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Heat shock transcription factor-1 inhibits H_2O_2-induced apoptosis via down-regulation of reactive oxygen species in cardiac myocytes

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Abstract Heat shock transcription factor-1 (HSF1) protects against cardiac diseases such as ischemia/reperfusion injury and myocardial infarction. However, the mechanisms have not yet been fully characterized. In this study, we investigated the effects of reactive oxygen species (ROS) and apoptosis signal-regulating kinase-1 (ASK1) in HSF1-regulated cardiomyocyte protection. Cultured cardiomyocytes of neonatal rats were transfected with *HSF1*, *ASK1* or both of them before exposure to H_2O_2, and the ROS generation, c-Jun N-terminal kinase (JNK) activity and apoptosis were examined. H_2O_2 significantly increased intracellular ROS generation and apoptotic cells as expected, and all these cellular events were greatly inhibited by overexpression of HSF1. However, H_2O_2-induced increases in JNK phosphorylation and cell apoptosis were largely enhanced by ASK1 overexpression whereas the similar transfection did not affect the ROS generation in the cells. Moreover, inhibition of H_2O_2-increased ROS generation, JNK phosphorylation, and cellular apoptosis by overexpression of HSF1 tended to be disappeared, when the cells were co-transfected with ASK1. These results suggest that HSF1 protects cardiomyocytes from apoptosis under oxidative stress via down-regulation of intracellular ROS generation and inhibition of JNK phosphorylation. Although ASK1 itself has no effect on intracellular ROS generation, it may affect the inhibitory effects of HSF1 on ROS generation, JNK activity, and cardiomyocyte injury.

Keywords Heat shock transcription factor-1 · Cardiomyocytes · Reactive oxygen species · Apoptosis signal-regulating kinase-1 · c-Jun N-terminal kinase

Introduction

A number of studies have shown that heat shock transcription factor-1 (HSF1), a transcription factor for heat shock proteins (HSPs), confers protection against cardiovascular diseases, such as ischemia/reperfusion injury, myocardial infarction, doxorubicin-induced cardiomyopathy, and atrial fibrillation [1–7]. Moreover, HSF1 can prevent cardiomyocytes from apoptosis induced by various stimulations. However, the mechanisms have not yet been fully characterized.

Reactive oxygen species (ROS) are thought to serve as second messengers to control a broad range of physiological and pathological processes, such as diabetes, atherosclerosis, Alzheimer's disease, and aging [8, 9]. The redox state of the cell is a consequence of the precise balance between the levels of ROS and endogenous antioxidants. Elevation of ROS in excess of the antioxidant-buffering

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capacity results in potentially cytotoxic oxidative stress, which leads to apoptosis as a final event [10]. c-Jun N-terminal kinase (JNK) involves in the pathways that regulate apoptosis of H_2O_2-stimulated human pulmonary vascular endothelial cells, and plays an important role in regulating left ventricular remodeling by promoting apoptosis [11, 12]. Moreover, elevated ROS can regulate the activity of mitogen-activated protein kinases (MAPKs) pathways that may be involved in apoptosis [13, 14], while JNK is one of the three major MAPKs [15]. Meanwhile, Yan [16] and Xiao [17] have found that HSF1 and HSPs are protective against oxidative damage, and HSF1 not only plays a role in regulating ROS levels during T cell activation [18], but also alleviates ischemia/reperfusion injury by prohibiting JNK activity [3]. Thus, it is reasonable to speculate that HSF1 may prevent cardiomyocytes from apoptosis under various stimulation via inhibition of intracellular ROS production and then JNK activity.

Apoptosis signal-regulating kinase-1 (ASK1), a member of the mitogen-activated protein kinase (MAP3K) family, triggers various biological responses, such as apoptosis, inflammation, differentiation, and survival in different cell types [19], and can be activated by various types of stress, including oxidative stress, ER stress, calcium overload, and inflammatory cytokines [10]. ASK1 mediates ROS-induced apoptosis signaling [20]. However, few studies detected whether ASK1 can feed back on ROS, and the interaction between HSF1, ASK1, and ROS is still unclear.

The aims of this study are to detect the effects of overexpressed HSF1 and ASK1 on H_2O_2-induced cardiomyocyte injury and to determine whether the effects are relative to co-regulation of intracellular ROS by the HSF1 and ASK1 under oxidative stress.

Materials and methods

Reagents

Dulbecco's modified Eagle's medium (DMEM), Fetal Bovine Serum (FBS), serum-free opti-MEM were obtained from Gibco (USA). Lipofectamine^{TM} 2000 transfection reagent, 5-(and-6)-carboxy-2′,7′-dichlorodihydrofluorescein diacetate (carboxy-H2DCFDA) were obtained from Invitrogen (USA). Plasmid Midi Kit was purchased from QIAGEN (Germany). Rabbit polyclonal antibody against the dually phosphorylated (Thr183/Tyr185) or non-phosphorylated JNK1/2 was purchased from Cell Signaling (USA), goat anti-rabbit horseradish peroxidase (HRP)-conjugated IgG was purchased from Jackson (USA). The enhanced chemiluminescence (ECL) western blot reagent kit was purchased from Pierce (USA). Cell death detection kit and RT-PCR kit were purchased from Roche (USA) and TOYOBO (Japan), respectively. All other chemicals used were of the highest grade commercially available.

Cell culture

Primary cultures of rat ventricular myocytes were obtained using one or two-day-old Sprague-Dawley rat pups, as described [21], with the approval of the Institutional Animal Care and Use Committee of Fudan University. Cells were incubated in low glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) FBS, 100 U/ml each of penicillin and streptomycin, and 20 mM HEPES (pH 7.2) at 37°C in humidified air with 5% CO_2. Confluent monolayers exhibiting spontaneous contractions were developed in culture within 2 days.

Plasmids and transfection

Construction of plasmids of human *HSF1* [22] and *ASK1* [10] has been previously described. In brief, the full-length *hHSF1* cDNA was modified by using PCR mutagenesis to introduce *Eco*RI sites and ligated into the pGEX-2T vector (Pharmacia) to create expressing vector pGEX2T-hHSF1. *ASK1* expression plasmid was constructed by inserting human ASK1 cDNA into the pcDNA3 (Invitrogen) expressing vector. The plasmids were amplificated with JM 109 bacteria, extracted, and purified by Plasmid Midi Kit. Cardiomyocytes were 90-95% confluent and the supernatant were replaced by serum-free Opti-MEM the day before transfection. Plasmids were transfected with lipofectamine^{TM} 2000. All the procedures were performed according to the manufacture's instructions. Briefly, for each transfection, liposomes were diluted with serum-free Opti-MEM and kept at room temperature for 5 min. They were then mixed with serum-free Opti-MEM containing plasmid DNA and the mixture (DNA-liposome complex) was left at room temperature for 20 min before added to the cultures, only vehicle was added to control. After incubation at 37°C in humidified air with 5% CO_2 for 6 h, the transfection medium were replaced with fresh complete medium containing 10% FBS. The transfection efficacy of this method in cultured cardiomyocytes of neonatal rats evaluated by transfection of the similar amounts of GFP-expressing vectors was 10–20% according to cell culture and transfection condition (Data not shown).

Oxidative stress treatment

The media were replaced by serum-free DMEM for synchronization the day before H_2O_2 stimulation. Cells were treated with 1 mM H_2O_2 for 30 min at 37°C 48 h later after transfection, and the following assessments were then performed.

Determination of cardiomyocyte apoptosis

Cardiomyocytes apoptosis were analyzed quantitatively by terminal deoxyribonucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining with an in situ cell death detection kit. Briefly, cells in culture were washed by PBS after H_2O_2 treatment, fixed with 4% formaldehyde, blocked with 3% H_2O_2, permeabilized with 0.1% Triton X-100, labeled with TUNEL reaction mixture containing tdt, stained with DAB and analyzed under microscopy. For negative control, only label solution was added.

ROS determination

ROS accumulation in the cardiomyocytes was detected before and after H_2O_2 stimulation, with a 5-(and-6)-carboxy-2′,7′-dichlorodihydrofluorescein diacetate (carboxy-H2DCFDA) staining method. This assay is based on the principle that the nonpolar, nonionic H2-DCFDA crosses cell membranes and is enzymatically hydrolyzed into nonfluorescent H2-DCF by intracellular esterases. In the presence of ROS, H2-DCF is rapidly oxidized to become highly fluorescent DCF. Cells were incubated at 37°C for 30 min with 5 μM carboxy-H2-DCFDA dissolved in the culture medium. 5×10^5 cells were resuspended in phosphate-buffered saline (PBS, pH7.4) and sent to flow cytometry analysis (Epics Altra, BECKMAN, USA). The percent of fluorescence-positive cells as a measure of ROS generation was recorded on a spectofluotometer using excitation and emission filters of 488 and 530 nm, respectively.

RNA isolation and reverse transcriptase polymerase chain reaction (RT-PCR)

The expression of *HSF1* and *ASK1* mRNA were analyzed by reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted from cultured cells using TRIzol reagent (Invitrogen, USA) according to the manufacturer's instructions. Concentration and purity of the extracted RNA were measured spectrophotometrically at A260 and A280. Total RNA (1.5 μg) from each sample was reverse transcribed for PCR using First Strand cDNA Synthesis Kit (TOYOBO, Japan) according to the manufacturer's instructions. The resulting cDNA was used as a template for PCR with specific primer pairs (Table 1). The PCR reactions were done using PCR Master Mix (Fermentas, USA) and *GAPDH* was used as internal control for RT-PCR. Reactions were followed by 30 cycles for HSF1 or 32 cycles for ASK1 of 95°C (denaturation) for 30 s, annealing (55°C for HSF1, 59°C for ASK1) for 30 s, 72°C (extension) for 30 s. Then it was extended at 72°C for 5 min. The PCR products were separated on a 1.5% agarose gel containing 0.5% ethidium bromide and densitometric analysis of the band was done by TotalLab software.

Protein isolation and western blot

The cells were washed with ice-cold PBS and then lysed using RIPA lysis buffer containing 1 mM phenylmethanesulfonylfluoride (PMSF, Beyotime biotechnology, China). The lysates were collected and centrifuged. The supernatants were harvested and frozen at −80°C until required.

Samples were separated on 12% SDS-polycrylamide gel, and after electroblotting onto polyvinylidene fluoride (PVDF) membranes (Millipore, USA), the membranes were blocked with blocking solution [5% (w/v) Bovine Serum Albumin (BSA, Roche, USA) in Tris-buffered solution plus Tween-20 (TBST): 50 mM Tris–HCl, 150 mM NaCl, pH = 7.5, 0.1% (v/v) Tween 20], incubated overnight at 4°C with primary antibody against phospho-JNK1/2 or non-phospho-JNK1/2, and then incubated with HRP-conjugated anti-rabbit IgG. Detection was performed by ECL using Supersingnal West Pico Chemiluminescent Substrate according to the manufacturer's instructions. Bands were quantified by densitometry using Image System (Bio-Rad).

Statistical analysis

The data are expressed as means ± SE. Statistical differences were analyzed by one-way ANOVA followed by multiple comparisons performed with post hoc Bonferroni test (SPSS version 11.5). Values of $P < 0.05$ were considered statistically significant. The significance of any

Table 1 Primer sequences

Gene	Forward (F) and reverse (R) primers	Size of PCR product (bp)
HSF1	F 5′CCCTGAAGAGTGAGGACATAA3′	367
	R 5′GCTGGAGATGGAGCTGAGTA3′	
ASK1	F 5′CCTGTGCTAATGACCTGCTTGTTG3′	159
	R 5′TGCTGCTGCTGGTGTCTTCTAC3′	
GAPDH	F 5′GGAAAGCTGTGGCGTGATGG3′	393
	R 5′GTAGGCCATGAGGTCCACCA3′	

PCR polymerase chain reaction, *HSF1* heat shock transcription factor-1, *ASK1* apoptosis signal-regulating kinase-1; *GAPDH* glyceraldehyde-3-phosphate dehydrogease

differences between two groups was tested using paired-samples *t* test when appropriated.

Results

Effects of *HSF1* and *ASK1* transfection on mRNA expression of *HSF1* and *ASK1*

We first confirmed the effects of *HSF1* and *ASK1* transfection on the expression of HSF1 and ASK1 in cardiomyocytes. Cultured cardiomyocytes were transfected with *HSF1*, *ASK1* or both of them. Forty-eight hours after transfection, mRNA expression of *HSF1* (Fig. 1a, b) and *ASK1* (Fig. 1c, d), evaluated by RT-PCR method, were significantly upregulated by *HSF1* and *ASK1* gene transfection, respectively. Transfection of *ASK1* and *HSF1* could not affect the expression of *HSF1* and *ASK1*, respectively. Also, co-transfection with *ASK1* or with *HSF1* could not interrupt upregulation of *HSF1* or that of *ASK1* by *HSF1* or *ASK1* transfection, respectively. These results suggested that transfection of *HSF1* and *ASK1* did induce overexpression of *HSF1* and *ASK1*, respectively, in cultured cardiomyocytes and that expression of *HSF1* or *ASK1* was independent of ASK1 or HSF1 regulation, respectively.

Effects of *HSF1* and *ASK1* overexpression on H_2O_2-induced cardiomyocyte apoptosis

We next examined protection of cardiomyocytes against oxidative stress by HSF1. Apoptosis was determined by TUNEL staining, as shown in Fig. 2, the number of apoptotic cells was significantly increased after treated with H_2O_2 for 30 min ($P < 0.05$). As expected, the increase in apoptosis was significantly inhibited by *HSF1* overexpression ($P < 0.05$), confirming the protective role of HSF1. On the other hand, overexpression of *ASK1* exacerbated H_2O_2-induced increase in cardiomyocyte apoptosis ($P < 0.05$), suggesting that ASK1 enhanced cellular injury by H_2O_2. However, in *ASK1*-co-transfected cells, the decrease of H_2O_2-stimulated cardiomyocyte death by *HSF1* overexpression was diapered ($P < 0.05$). These results indicated that *ASK1* overexpression not only enhances H_2O_2-induced cardiomyocyte injury, but also weakens HSF1-based cardiomyocyte protection after oxidative stress.

Effects of *HSF1* and *ASK1* transfection on H_2O_2-induced intracellular ROS generation

To explore the reason for the effects of HSF1 and ASK1 on cardiomyocytes, we examined the ROS generation in cardiomyocytes since ROS is important for H_2O_2-induced

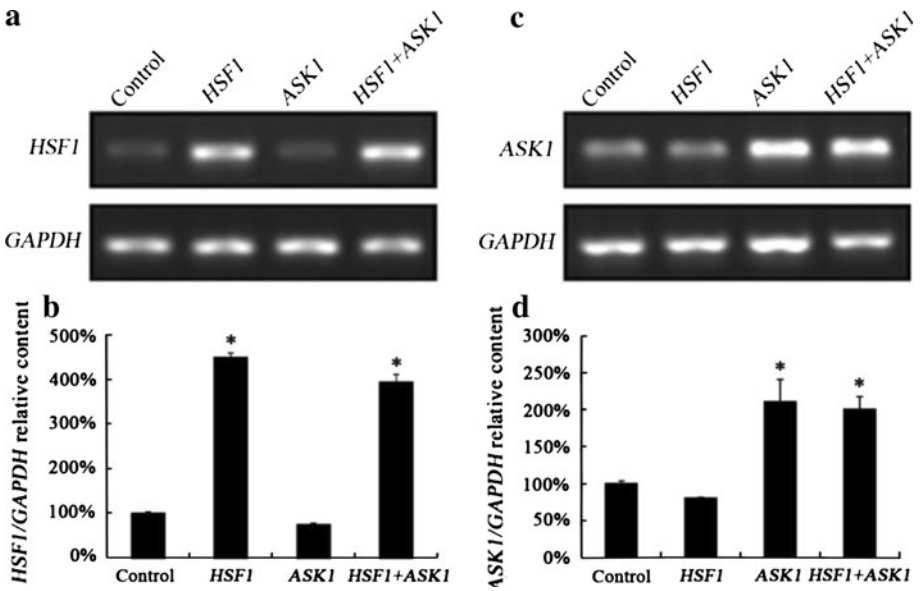

Fig. 1 Detection of *HSF1* and *ASK1* mRNA expression in cultured cardiomyocytes. Cultured cardiomyocytes of neonatal rats were transfected with empty vector (control), *HSF1*, *ASK1* or *HSF1* plus *ASK1*. After 48 h of transfetion, cells were harvested and total RNA was extracted. mRNA expression of *HSF1* and *ASK1* was detected by RT–PCR. *GAPDH* was used as a loading control. **a** *HSF1* expression. **c** *ASK1* expression. Representative photograms from three independent experiments are shown. **b**, **d** Expression of *HSF1* and *ASK1* was quantified as the ratio of *HSF1* or *ASK1* to *GAPDH*, respectively and expressed as % of control. Data are shown as mean ± SE from three individual experiments. * $P < 0.05$ versus respective control

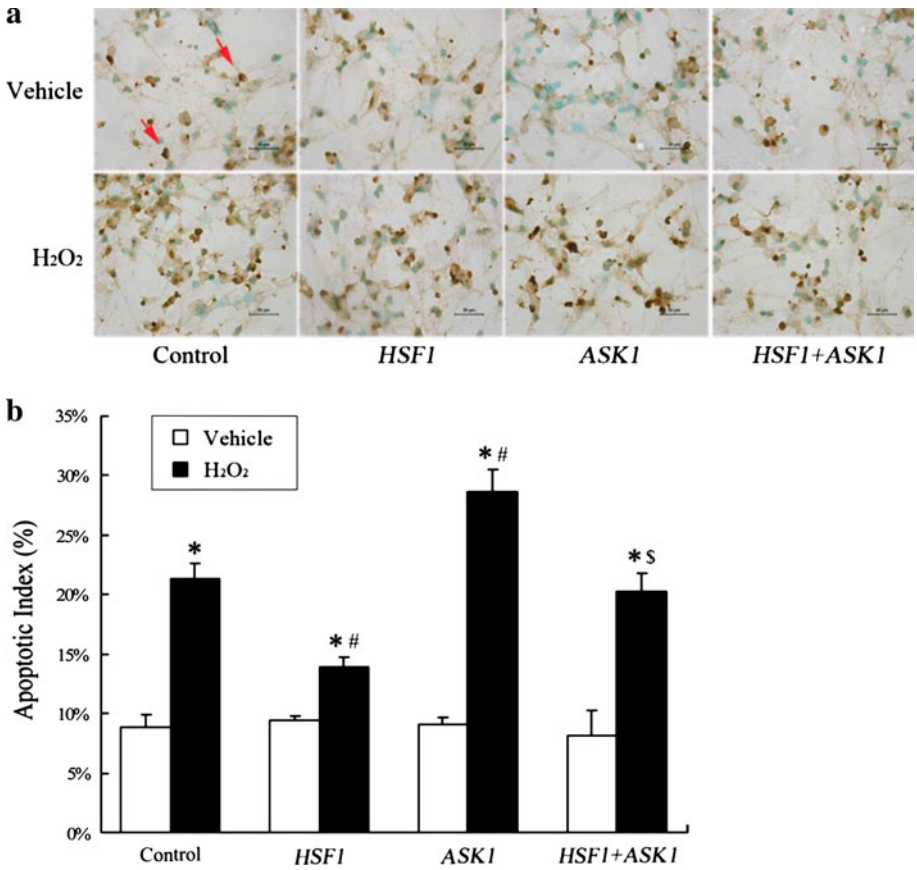

Fig. 2 Detection of cardiomyocyte apoptosis by TUNEL method. Cardiomyocytes transfected with empty vector (control), *HSF1*, *ASK1* or *HSF1* plus *ASK1* were incubated with vehicle (*upper*) or H_2O_2 (*lower*) for 30 minutes. Apoptosis was detected by TUNEL staining. Representative photographs from three independent experiments are shown. Methyl green was used for counterstaining of nuclei; DAB was used for detection of TUNEL-positive cells. *Scale bar* = 20 μm. Apoptotic cells was quantified as the % of total cells. Data are shown as mean ± SE of three independent experiments ($n = 3$). * $P < 0.05$ versus respective vehicle; # $P < 0.05$ versus control with H_2O_2; $ $P < 0.05$ versus *HSF1* with H_2O_2

cell death. H_2O_2 significantly increased intracellular ROS generation ($P < 0.01$), which was significantly reduced by *HSF1* overexpression ($P < 0.05$) (Fig. 3a, b). Although *ASK1* transfection alone did not further elevate H_2O_2-increased ROS generation, it tended to abolish the inhibitory effects of *HSF1* on H_2O_2-elevated ROS generation, when *ASK1* was co-transfected with *HSF1* ($P = 0.22$).

Effects of *HSF1* and *ASK1* transfection on JNK phosphorylation

We have previously shown that HSF1 exerts protective effects on ischemic injury of cardiomyocytes at least in part through suppression of JNK activation [3]. We here tested the effects of *HSF1* and *ASK1* transfection on JNK phosphorylation. The JNK1/2 protein phosphorylation was unchanged after transfection alone (Fig. 4a, b). After stimulation with H_2O_2, the phosphorylation of JNK1/2 was elevated in vector-transfected cardiomyocytes, which was significantly lowered by *HSF1*-transfection ($P < 0.05$) (Fig. 4c, d). However, *ASK1*-transfection itself furtherly enhanced the H_2O_2-induced elevation of JNK1/2 phosphorylation levels ($P < 0.05$) and, when co-transfected with *HSF1*, abrogated the inhibitory effect of *HSF1* on H_2O_2-elevated JNK1/2 phosphorylation ($P < 0.05$) (Fig. 4c, d).

Discussion

ROS, mainly generated by mitochondria, are by-products of all aerobic metabolisms. At high concentrations, ROS are cytotoxic for cells, leading to irreversible damage to DNA, proteins and lipids [16]. Moreover, ROS can directly function on unsaturated fatty acid within cardiomyocyte membrane, influencing its fluidity and permeability, which finally result in the dysfunction of ion transportation and destruction of membrane structure. Besides, ROS induce aqtocytolysis and dysfunction of energy metabolism by destroying lysosomal membrane, sarcoplasmic reticulum, and mitochondria [23]. Thus, cellular homoeostasis requires the intracellular concentrations and toxic activities of ROS to be finely balanced with the co-ordinated induction of proteins possessing radical-scavenging properties and protective activities, such as superoxide dismutase, ferritin, haem oxygenase, and thioredoxin [24]. Studies have showed that many cardiovascular diseases such as chronic heart failure are accompanied by intensified oxidative stress, while ROS promote heart failure in turn. Yet cardiac and mitochondrial function can be preserved by preventing ROS generation [25, 26].

Among three HSFs (HSF1, HSF2, and HSF4) in mammals, HSF1 plays a crucial role in inducing HSPs under various stimuli [27]. There have been many reports that

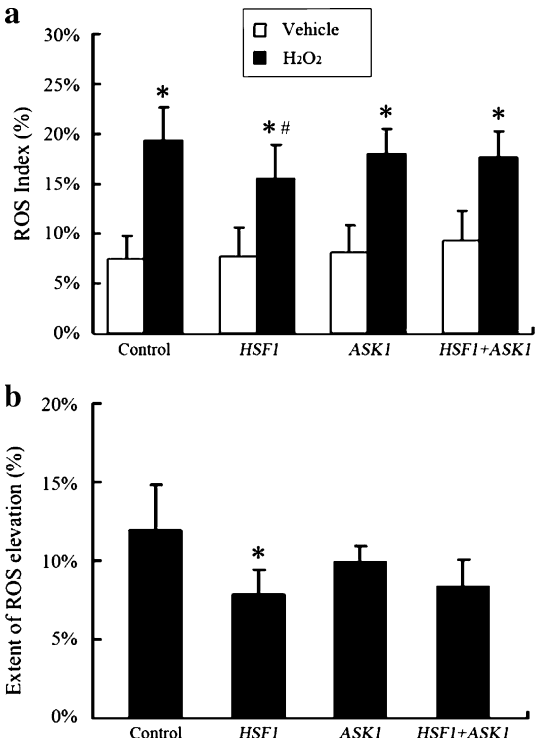

Fig. 3 Detection of ROS generation in cardiomyocytes by carboxy-H2DCFDA. Cardiomyocytes transfected with empty vector (control), *HSF1*, *ASK1* or *HSF1* plus *ASK1* were incubated with vehicle or H_2O_2 for 30 min. **a** ROS levels were measured by FACS as described in "Materials and methods" section and expressed as "ROS index" (% of fluorescence-positive cells). Data are shown as mean ± SE for six individual experiments. * $P < 0.01$ versus Vehicle; # $P < 0.05$ versus control with H_2O_2. **b** The extent of elevation of ROS levels after incubation with H_2O_2. * $P < 0.05$ versus control

HSF1 has beneficial effect in animal models of cardiovascular disease, such as reducing the size of myocardial infarcts after ischemia/reperfusion and regulating cardiac hypertrophy [3, 28], but the mechanisms have not yet been fully characterized.

The activation of HSFs by oxidants is selective for the type of oxidant, H_2O_2 being effective while superoxide is not [29]. H_2O_2 leads to HSFs activation in human cells, and specifically, to the activation of HSF1 by favouring its nuclear translocation [24, 30]. HSF1 thus belongs to ROS-modulated transcription factor. Furthermore, it was reported that HSF1 can regulate ROS levels during T cell activation [18]. Based on these facts, we hypothesized that HSF1 can not only be activated by ROS, but also negatively feed back on it under various stress. Thus, we transfected *HSF1* plasmid into cardiomyocytes before inducing cell apoptosis by H_2O_2. Consistent with our hypothesis, the results showed that *HSF1* overexpression significantly suppressed intracellular ROS generation as well as apoptosis under oxidative stress in comparison to control. Actually, the same scene was observed in cardiac microvascular endothelial cells (data not shown). Since cellular responses shift accordingly to increases in the level of ROS [10], it is not difficult to understand that HSF1 may protect cardiomyocytes from apoptosis via inhibiting intracellular ROS generation. Moreover, Mehlen [31] have found that HSP27 protects cells from death through its conserved ability to raise the pool of reduced glutathione (GSH), which decreases the intracellular ROS level, and HSP25 plays a crucial role in both kidney and heart for cellular redox homeostasis [16]. Thus, we can infer that HSF1 may down-regulate ROS by inducing small HSPs such as HSP27 and HSP25.

The major groups of MAPKs found in cardiac tissue include the extracellular signal-regulated kinases (ERKs), the stress-activated protein kinases (SAKs)/JNKs, p38-MAPK, and ERK5/big MAPK 1 (BMK1). Activation of MAPKs family plays a key role in the pathogenesis of various processes in the heart [32]. Moreover, JNK can be activated by various prooxidants such as H_2O_2, and contributes to determination of cell fate [33, 34]. To further determine whether JNK, one member of MAPKs family, could be influenced on condition of ROS inhibition by HSF1 in H_2O_2-treated cardiomyocytes, we detected phosphorylated JNK expression, which stands for JNK activity. Our study showed that overexpressed *HSF1* significantly reduced p-JNK in cardiomyocytes under oxidative stress. Matsuzawa [10] have found that TNFα-induced apoptosis requires activation of the ASK1-JNK/p38 pathway mediated by ROS as second messengers. In view of this, we presume that HSF1 may prohibit JNK activity on account of messengers' suppression.

ASK1 is a key element in cytokine and stress-induced apoptosis, and can be activated in cells either treated with inflammatory cytokines or exposed to various types of stress (e.g., oxidative stress). Moreover, ASK1 can serve as an initial sensor of generation of ROS or fluctuation of the cellular redox, to modulate the downstream signal transductions for maintenance of homeostasis [10]. We also tried to determine whether ASK1 could influence ROS generation in turn by ovexpressing *ASK1* in cardiomyocytes. The data showed that unlike *HSF1*, *ASK1* overexpression had no impact on intracellular ROS generation. Nevertheless, ovexpressed *ASK1* significantly increased the rate of cell apoptosis under oxidative stress, which is compatible with former study [35]. In this case, ASK1 might be activated by oxidative stress, and in turn, enhanced JNK activity (Fig. 4c, d), which finally led to apoptosis [36]. Surprisingly, we found that the beneficial role of HSF1 in prohibiting apoptosis, inhibiting JNK activity, and even down-regulating ROS tended to be vanished, when *ASK1* and *HSF1* were co-transfected in cardiomyocytes. Such consequence may explained by the interaction between HSF1 and ASK1. On one hand, ASK1

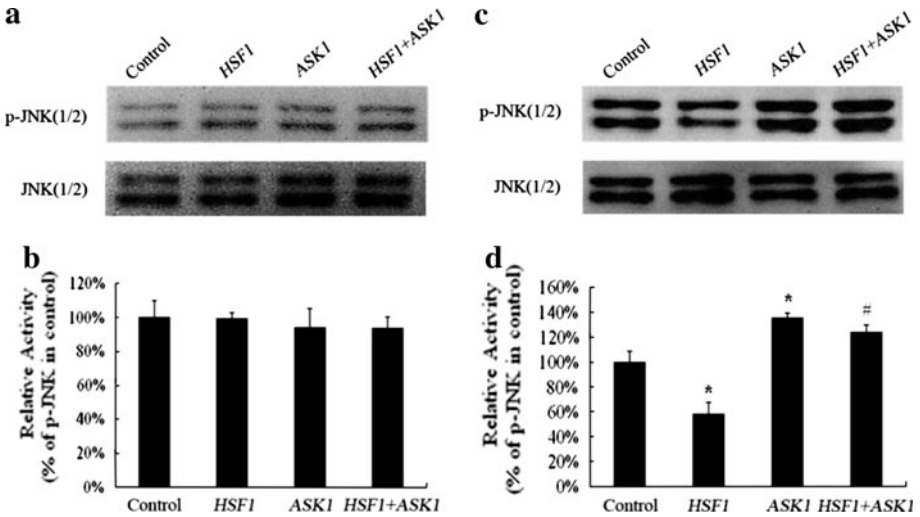

Fig. 4 Phosphorylation of JNK1/2 in cardiomyocytes. Cardiomyocytes were transfected with empty vector (control), *HSF1*, *ASK1*, or *HSF1* plus *ASK1* and incubated with vehicle (**a**) or H_2O_2 (**c**) for 30 min. Total proteins were extracts and subjected to SDS–PAGE. Immunoblot analysis was performed using an anti phospho JNK1/2 (p-JNK1/2) or non-phospho JNK1/2 (JNK1/2) antibody as a loading control. **a**, **c** Representative photograms from three independent experiments are shown. **b**, **d** Quantitative analysis. The intensities of p-JNK1/2 and JNK1/2 bands were measured separately by densitometric scanning of the autoradiograms. Amounts of p-JNK1 and 2 were normalized to JNK1 and 2, respectively, and the value represented the percentage of both p-JNK1 and 2 (p-JNK) in control. Data are shown as mean ± SE from three individual experiments. No significant difference was shown in vehicle-treated cells (**a**, **b**). After H_2O_2 stimulation (**c**, **d**), however, p-JNK was significantly lower in cardiomyocytes transfected with *HSF1* but was significantly higher in cardiomyocytes transfected with *ASK1* than in control. Moreover, p-JNK was significantly higher in co-transfected cardiomyocytes than in *HSF1*-transfected cells. $^{*}\ P < 0.05$ versus control; $^{\#}\ P < 0.05$ versus *HSF1*

may either directly or indirectly influence HSF1 activity and its downstream effects, such as p-JNK and apoptosis inhibition; on the other hand, HSF1 may influence ASK1 activity and its downstream effects, which is manifested by the similar percentage of apoptotic cells between co-transfection group and control group.

In conclusion, this study demonstrates that overexpressing HSF1 may protect cardiomyocytes from apoptosis under oxidative stress via down-regulation of intracellular ROS generation and thereby inhibition of JNK activity. Elevation of ASK1 itself has no effect on intracellular ROS generation, but may affect the function of HSF1 on ROS down-regulation and cardiomyocytes protection.

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Disclosures None.

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