In brief, H9c2 cells were lysed in ice-cold lysis buffer, and the supernatant was collected after centrifugation at 4 °C for 10 min at 12,000 rpm. Then, 10 μL of Ac-DEVD-pNA (2 mM) was added to the mixture of buffer solution, supernatant, and lysis buffer. After 2 h of incubation at 37 °C, the optical density was detected with a spectrophotometer (Beckman Coulter DU-800, Fullerton, CA, USA) at 405 nm.

2.7. Flow cytometry

The apoptosis of H9c2 cells was assessed by flow cytometry using stained with Annexin V-FITC/propidium iodide (PI) kit (Sigma, St. Louis, MO, USA). In brief, after washed with PBS and resuspended with the kit binding buffer, cells were incubated with 10 μL Annexin V-FITC and 5 μL PI for 15 min in dark. The fluorescence was detected using a flow cytometer (Becton Dickinson, San Jose, CA, USA).

2.8. Quantitative real-time PCR

Total RNA of H9c2 cells was extracted with TRIzol (Invitrogen), and aliquots of RNA (1 μg) was reverse-transcribed to cDNA using a PrimeScriptTM RT reagent Kit (TaKaRa, Dalian, China). The primers used were FABP4 forward 5′-TCCCTACTTGTGTGGCGTGAA-3′ and reverse 5′-TCACCCGAGTGGTAGTCACAATG-3′ and internal control β-actin forward 5′-GACTACCTCATGAAGATCCT-3′ and reverse 5′-CCAC ATCTGCTGGAAGGTGG-3′. The qPCR analysis was performed using a SYBR Premix Ex TaqTM II (TaKaRa). The relative mRNA level was quantified by using the $2^{-\Delta\Delta Ct}$ method.

2.9. Western blot

After washing with PBS, cells were lysed in lysed buffer on ice for 30 min and then centrifuged (12,000 rpm/min) for 10 min at 4 °C. Subsequently, bicinchoninic acid assays (Pierce, Rockford, IL, USA) were performed to determine protein concentrations. Equal amounts (20 μg) of proteins were separated by SDS-PAGE, transferred to a PVDF membrane (Bio-Rad), and blocked in 5% non-fat milk for 1 h, followed by incubation with primary antibodies at 4 °C overnight. The primary antibodies were rat anti-rabbit FABP4 (1:1000; ab92501; Abcam, Cambridge, UK), glucose-regulated protein 78 (GRP78) (1:1000; ab108613; Abcam), transcription factor C/EBP homologous protein (CHOP) (1:250; ab10444; Abcam), p-Akt1 (phospho S473) (1:5000; ab81283; Abcam), p-Akt (phospho T308) (1:500; ab38449; Abcam), Akt (1:500; ab8805; Abcam), phosphoinositide 3-kinase (PI3K) p85 alpha (1:1000; ab40755; Abcam), p-PI3K p85 (1:500; ab182651; Abcam), protein kinase RNA-like endoplasmic reticulum kinase (PERK) (1:1000; 3192; Cell Signaling Technology, Inc., Danvers, MA, USA), p-PERK (Phospho-PERK (Thr980) (1:1000; 3179; Cell Signaling Technology), c-Jun N-terminal kinase (JNK) (1:1000; sc-572; Santa Cruz Biotechnology), p-JNK (1:500; sc-135642; Santa Cruz Biotechnology), Bcl-2 (1:1000; sc-783; Santa Cruz Biotechnology), Bax (1:250, Sc6236; Santa Cruz Biotechnology), caspase-12 (1:75; sc-5627; Santa Cruz Biotechnology), activating transcription factor 6-α (ATF6-α) (1:1000; sc-22799, Santa Cruz Biotechnology), and β-actin (1:000; ab8227; Abcam). After that, the membranes were incubated with a secondary antibody at room temperature for 1 h. Bands were visualized by enhanced chemiluminescence detection and analyzed with Image-Pro Plus 6.0 software.

2.10. Statistical analysis

All experiments were repeated at least three times. Data are presented as mean ± SD and analyzed by one-way analysis of variance followed by Dunnett's test among multiple groups and a two-tailed *t*-test between two groups using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). Differences were considered significant if $P < 0.05$.

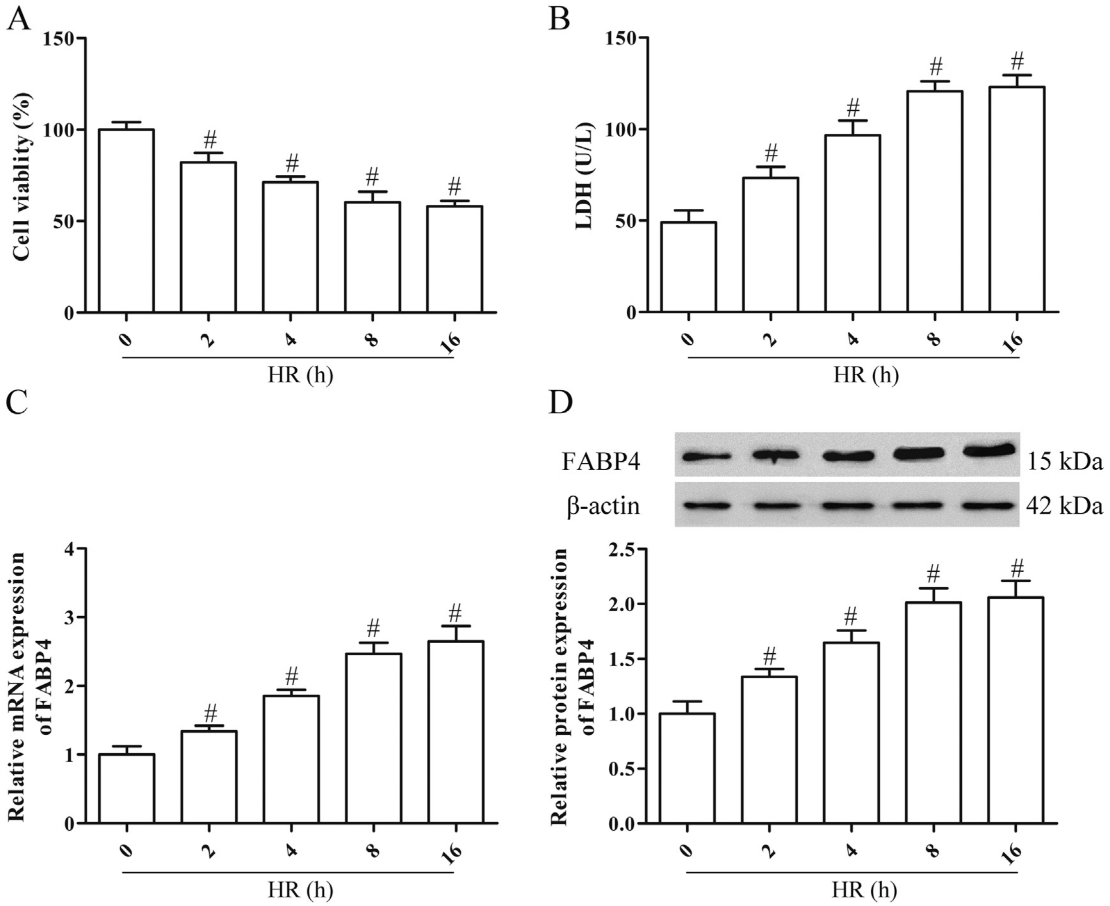

Fig. 1. Upregulation of FABP4 in H9c2 cells after stimulation with HR. (A) Cell viability of H9c2 cells after HR detected by CCK-8. (B) LDH release in culture medium after HR. (C) Detection of FABP4 mRNA expression by qPCR in H9c2 cells after HR induction. (D) Determination of FABP4 protein level by Western blot in H9c2 cells after HR treatment. $^{\#}P < 0.05$ *vs* control.

3. Results

3.1. FABP4 was overexpressed in H9c2 cells under HR condition

To investigate whether FABP4 participates in IR injury, H9c2 cells were induced with hypoxia for 6 h and re-oxygenated at 2 h, 4 h, 8 h, and 16 h to mimic myocardial IR. The cells that underwent reoxygenation for 2 h, 4 h, 8 h, or 16 h showed decreased cell viability (Fig. 1A) and increased LDH release (Fig. 1B) compared to the cells that underwent hypoxia alone (control group) ($P < 0.05$). These demonstrated that HR caused severe cell damage. Moreover, a dramatic upregulation of FABP4 mRNA and protein expression was observed in HR treated H9c2 cells ($P < 0.05$; Fig. 1C and D) with prolonged reoxygenation times. No differences in cell viability or LDH release were observed for reoxygenation times of 8 h and 16 h. Thus, reoxygenation for 8 h was selected for the following experiments.

3.2. FABP4 silencing alleviated injury caused by HR in H9c2 cells

To explore the effect of FABP4 on HR injury in H9c2 cells, we knocked down FABP4 expression *via* siRNA transfection. As shown in Fig. 2A and B, mRNA and protein of FABP4 were notably downregulated after siFABP4-1 or siFABP4-2 transfection ($P < 0.05$), especially in the siFABP4-2 group. Therefore, siFABP-2 was used for subsequent experiments. SiFABP4-2 significantly inhibited the upregulation of FABP4 protein expression caused by HR ($P < 0.05$; Fig. 2C). The CCK-8 results showed that decreased cell viability induced by HR was significantly increased by siFABP-2 ($P < 0.05$; Fig. 2D). Compared with the HR group, FABP4 silencing markedly reduced LDH activity ($P < 0.05$; Fig. 2E).

3.3. FABP4 silencing attenuated apoptosis elicited by HR in H9c2 cells

Then we investigate the effect of FABP4 on the apoptosis of H9c2 cardiomyocytes after HR. H9c2 cells exposed to HR displayed a profound increase in apoptotic cells, compared with untreated cells (Fig. 3A and B). Compared with HR-treated cells, FABP4 downregulation resulted in a decrease in apoptotic cells ($P < 0.05$; Fig. 3A and B). To further confirm the anti-apoptotic effect of siFABP4, caspase-3 activity in H9c2 cells following HR injury was detected. The results showed that, compared with the HR group, caspase-3 activity in siFABP4 group was significantly decreased ($P < 0.05$; Fig. 3C), indicating that siFABP4 significantly reduced activation of caspase-dependent pathways.

3.4. Downregulation of FABP4 inhibited ER stress–apoptotic pathway during HR

ER stress and the induced apoptosis are associated with myocardial injury during IR [38]. To investigate the effect of FABP4 silencing on ER stress, the ER-stress-related markers were examined. As shown in Fig. 4A–C, HR treatment activated ER stress, as indicated by increases in PERK, GRP78, and ATF6 protein levels. After transfection with siFABP4, expression of p-PERK, GRP78, and ATF6 was significantly attenuated ($P < 0.05$; Fig. 4A–C), demonstrating that FABP4 silencing appears to inhibit HR-induced ER stress.

To further investigate the effect of siFABP4 on ER stress-induced apoptosis during HR injury, we measured ER-stress-associated

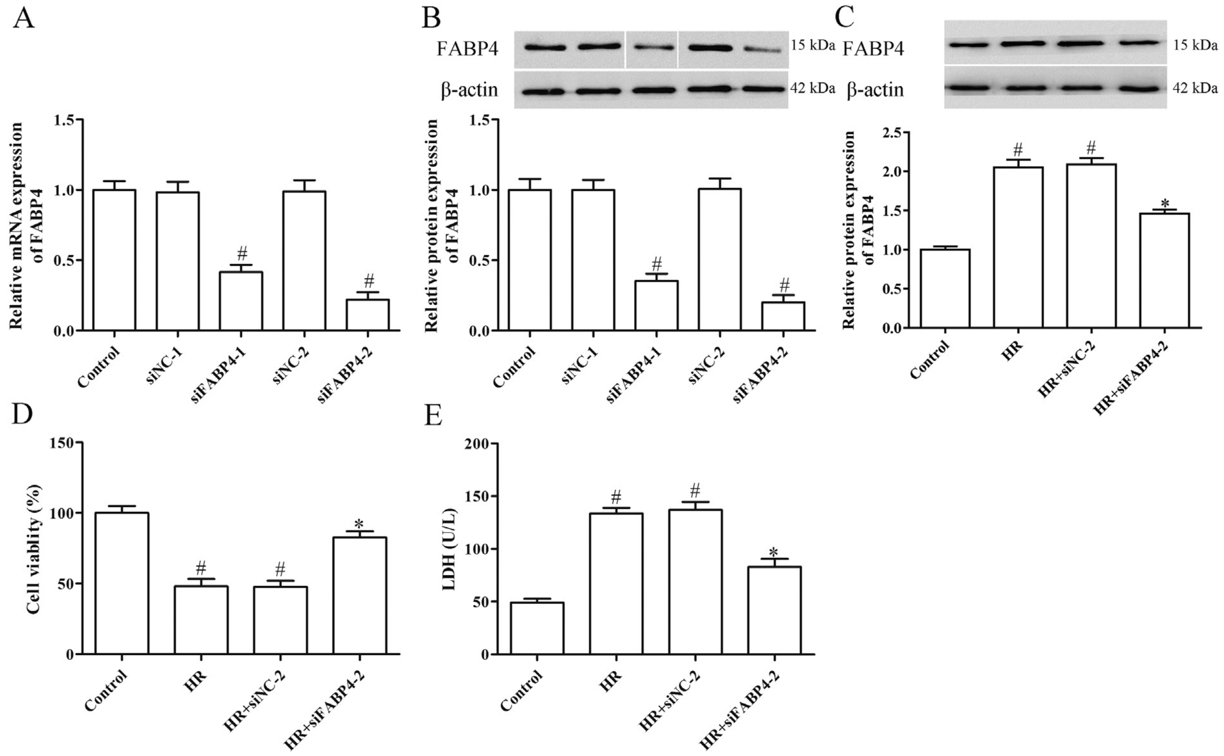

Fig. 2. FABP4 silencing protects against myocardial cell injury caused by HR. The (A) mRNA and (B) protein level of FABP4 in H9c2 cells after FABP4 siRNA transfection. (C) The protein expression of FABP4 in HR treated H9c2 cells. (D) Cells were transfected with FABP4 siRNA followed by hypoxia for 6 h and reoxygenation for 12 h. (E) FABP4 silencing increased HR decreased cell viability. FABP4 silencing decreased LDH release induced by HR. *N* = 6. $^{\#}P < 0.05$ *vs* control, $^{*}P < 0.05$ *vs* HR.

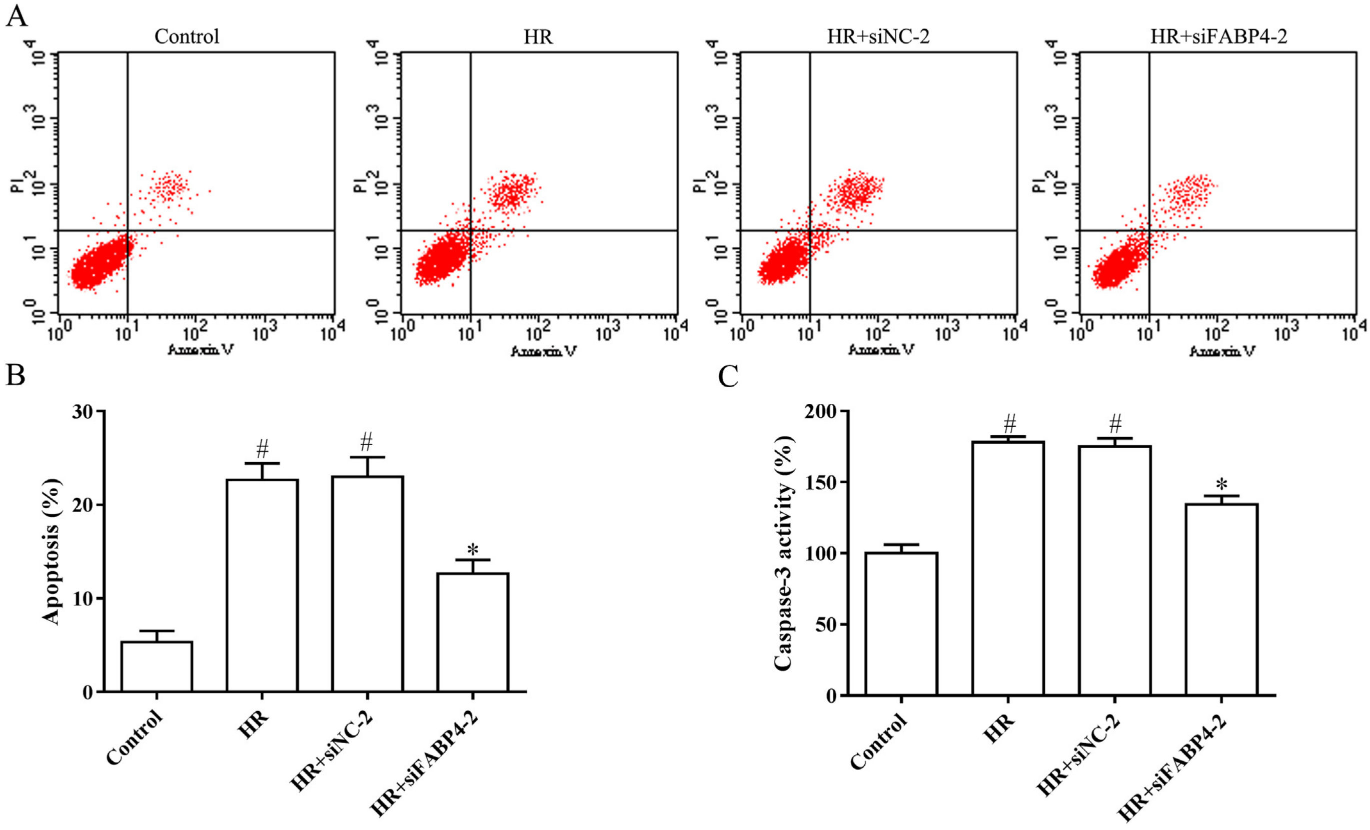

Fig. 3. Downregulation of FABP4 alleviated H9c2 cell apoptosis induced by HR. (A) Flow cytometry analysis of apoptotic H9c2 cells. (B) Quantitative analysis of apoptotic H9c2 cells. Cells in the upper-right quadrant (represent late apoptotic cells) and lower-right quadrant (represent early apoptotic cells) were quantificated. (C) Measurement of caspase-3 activity. N = 6. $^{\#}P < 0.05$ *vs* control, $^{*}P < 0.05$ *vs* HR.

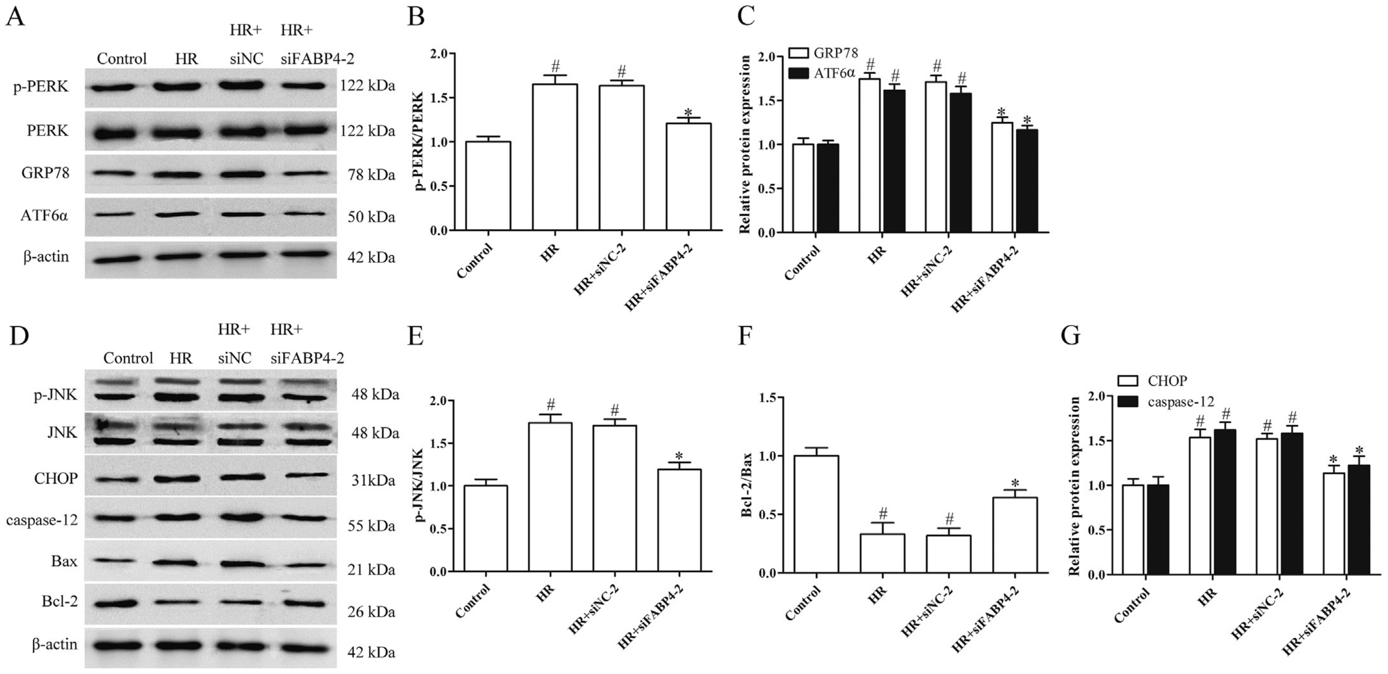

Fig. 4. Effects of FABP4 silencing on ER stress and mediation of apoptosis in H9c2 cells with HR. (A) Protein expression of p-PERK, PERK, GRP78, and ATF6α was detected by Western blot. (B) The normalized ratio of p-PERK to PERK. (C) The normalized ratio of GRP78 and ATF6α to β-actin. (D) Western blot analysis of p-JNK, JNK, CHOP, Bcl-2, Bax, and caspase-12. Quantitative analysis of (E) p-JNK to JNK, (F) Bcl-2 to Bax, and (G) CHOP and caspase-12 to β-actin. N = 6. $^{\#}P < 0.05$ *vs* control, $^{*}P < 0.05$ *vs* HR.

apoptosis proteins p-JNK, JNK, CHOP, Bcl-2, Bax, and caspase-12. Western blot results showed that, following HR injury, protein expression of p-JNK, CHOP, Bax, and caspase-12 increased and that expression of Bcl-2 decreased ($P < 0.05$; Fig. 4D–G). Downregulation of FABP4 in H9c2 cells evidently counteracted the effects of HR ($P < 0.05$; Fig. 4D–G), suggesting that downregulation of FABP4 also suppressed ER stress-mediated apoptosis during HR.

FABP4 silencing protected cardiomyocytes against HR injury partly through the attenuation of ER stress-induced apoptosis *via* the activation of the PI3K/Akt pathways.

The PI3K/Akt pathway regulates cell survival in myocardial IR injury [35]. To determine whether the cardioprotective effect of FABP4 silencing is related to PI3K/Akt signal pathway, the protein expression of PI3K/Akt pathway was detected by Western blot. Expression of p-PI3K and p-Akt was elevated markedly in the siFABP4 group, compared with the HR group ($P < 0.05$; Fig. 5A–C), indicating that siFABP4 activated the PI3K/Akt signal pathway.

To determine whether PI3K/Akt participated in the protective effect of FABP4 silencing against the ER-stress-mediated apoptotic pathway, the PI3K inhibitor LY294002 was used to block the PI3K/Akt pathway. After HR treatment, LY294002 inhibited activation of PI3K/Akt by siFABP4 (Fig. 5A–D). Moreover, LY294002 treatment partially abolished the inhibitory effect of siFABP4 on ER stress induced apoptosis of H9c2 cells during HR, as indicated by suppressing the decreased expression of p-JNK, CHOP, Bax, caspase-12 and the increased expression of PERK, GRP78, ATF6 and Bcl-2 induced by siFABP4 ($P < 0.05$; Fig. 5E–I). LY294002 also partially counteracted the effects of siFABP4 on cell viability (Fig. 5J), LDH release (Fig. 5K), and apoptosis (Fig. 5L) during HR ($P < 0.05$). Taken together, these results indicate that the PI3K/Akt signaling pathway was participates in the cardioprotective effect of FABP4 silencing against HR injury by regulating the ER stress process.

4. Discussion

Ischemic heart disease is a serious threat to human health. Although restoring myocardial blood supply is important in ischemic heart disease treatment, myocardial reperfusion after myocardial ischemia aggravates myocardial injury. Therefore, it is important to explore the underlying mechanism of IR progression and find new treatment strategies to protect cardiomyocytes from myocardial IR injury. In this study, FABP4 was found to increase in a time-dependent manner in H9c2 following HR treatment. These findings are consistent with the previous report in liver [17]. FABP4 inhibition increased cell viability and decreased LDH release and apoptotic cells. Importantly, downregulation of FABP4 restrained the ER stress response and resulting apoptosis. Furthermore, we demonstrated that PI3K/Akt activation by FABP4 silencing regulates ER-stress-induced apoptosis in H9c2 cells during HR.

Cardiac muscle cells under myocardial IR injury show excessive ER stress. IR injury can induce cardiomyocyte apoptosis by triggering ER stress [2,34]. Therefore, inhibition of ER stress has become a major strategy for preventing and treating myocardial IR injury. ER homeostasis disorder activates the unfolded protein response (UPR), which can reduce the burden on ER and maintain normal cells by regulating ER protein synthesis, processing, and transportation. UPR signal system includes three main responses: inositol-requiring kinase/endoribonuclease 1 (IRE1), ATF6, and PERK pathways. Upon ER stress, GRP78, which is a regulator of ER stability, dissociates from IRE1, ATF6, and PERK and activates them to trigger the UPR. Activated IRE1 pathway splices introns of 26-bp nucleotide from the X-box-binding protein-1 (XBP-1) mRNA and change the open reading frame of XBP-1 leading to the expression of a spliced XBP1 (sXPB1). Its translation product XBP1 can induce ER related degradation gene expression and regulate the degradation of unfolded and misfolded proteins in ER, thereby reducing ER pressure and restoring homeostasis of the intracellular environment. IRE1-XBP1 pathway seems to promote survival by activating ER chaperones transcription. However, sustained ER stress accompanied by adaptive response failure may eventually lead to apoptosis. Activated PERK phosphorylates eukaryotic translation initiation factor 2 subunit α (eIF2α), leading to suppression of global protein synthesis. Activated ATF6 can regulate the function of ER and

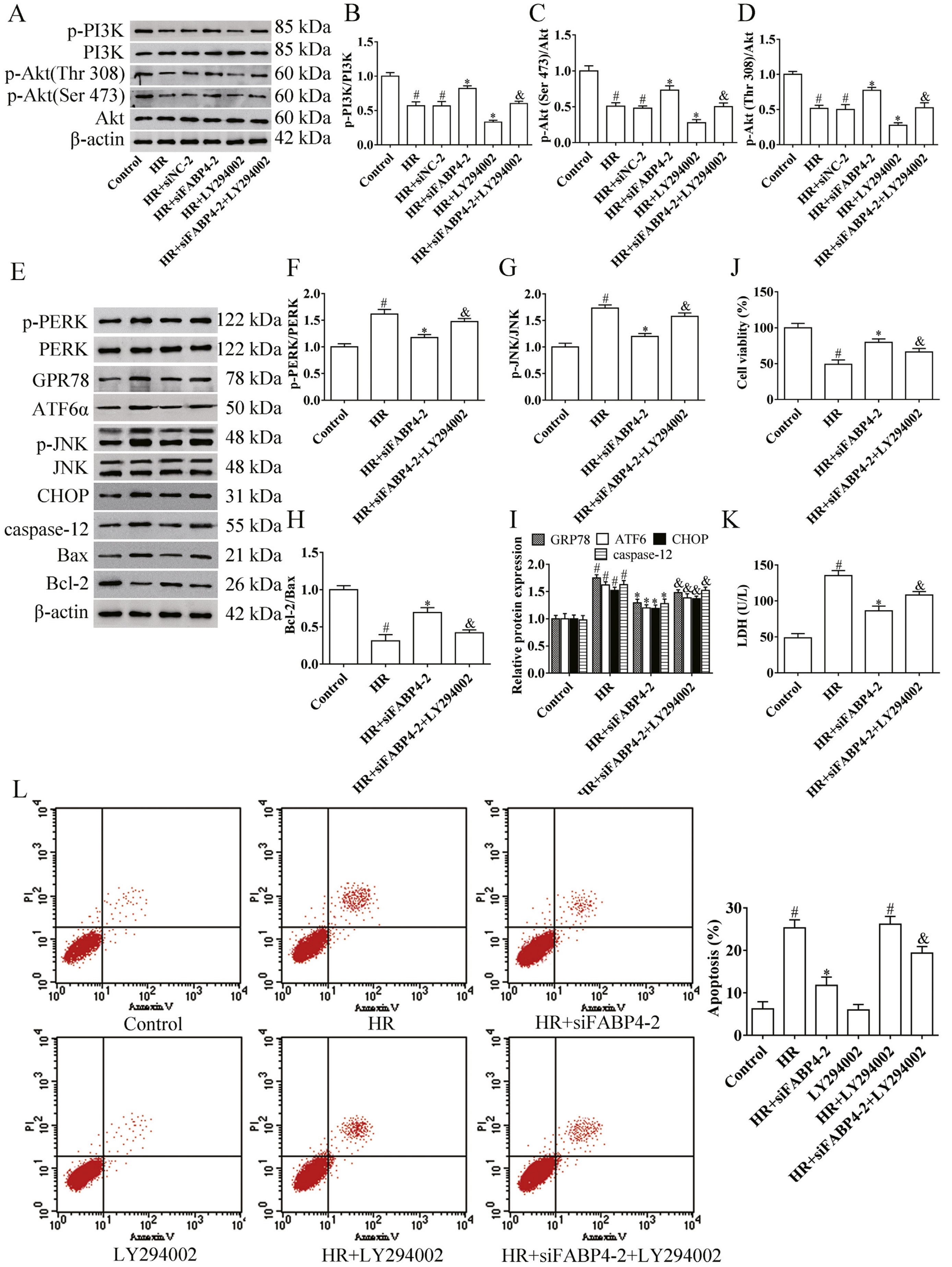
A
p-PI3K 85 kDa
PI3K 85 kDa
p-Akt(Thr 308) 60 kDa
p-Akt(Ser 473) 60 kDa
Akt 60 kDa
β-actin 42 kDa
Control
HR
HR+siNC-2
HR+siFABP4-2
HR+LY294002
HR+siFABP4-2+LY294002
B
p-PI3K/PI3K
C
p-Akt (Ser 473)/Akt
D
p-Akt (Thr 308)/Akt
E
p-PERK 122 kDa
PERK 122 kDa
GPR78 78 kDa
ATF6α 50 kDa
p-JNK 48 kDa
JNK 48 kDa
CHOP 31 kDa
caspase-12 55 kDa
Bax 21 kDa
Bcl-2 26 kDa
β-actin 42 kDa
F
p-PERK/PERK
G
p-JNK/JNK
J
Cell viability (%)
H
Bcl-2/Bax
I
GRP78
ATF6
CHOP
caspase-12
Relative protein expression
K
LDH (U/L)
L
PI
Annexin V
Control
HR
HR+siFABP4-2
LY294002
HR+LY294002
HR+siFABP4-2+LY294002
Apoptosis (%)
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Fig. 5. FABP4 silencing exerts inhibitory effect on ER stress induced apoptosis by activating PI3K/Akt pathway in H9c2 cells with HR. Cells were pretreated with PI3K inhibitor LY294002 (20 μM) for 30 min and then exposed to HR stimulation. (A) Western blot analysis of the PI3K/Akt pathway. The normalized ratio of (B) p-PI3K to PI3K, (C) p-Akt (Ser 473)/Akt, and (D) p-Akt (Thr 308)/Akt. (E) The protein expression of p-PERK, PERK, GRP78, ATF6α, p-JNK, JNK, CHOP, Bax, caspase-12, and Bcl-2. The normalized ratio of (F) p-PERK to PERK; (G) p-JNK to JNK; (H) Bcl-2 to Bax; and (I) CHOP, caspase-12, GRP78, and ATF6α to β-actin. LY294002 reversed the effect of siFABP4 on (J) cell viability, (K) LDH release, and (L) cell apoptosis. N = 6. $^{\#}P < 0.05$ *vs* control, $^{*}P < 0.05$ *vs* HR, $^{\&}P < 0.05$ *vs* HR + siFABP4.

induce the expression of downstream genes, protecting cells from chronic stress damage. ATF6α, an integral membrane protein, is converted to a cleaved transcription factor, resulting in nuclear translocation. Previous studies showed that ATF6 activation has a protective effect on IR damage in mice [21]. Increased GRP78 expression often indicates ER stress activation and UPR [15]. During IR injury, the expression of ER stress protein markers GRP78, PERK, and ATF6 are increased [29,43]. In our results, the protein levels of GRP78, PERK, and ATF6 were elevated in HR-treated H9c2 cells. Some studies confirm the critical role of FABP4 in mediating ER stress. Studies from Bosquet et al. show that exogenous FABP4 induced ER stress in HepG2 liver cells [5]. FABP4 also mediates apoptosis *via* ER stress in mesangial cells of diabetic nephropathy [41]. We found that FABP4 silencing inhibited the excessive ER response during HR in myocardial cells. Persistent or excessive ER stress can activate the related apoptotic signals to accelerate abnormal apoptosis of myocardial cells [40]. Activation of caspase-12 and expression of CHOP are two major downstream molecules that mediate apoptosis during persistent ER stress. When caspase-12 is activated during ER stress, it can activate the ER-related, cascading, apoptotic signaling pathway, including caspase-3 signaling [31]. CHOP is an important apoptosis-specific transcription factor of the ER stress response [27]. During ER stress, the apoptosis signaling pathways of ER-IRE1, ATF6, and PERK can induce CHOP transcription and expression, which then translocate to the nucleus to accelerate apoptosis by regulating the balance between the anti-apoptosis protein Bcl-2 and pro-apoptosis Bax [10,19].We found that FABP4 silencing reduced the protein expression of p-JNK, CHOP, and caspase-12. However, we also found that it modulated the balance of Bcl-2 and Bax during HR. These results indicate that downregulation of FABP4 effectively inhibited ER stress-mediated apoptotic pathways to protect myocardial cells from HR injury.

The PI3K/Akt signaling pathway plays an essential role in promoting cell survival after IR injury [22]. PI3K activates its downstream protein Akt at T308 by PDK1 and at S473 by mTORC2 complex. Previous evidence indicates that the PI3K/Akt signaling pathway regulates ER stress and ER-stress-related apoptotic pathways [38]. PI3K/Akt pathway activation is a useful strategy for regulating ER-stress-induced apoptosis during HR [36,38]. FABP4 could inhibit activation of Akt [1,30]. In this study, we demonstrated that FABP4 silencing activated the PI3K/Akt signaling pathway during myocardial HR injury. To further assess this pathway's involvement in the protective role of FABP4 silencing, H9c2 cells were pretreated with the PI3K/Akt inhibitor LY294002. The protective effect of FABP4 silencing on ER-stress-mediated cell apoptosis during HR was counteracted by LY294002. These results demonstrated that FABP4 silencing alleviated HR injury by inhibiting ER-stress-induced cell apoptosis *via* activation of the PI3K/Akt pathway.

In conclusion, we demonstrated the significant protective effect of FABP4 silencing against HR injury in H9c2 cells. FABP4 silencing reduced ER-stress-mediated apoptosis *via* activation of PI3K/Akt signaling pathways. Our study elucidated that FABP4 may act as a therapeutic target against IR injury.

Conflict of interest

The authors declare there are no conflicts of interest.

Acknowledgement

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