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PI3K/Akt/FoxO3a signaling mediates cardioprotection of FGF-2 against hydrogen peroxide-induced apoptosis in H9c2 cells

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Abstract Cardiovascular disease is a growing major global public health problem. Oxidative stress is regarded as one of the key regulators of pathological physiology, which eventually leads to cardiovascular disease. However, mechanisms by which FGF-2 rescues cells from oxidative stress damage in cardiovascular disease is not fully elucidated. Herein this study was designed to investigate the protective effects of FGF-2 in H_2O_2-induced apoptosis of H9c2 cardiomyocytes, as well as the possible signaling pathway involved. Apoptosis of H9c2 cardiomyocytes was induced by H_2O_2 and assessed using methyl thiazolyl tetrazolium assay, Hoechst, and TUNEL staining. Cells were pretreated with PI3K/Akt inhibitor LY294002 to investigate the possible PI3K/Akt pathways involved in the protection of FGF-2. The levels of p-Akt, p-FoxO3a, and Bim were detected by immunoblotting. Stimulation with H_2O_2 decreased the phosphorylation of Akt and FoxO3a, and induced nuclear localization of FoxO3a and apoptosis of H9c2 cells. These effects of H_2O_2 were abrogated by pretreatment with FGF-2. Furthermore, the protective effects of FGF-2 were abolished by PI3K/Akt inhibitor LY294002. In conclusion, our data suggest that FGF-2 protects against H_2O_2-induced apoptosis of H9c2 cardiomyocytes via activation of the PI3K/Akt/FoxO3a pathway.

Keywords FGF-2 · Forkhead transcription factor · Apoptosis · Cardiomyocytes · Akt

Mi-Hua Liu, Guo-Hua Li, and Li-Jun Peng have contributed equally to this work.

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Introduction

Cardiovascular disease is a growing public health concern, mainly because of the aging population and the increase in the prevalence of cardiovascular disease in the elderly. Oxidative stress is defined as an excess production of reactive oxygen species (ROS) relative to the endogenous antioxidant reserve to counteract the effects of ROS [1, 2]. Several studies have indicated that ROS induces apoptosis of cardiac myocytes and plays a pivotal role in cardiac pathophysiology responsible for the development and progression of ischemic heart disease [3]. Therefore, to clarify the mechanism of myocardial cell apoptosis after oxidative stress would be helpful, and might provide new targets for the treatment of ischemic heart disease.

Fibroblast growth factor 2 (FGF-2) is produced by both cardiac fibroblasts and myocytes, as well as in other tissues [4, 5]. Several studies suggest that FGF-2 protects the heart from ischemia and reperfusion injury [6–9]. Our laboratory and House et al. have shown that the protective effects of FGF-2 are mediated by PKC and MAPK activation [7, 8, 10–14]. However, the cardiprotection by FGF-2 cannot be explained completely by activation of PKCs and MAPKs, because of the diversity of PKC and MAPK subtypes and the inconsistencies of their subcategory features [7, 8, 11, 13, 14]. For example, we found that the protection effect of FGF-2 cannot be completely blocked by PKC inhibitors chelerythrine and MEK1/2 inhibitors PD-98059 [7, 8, 11, 13, 14], suggesting that the myocardial protective effect of FGF-2 may involve additional unclarified mechanisms.

PI3K/Akt pathway is independent of PKC, PKA, and MAPK signaling pathway, and plays a vital role in the process of cell survival. Activation of PI3K/Akt pathway by FGF-2 prevents ROS-induced apoptosis in human umbilical vein endothelial cells [15], retinal photoreceptor cells [16], and PC12 cells [17–19]. Forkhead box O3 (FoxO3a) transcription factor is one of the most important downstream targets of PI3K/Akt signaling and a crucial regulator of cell apoptosis [20]. Li et al. [21] found that the PI3K/Akt/FoxO3a pathway is involved in neuronal apoptosis in the developing rat brain. In addition, sodium tanshinone IIA sulfonate [22] and bromelain [23] protects the rat heart from ischemia–reperfusion injury via the activation of PI3K/Akt/FoxO3a pathway. Therefore, we hypothesized that FGF-2 was able to protect H9c2 cardiomyocytes from H_2O_2-induced cell apoptosis by activating the PI3K/Akt/FoxO3a pathway. The present study was designed to test this hypothesis and our results may have implications in the treatment of cardiovascular disease.

Materials and methods

Materials

Recombinant rat FGF-2 protein, methyl thiazolyl tetrazolium (MTT), Hoechst 33342, TUNEL, and H_2O_2 were purchased from Sigma-Aldrich (St. Louis, MO, USA). LY294002 was purchased from Calbiochem. All cell culture medium components were purchased from Thermo Fisher Scientific unless otherwise noted. The H9c2 cardiac myocytes were supplied from the Shanghai cell library of China (originally from ATCC, Manassas, VA, USA).

Cell culture

H9c2 cardiac myocytes were cultured in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS), 100 μg/mL streptomycin (Gibco, USA), and 100 U/mL penicillin streptomycin (Gibco, USA) in a humidified 5 % CO_2 atmosphere at 37 °C. H9c2 cardiac myocytes were passaged every 2 days. H9c2 cardiac myocytes were seeded at a density of 2×10^6 cells/dish in 100 mm dishes with 10 % calf serum incubated for 24 h and changed to 0.5 % FBS DMEM for 24 h starvation. In order to determine the degree of apoptotic cells, H9c2 cardiac myocytes were pretreated with recombinant rat FGF-2 protein for 30 min before H_2O_2 for 6 h. In some experiments, H9c2 cells were pretreated with phosphatidylinositol-3-kinase (PI3K) inhibitor LY294002 (20 μM) before FGF-2 stimulation.

MTT assay

The MTT assay is a standard method used to assess cell viability. Before each experiment, H9c2 cardiac myocytes (5000 cells/well) were seeded in 96-well microtitre plates. Following incubation with LY294002 (20 μM) for 60 min and/or FGF-2 for 30 min, H9c2 cells were treated with 100 μM H_2O_2 for a further 6 h. Subsequently, 10 μl MTT solution was added to each of the wells, and the plates were incubated for 4 h at 37 °C. The absorbance was measured at 470 nm and used to calculate the relative ratio of cell viability. Three independent experiments were performed for each experimental condition.

Assessment of cardiomyocyte cell apoptosis

Apoptotic cardiomyocytes was evaluated by terminal dUTP nick end-labeling (TUNEL) single staining. The TUNEL staining was detected using the in situ cell apoptosis detection kit (Roche Diagnostics), according to the manufacturer's instructions. Briefly, after the cells were fixed in ice-cold 4 % paraformaldehyde dissolved in phosphate-buffered saline at room temperature for 20 min, and permeabilized with 0.1 % Triton X-100, 50 μl TUNEL reaction mixture was added on samples for 60 min at 37 °C in the dark. The cells were washed with PBS three times, and then observed with a fluorescence microscopy.

Apoptotic cell death was detected by nuclear condensation. After various treatments, the cells were fixed in ice-cold 4 % paraformaldehyde for 20 min. Nonspecific binding was blocked using 5 % normal goat serum. Cells were washed with PBS three times and 10 μg/ml Hoechst 33258 was added on samples for 10 min in the dark, and then observed with a fluorescence microscopy.

Flow cytometry analysis

After various treatments, H9c2 cells were fixed in 70 % ethanol at −20 °C overnight. H9c2 cells were then

suspended in 0.5 ml of PI solution. Cellular fluorescence was then measured by FCM analysis with a FACS can apparatus (Becton–Dickinson, Heidelberg, Germany). The percentage of Sub-G1 DNA content was used to measure the apoptosis of cells.

Western blot analysis

Cells were homogenized directly into cell lysis buffer (Cell signaling, USA) and phosphatase inhibitor cocktail (Sigma-Aldrich), lysates were centrifugated at 14,000 rpm for 10 min at 4 °C. Protein concentration was determined with the use of a BCA protein assay kit following the manufacturer's instruction. The extracted proteins were mixed with 5 % sodium dodecyl sulfate (SDS)—PAGE sample buffer, then boiled at 100 °C for seven minutes and separated by electrophoresis. After electrophoresis, proteins were transferred to polyvinylidene difluoride membranes. The membranes were blocked in 5 % non-fat dry milk for 2 h with rotation. After blocking, the membranes were incubated with the following antibodies: rabbit anti-Akt polyclonal antibody (Cell Signaling, 1:2000), rabbit anti-p-Akt (Ser 473) monoclonal antibody (Cell Signaling, 1:2000), rabbit anti-FoxO3a polyclonal antibody (Cell Signaling, 1:2000), rabbit anti-p-FoxO3a (ser 253) polyclonal antibody (Cell Signaling, 1:1000), and rabbit anti-Bim polyclonal antibody (Abcam, 1:200). Then, membranes were incubated in 5 % milk overnight at 4 °C. Primary antibody was removed after washing the membranes 3 times in TBS-T, and incubated with horseradish peroxidase-conjugated secondary antibodies for 2 h. Following 3 times of washing in TBS-T, The antigen–antibody bands were detected using enhanced chemiluminescence reagent kit and visualized using X-ray film.

Statistical analysis

Quantitative data are presented as mean ± SD. Comparison between the groups was performed by Student's *t* test. In all cases, a value of $P < 0.05$ was accepted as statistically significant.

Results

FGF-2 protects H9c2 cardiomyocyte against H_2O_2-induced apoptosis involving the PI3K/Akt pathway

To investigate the effects of FGF-2 protects H_2O_2-induced apoptosis, the viability of H9c2 cells was first examined by treatment with H_2O_2 through the MTT assay. H9c2 cells were treated with H_2O_2 at different doses (Fig. 1a). After incubating with H_2O_2 for 6 h, a concentration-dependent decrease in cell viabilities was observed in 100–400 μM H_2O_2 treatment groups ($P < 0.05$). Based on this result, we selected 100 μM of H_2O_2 for subsequent studies. Next, we studied the effect of FGF-2 in preventing H_2O_2-induced cell apoptosis. Figure 1b showed that cell viability fell to 62 % after exposure to H_2O_2 (100 μM) for 6 h ($P < 0.01$, vs. control). However, cell pretreatment with 10 and 20 ng/ml FGF-2 for 30 min prior to exposure to H_2O_2 significantly ameliorated the H_2O_2-induced cytotoxicity, as evidenced by an increase in cell viability ($P < 0.01$). Figure 1c showed that cell viability was reduced to 61.23 % by H_2O_2 while 10 ng/ml FGF-2 pretreatment significantly increased cell viability to 89.13 %. Compared with FGF-2 group, the cell viability of FGF-2 + H_2O_2 + LY294002 group was decreased to 69.72 % ($P < 0.01$). Thus, pretreatment with phosphatidylinositol-3-kinase (PI3K) inhibitor LY294002 prevented the protective effect of FGF-2 against H_2O_2-induced cell apoptosis.

Under fluorescence microscope, Hoechst and TUNEL staining showing that H_2O_2 resulted in a significant increase in the number of apoptotic cardiomyocytes (Fig. 1d). However, FGF-2 obviously inhibited the H_2O_2-induced apoptosis. Pretreatment of the H9c2 cells with LY294002 attenuated the protective effects of FGF-2.

Flow cytometric analysis showed the protective effects of FGF-2 on H_2O_2-induced apoptosis (Fig. 1e, f). The percentage of cells stained by PI revealed that 26.89 % of cells were in the early stages of apoptosis at 6 h of exposure to H_2O_2, FGF-2 treatment cardiomyocyte apoptosis significantly decreased to 7.56 %, compared to H_2O_2-induced control group. However, apoptosis was significantly increased to 17.81 % by co-treatment with FGF-2 and LY294002 compared to FGF-2 treatment alone ($P < 0.05$). These data strongly suggested that FGF-2 has a protective role in H_2O_2-induced apoptotic cell death of H9c2 cells, which may involve the PI3K/Akt pathway.

Posphorylation of Akt and FoxO3a protein as well as FoxO3a translocation and Bim induction after H_2O_2-induced injury

To investigate the role of PI3K/Akt/FoxO3a in mediating the effect of H_2O_2 in the apoptosis of H9c2 cells, the phosphorylation of Akt and FoxO3a in H9c2 cell was studied after exposure to H_2O_2 (Fig. 2).

First, H9c2 cells were treated with 100 μM H_2O_2 in different times, and the phosphorylation of FoxO3a and Akt was determined by Western blotting. As shown in Fig. 2, H_2O_2 decreased the phosphorylation of Akt and FoxO3a in a time-dependent manner in H9c2 cells. A

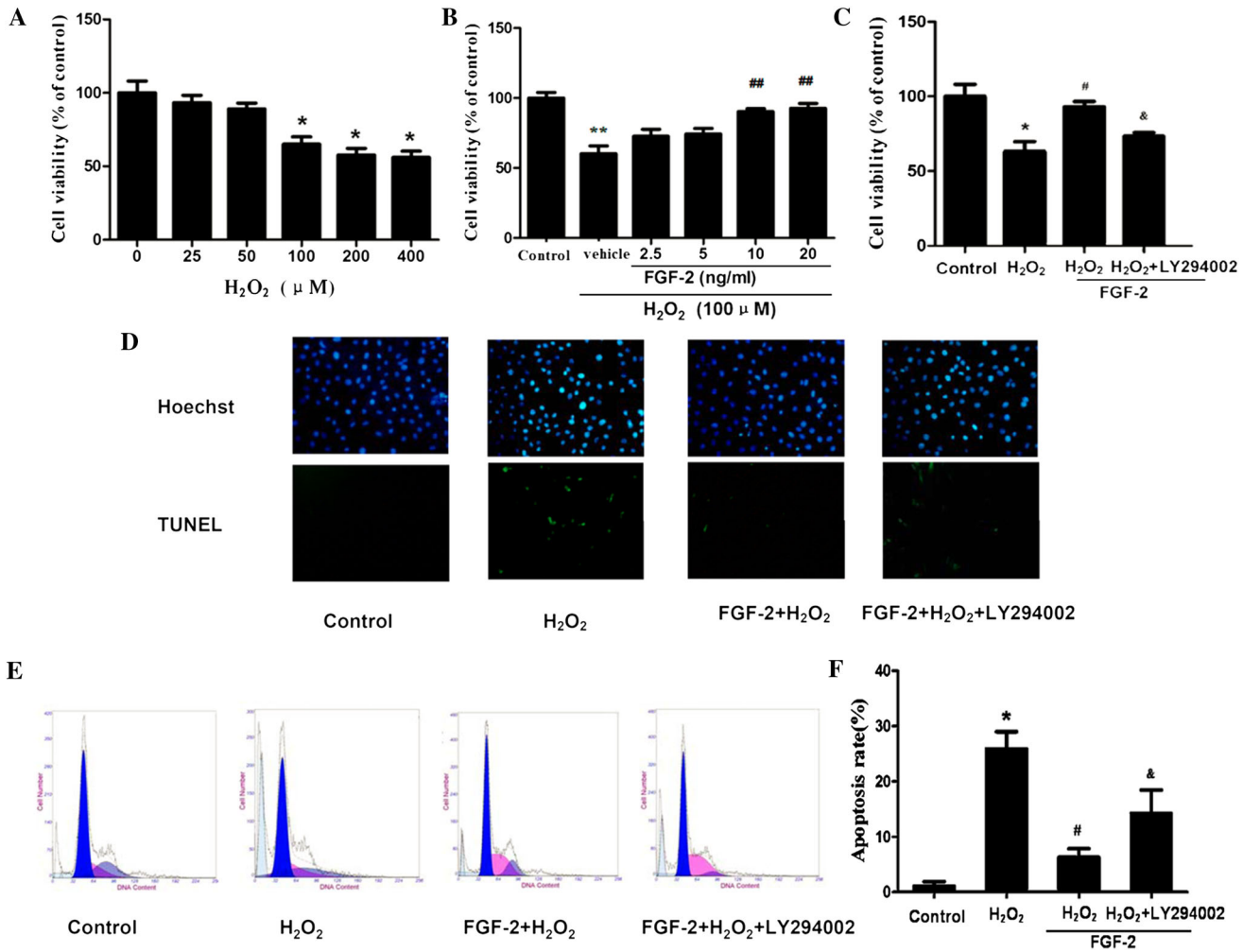

Fig. 1 Protective effects of FGF-2 on H_2O_2-induced apoptosis in H9c2 cardiomyocytes. **a** H9c2 cardiomyocytes cultured with different concentrations of H_2O_2 for 6 h. **b** FGF-2 significantly increased cell viability in H_2O_2 (100 μM)-treated H9c2 cardiomyocytes. **c** LY294002, a PI3K inhibitor, attenuated the protective effect of FGF-2. Cell viability was measured by MTT assay. **d** Hoechst 33258 nuclear staining and TUNEL staining. **e-f** Pretreatment with LY294002 antagonized the protective effect of FGF-2 against H_2O_2-induced cell apoptosis. Apoptosis was assessed by flow cytometry. *Data* are shown as the mean ± SD ($n = 3$). $^{*}P < 0.05$, compared with control group; $^{\#\#}P < 0.01$, compared with only the H_2O_2 treatment group; $^{**}P < 0.01$, compared with control; $^{\#}P < 0.05$ compared with the H_2O_2 group, $^{\&}P < 0.05$ compared with the H_2O_2 + FGF-2 group

significant decrease in the levels of phosphorylated FoxO3a and Akt was observed at 45 min of H_2O_2 stimulation.

Because dephosphorylation of FoxO3a could induce Bim expression and result in cell apoptosis, we studied whether H_2O_2 would promote FoxO3a translocation and upregulate Bim transcription (Fig. 3a). We found that nuclear protein FoxO3a was significantly increased, and cytoplasmic protein was obviously decreased. In addition, FoxO3a translocation induces the expression of Bim, a pro-apoptotic protein [24]. Our data showed that Bim was increased from 15 to 60 min in a time-dependent manner (Fig. 3c).

FGF-2 increases the phosphorylation of Akt and FoxO3a, attenuates FoxO3a translocation and Bim expression

To further determine the effects of FGF-2 protects the apoptosis induced by H_2O_2, levels of phosphorylated Akt and FoxO3a were measured by Western blot analysis. In Fig. 4, the expression of phosphorylated Akt and FoxO3a was evidently reduced in the H_2O_2-treated group. Treatment with FGF-2 evidently increased the phosphorylated FoxO3a and Akt levels in H9c2 cells. Furthermore, FGF-2 treatment alone also significantly increased the

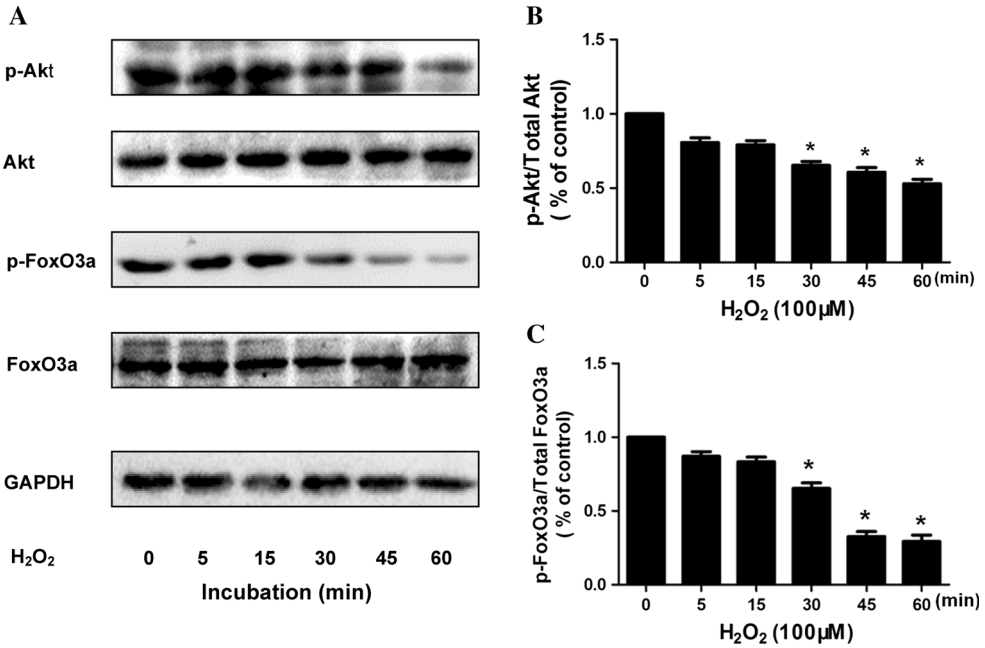

Fig. 2 Effect of H_2O_2 on the phosphorylation of Akt and FoxO3a. **a** Phosphorylation of Akt and FoxO3a as analyzed by immunoblotting. **b**, **c** Relative levels of p-FoxO3a versus total FoxO3a and p-Akt versus total Akt in each sample as determined by blot densitometry. Densitometric analysis of the immunoblot was expressed as a percentage of control. *Data* are shown as the mean ± SD ($n = 3$). $^*P < 0.05$ compared with control group

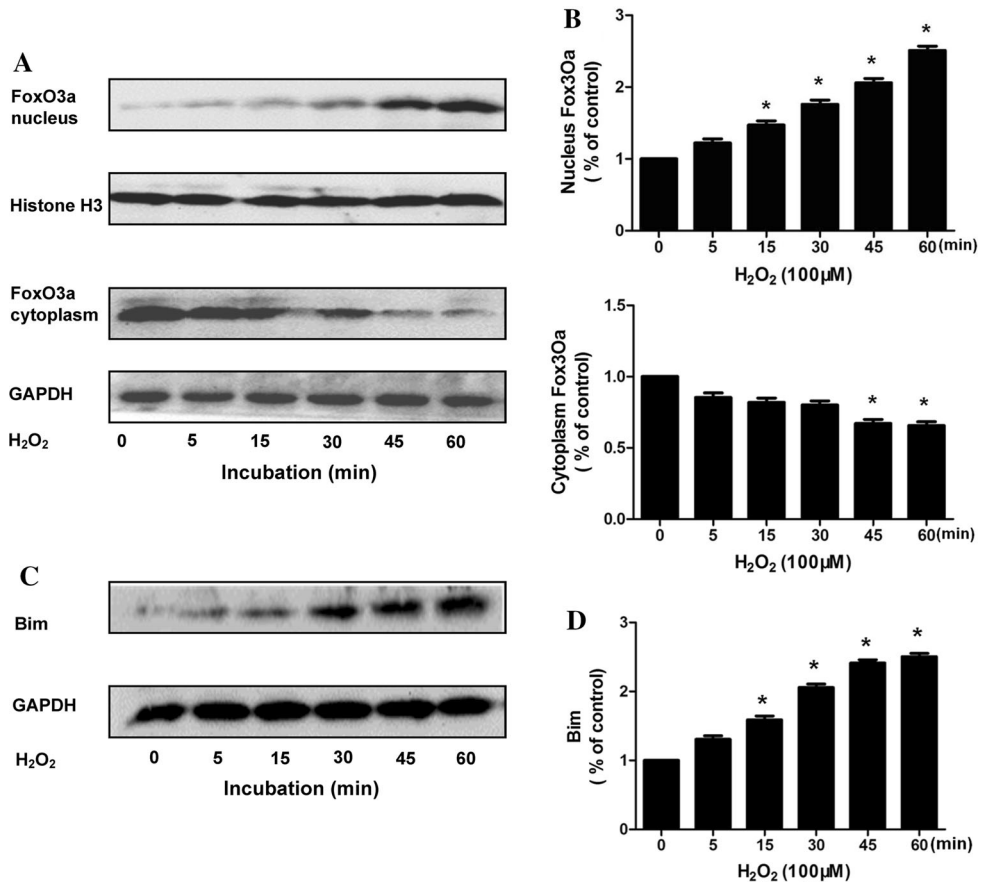

Fig. 3 H_2O_2 promotes FoxO3a translocation from the cytoplasm to the nucleus as well as the induction of Bim. **a**, **c** The nuclear and cytosolic protein of FoxO3a as analyzed by immunoblotting. **b**, **d** Quantification of the nuclear and cytosolic protein of FoxO3a and Bim expression. *Data* are shown as the mean ± SD ($n = 3$). $^*P < 0.05$ compared with control group

phosphorylated Akt and FoxO3a levels. The total Akt levels (t-Akt) and FoxO3a (t-FoxO3a) were unchanged among the four groups (Fig. 4).

As FGF-2 treatment rescued the expression of p-Akt, and p-FoxO3a, we further investigated whether FGF-2 was involved in the regulation of FoxO3a translocation from the

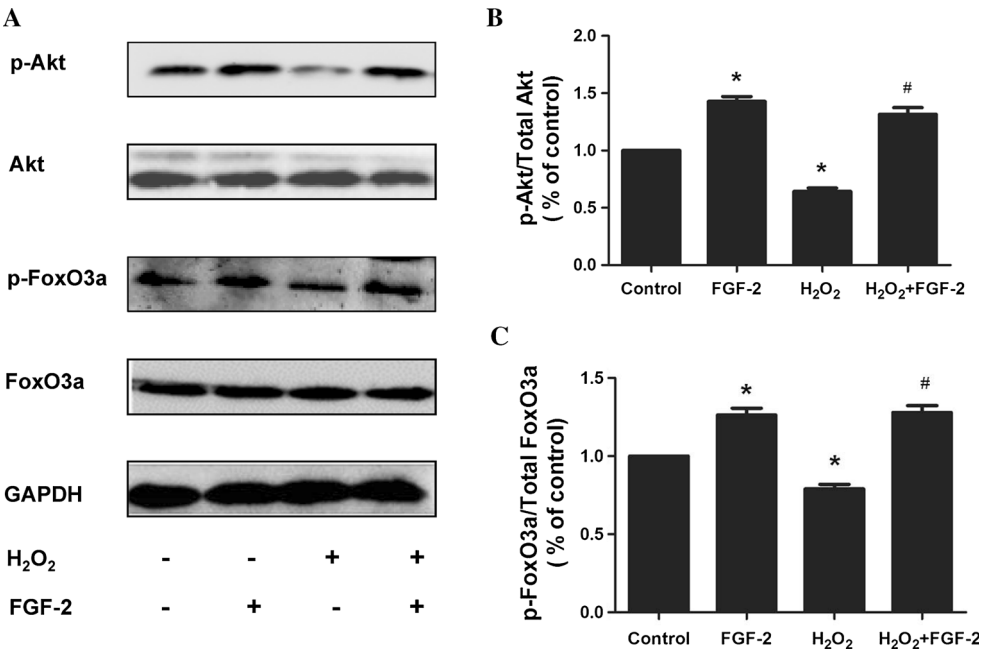

Fig. 4 Effect of FGF-2 on FoxO3a and Akt phosphorylation. H9c2 cardiac cells were treated with FGF-2 (10 ng/ml) for 30 min prior to exposure to H_2O_2 (100 μM) for 45 min. **a** Phosphorylation of Akt and FoxO3a as analyzed by immunoblotting. **b**, **c** Relative levels of p-FoxO3a versus total FoxO3a and p-Akt versus total Akt in each sample as determined by blot densitometry. *Data* are shown as the mean ± SD ($n = 3$). $^{*}P < 0.05$ compared with control group, $^{\#}P < 0.05$ compared with H_2O_2 group

cytoplasm to the nucleus after stimulation with H_2O_2. We measured FoxO3a protein expression separately in the nucleus and cytoplasm using Western blot analysis (Fig. 5a). We found that H_2O_2 enhanced the nuclear localization of FoxO3a, whereas FGF-2 blocked the effect of H_2O_2. However, FGF-2 treatment alone had no effect on the translocation of FoxO3a.

To further understand whether FGF-2 could regulate the expression of Bim in this model, Bim protein levels were analyzed (Fig. 5c). We observed if Bim protein levels were upregulated in H_2O_2 group. Moreover, pretreatment with FGF-2 reduced the protein levels of Bim.

FGF-2 attenuates FoxO3a translocation and Bim induction via the PI3K/Akt/FoxO3a pathway

In order to confirm the role of the PI3K/Akt/FoxO3a pathway in the protective effects of FGF-2, H9c2 cells were pretreated with the PI3K inhibitor LY294002 before treatment of FGF-2 (Fig. 6). LY294002 inhibited the phosphorylation of Akt and FoxO3a, when compared with the FGF-2 group. In addition, LY294002 upregulated FoxO3a in the nucleus, downregulated FoxO3a in the cytoplasm, and increased Bim protein levels compared to FGF-2 group (Fig. 7). All of these results suggest that the protective effect of FGF-2 is mediated by the PI3K/Akt/FoxO3a signaling pathway.

Discussion

Oxidative stress is considered to be a major mediator of the biochemical changes leading to cardiomyocyte apoptosis in cardiovascular disease [25]. H_2O_2 is a representative ROS produced during the redox process and plays a key role in intracellular signaling in pathophysiological conditions [26]. Given that oxidative stress is a crucial initiator of cardiomyocyte apoptosis, blocking the apoptosis process induced by oxidative stress may help to slow down or even prevent the onset and progression of cardiovascular disease. We therefore studied the signaling pathway underlying the effect of FGF-2 to protect H9c2 cardiomyocytes against H_2O_2-induced apoptosis.

Our laboratory previously showed that FGF-2 protects the heart from myocardial dysfunction and injury following ischemia–reperfusion [6–9]. Hearts deficient of FGF-2 displayed a significant decrease in percent recovery of postischemic contractile function, while overexpression of FGF-2 resulted in a significant increase cell survival and functional recovery in the ischemic rat heart [12–14]. FGF-2 also has anti-apoptotic effect in different cell types. For example, FGF-2 protects neurons from radiation injury via the ERK1/2 signal transduction pathways [19]. Li et al. [27] reported that FGF-2 prevents HepG2 and MCF-7 cells from ER stress-mediated apoptosis via enhancing proteasome-mediated Nck degradation. In ATDC5 [28] and endothelial cells [29], FGF-2 up-regulates the expression of Bcl-2 and prolongs their survival. Furthermore, FGF-2 stimulates endothelial nitric oxide synthase expression and increases NO levels in Nb2 lymphoma cells [30].

Multiple potential mechanisms may mediate the cardioprotection of FGF-2 during ischemia–reperfusion injury. Our laboratory has demonstrated that both mitogen-activated protein kinase (MAPK) and protein kinase C (PKC) pathways are involved in postischemic recovery of contractile function when hearts were treated with FGF-2

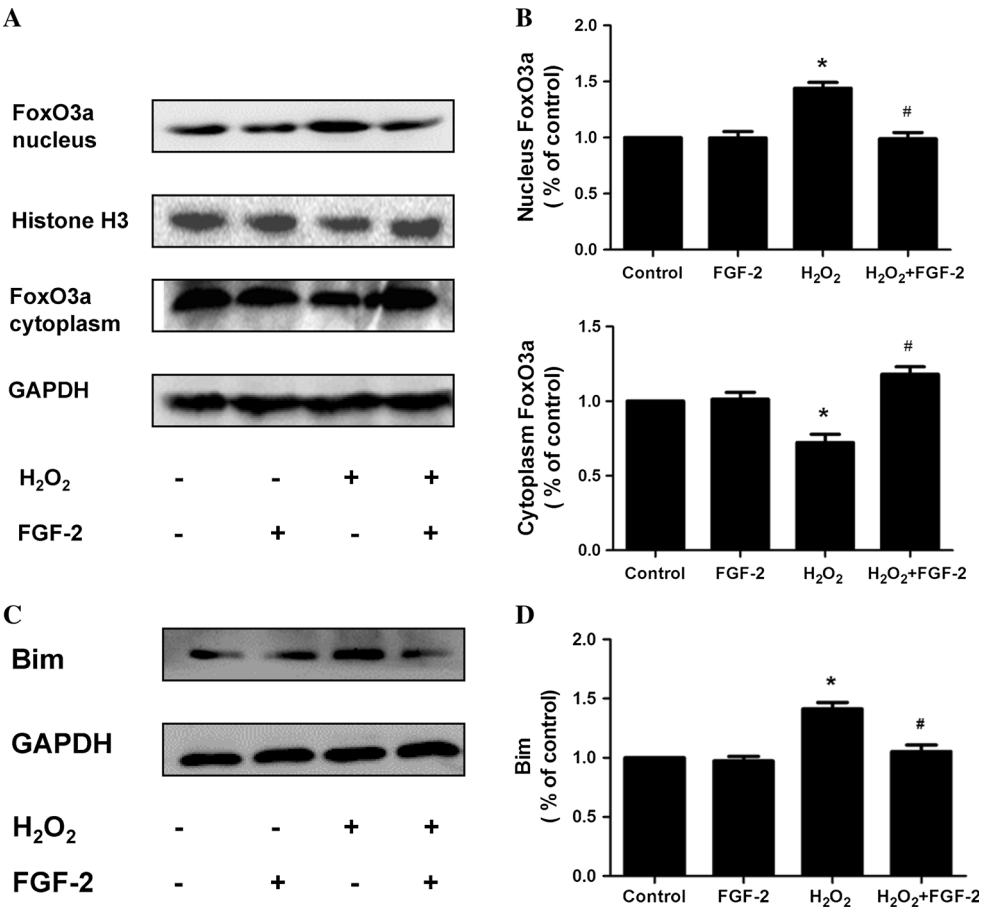

Fig. 5 Effects of FGF-2 on FoxO3a nuclear translocation and Bim induction. H9c2 cardiac cells were treated with FGF-2 (10 ng/ml) for 30 min prior to exposure to H_2O_2 (100 μM) for 45 min. **a**, **c** The nuclear and cytosolic protein of FoxO3a as analyzed by immunoblotting. **b**, **d** Quantification of the nuclear and cytosolic protein of FoxO3a and Bim expression. *Data* are shown as the mean ± SD ($n = 3$). $^{*}P < 0.05$ compared with control group, $^{\#}P < 0.05$, compared with H_2O_2 group

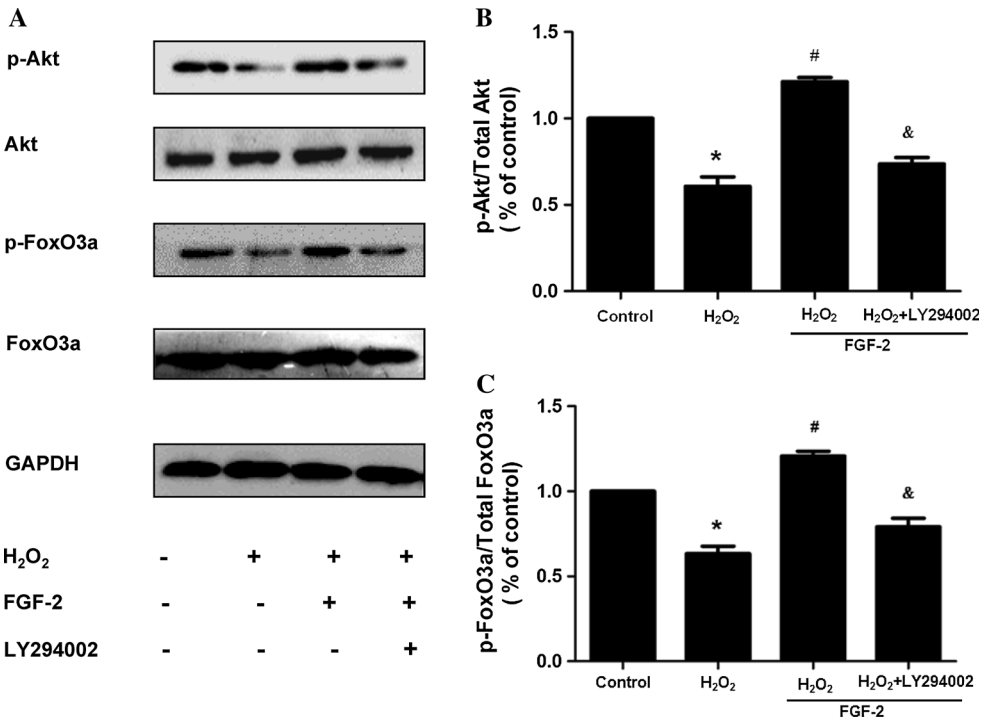

Fig. 6 Effects of LY294002 on the phosphorylation of Akt and FoxO3a induced by FGF-2 in H9c2 cardiomyocytes. H9c2 cardiac cells were treated with LY294002 (20 μM) for 60 min prior to stimulation with FGF-2, followed by a 45-min culture with H_2O_2. **a** Phosphorylation of Akt and FoxO3a as analyzed by immunoblotting. **b**, **c** Relative levels of p-FoxO3a versus total FoxO3a and p-Akt versus total Akt in each sample as determined by blot densitometry. *Data* are shown as the mean ± SD ($n = 3$). $^{*}P < 0.05$ compared with control group, $^{\#}P < 0.05$ compared with H_2O_2 group, $^{\&}P < 0.05$ compared with H_2O_2 + FGF-2 group

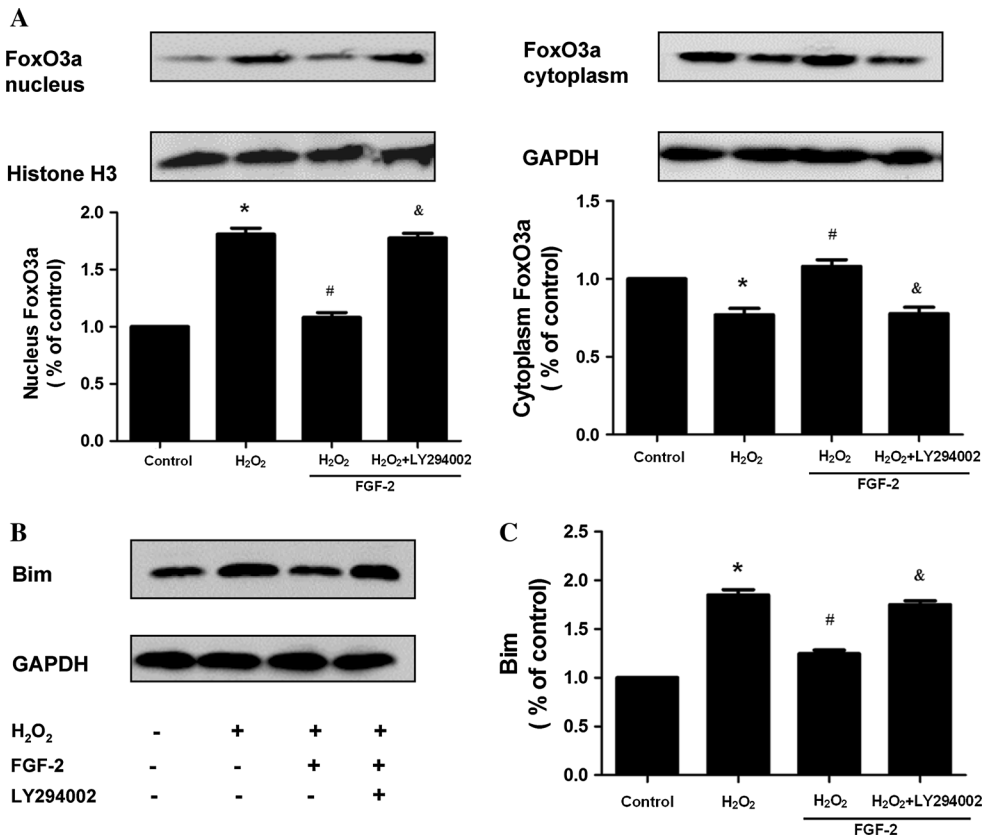

Fig. 7 LY294002 inhibited the effect of FGF-2 on FoxO3a nuclear translocation and Bim induction. H9c2 cardiac cells were treated with LY294002 (20 μM) for 60 min prior to stimulation with FGF-2, followed by a 45-min culture with H_2O_2. **a** Nuclear and cytosolic protein of FoxO3a as analyzed by immunoblotting. **b**, **c** Protein of Bim expression as analyzed by immunoblotting (**b**) and quantification (**c**). *Data* are shown as the mean ± SD ($n = 3$). $^{*}P < 0.05$ compared with control group, $^{\#}P < 0.05$ compared with H_2O_2 group, $^{\&}P < 0.05$ compared with H_2O_2 + FGF-2 group

[7, 10]. House et al. [13, 14] demonstrated that FGF-2 overexpression causes activation of PKC and ERK, and inhibition of p38 during ischemia–reperfusion injury, leading to cardioprotection from postischemic contractile dysfunction and myocardial infarction. In addition, the ERK pathway has been implicated in FGF-2-induced cytoprotection against iNOS-mediated apoptosis [31]. Furthermore, FGF-2-induced cardioprotection against myocardial infarction occurs via protein kinase-NOS pathways and ATP-sensitive potassium channel activity [32]. However, additional pathways, which may also play a role in the mediation of the protective effect of FGF-2.

PI3K/Akt signaling pathway has been shown to play a vital role in promoting cardiomyocyte survival and function [33]. Previous studies have shown that FGF-2 activates the PI3K/Akt signaling pathway to promote survival in several cell types [18, 34]. In the injuries of spinal cord injury model rat, FGF-2 inhibits ER stress-induced apoptosis via the activation of PI3K/Akt/GSK-3β and ERK1/2 signal pathway [18]. In addition, FGF-2 activates PI3K/Akt pathway and inhibits radiation-induced apoptosis of primary cultured human umbilical vein endothelial cells (HUVECs) [35]. Recently, Wang et al. [36] found that FGF-2 treatment significantly reduced the apoptosis induced by acute myocardial I/R injuries, which is related to the inhibition of ER stress- and mitochondrial-related protein expression via both the PI3K/Akt and ERK1/2 signaling pathways. Moreover, the role of FGF-2 in myocardial I/R recovery is related to the inhibition of excessive autophagy and increased ubiquitinated protein clearance via the activation of PI3K/Akt/mTOR signaling [37]. In the present study, H9c2 cells were challenged with H_2O_2 to induce apoptosis. FGF-2 pretreatment before H_2O_2 significantly protected H9c2 cardiomyocytes from H_2O_2-induced apoptosis. This effect was abolished by LY294002, a highly specific inhibitor of PI3K/Akt. This data suggests that activation of PI3K/Akt signaling pathway plays an important role in the protective effects of FGF-2 against H_2O_2-induced apoptosis in cardiomyocytes.

FoxO3a transcription factor is an important downstream target of PI3K/Akt pathway, and activated Akt phosphorylates FoxO3a, and leads to cytoplasmic localization of FoxO3a and inhibition of apoptosis [38]. Inhibition of the

PI3K/Akt pathway increases the nuclear translocation of FoxO3a and promotes neuronal apoptosis [24, 39]. Wang et al. [40] reported that venlafaxine protects PC12 cells against corticosterone-induced cell death by modulating the activity of the PI3K/Akt/FoxO3a pathway. In addition, erythropoietin activates PI3K/Akt/FoxO3a signaling pathway and protects neurons from 6-hydroxydopamine (6-OHDA)-induced apoptosis [41]. Cardiac microvascular endothelial cells (CMECs) are some of the predominant cells damaged immediately after myocardial I/R injury. High glucose [42] and hypoxia [43] decrease the phosphorylation of Akt and FoxO3a, induce FoxO3a activation, leading to ROS production and apoptosis in CMECs. Furthermore, simvastatin inhibits rapamycin-induced CMECs dysfunction and apoptosis, probably through activation of PI3K/Akt/FoxO3a signaling pathway [44].

In order to reveal the role of the PI3K/Akt/FoxO3a signaling pathway in the cytoprotective actions of FGF-2, we evaluated its ability to induce Akt and FoxO3a phosphorylation and activation in H9c2 cells. In this study, we found that the phosphorylation of Akt and FoxO3a protein was decreased, FoxO3a translocation into the nucleus and Bim transcription was increased after H_2O_2 stimulation. These effects were prevented by FGF-2. Furthermore, the action of FGF-2 was blocked by a PI3K/Akt-specific inhibitor LY294002, suggesting that effects of FGF-2 on FoxO3a nuclear translocation and Bim expression are mediated by the PI3K/Akt pathway.

In conclusion, the present study showed that FGF-2 protects H9c2 cardiomyocytes from apoptosis induced by H_2O_2. Furthermore, we showed that activation of the PI3K/Akt/FoxO3a pathway is involved in this protective process. To our knowledge, this is the first study showing that FGF-2 is capable of acting on PI3K/Akt/FoxO3a pathway to enhance cardiomyocyte survival, suggesting that the FoxO3a pathway may be a novel therapeutic target in cardiovascular disease.

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