Small Heat-Shock Protein Hsp20 Attenuates 
β-Agonist–Mediated Cardiac Remodeling Through Apoptosis Signal–Regulating Kinase 1

Guo-Chang Fan, Qunying Yuan, Guojie Song, Yigang Wang, Guoli Chen, Jiang Qian, Xiaoyang Zhou, Yong J. Lee, Muhammad Ashraf, Evangelia G. Kranias

Abstract—Chronic stimulation of the β-adrenergic neurohormonal axis contributes to the progression of heart failure and mortality in animal models and human patients. In cardiomyocytes, activation of the β-adrenergic pathway has been shown to result in transiently increased expression of a cardiac small heat-shock protein Hsp20. The present study shows that cardiac overexpression (10-fold) of Hsp20 may protect the heart against β-agonist–induced cardiac remodeling, associated with isoproterenol (50 μg/g per day) infusion for 14 days. Hsp20 attenuated the cardiac hypertrophic response, markedly reduced interstitial fibrosis, and decreased apoptosis. Contractility was also preserved in hearts with increased Hsp20 levels. These beneficial effects were associated with attenuation of the ASK1-JNK/p38 (apoptosis signal–regulating kinase 1/c-Jun NH₂-terminal kinase/p38) signaling cascade triggered by isoproterenol, whereas there was no difference in either extracellular signal-related kinase 1/2 or Akt activation. Parallel in vitro experiments supported the inhibitory role of Hsp20 on enforced ASK1-JNK/p38 activation in both H9c2 cells and adult rat cardiomyocytes. Immunostaining studies also demonstrated that Hsp20 colocalizes with ASK1 in cardiomyocytes. Taken together, our findings indicate that (1) β-agonist–induced cardiac injury is associated with activation of the ASK1-JNK/p38 cascade; (2) increased expression of Hsp20 attenuates the induction of remodeling, dysfunction, and apoptosis in response to sustained β-adrenergic stimulation; and (3) the beneficial effects of Hsp20 are at least partially attributable to inhibition of the ASK1-signaling cascade. (Circ Res. 2006;99:0-0.)

Key Words: small heat-shock protein Hsp20 ■ apoptosis ■ β-adrenergic receptor ■ apoptosis signal–regulating kinase 1 (ASK1)

A poptosis signal–regulating kinase 1 (ASK1) is a ubiquitously expressed mitogen-activated protein kinase (MAPK) kinase (MKK), which activates the c-Jun NH₂-terminal kinase (JNK) (also known as stress-activated protein kinase [SAPK]) and p38 MAPK signaling cascades.1 Overexpression of wild-type (WT) or constitutively active ASK1 induced apoptosis in isolated rat neonatal cardiomyocytes, whereas rat neonatal ASK1-deficient cardiomyocytes were resistant to H₂O₂-induced apoptosis.2 Recent studies have indicated that ablation of ASK1 was associated with inhibition of angiotensin II– and G protein–coupled receptor (GPCR) agonist-induced heart dysfunction and dilatation.3,4; (2) attenuation of cardiac remodeling mediated by myocardial infarction and pressure overload; and (3) rescue of Raf-1 knockout–induced cardiac dysfunction and apoptosis.5 These observations suggest that inhibition of ASK1 may be a promising target for prevention of cardiac remodeling and, thus, suppression of heart failure onset and progression.

Heart failure is characterized by enhanced adrenergic stimulation to correct systolic and diastolic dysfunction, but chronic elevation of sympathetic drive can exert deleterious effects on the myocardium, promoting the development of fibrotic cardiomyopathy.6,7 The mechanisms underlying the transition between the contractile benefits and the maladaptive processing, including cell death, are still heavily debated.8,9 The MAPKs, mainly including JNKs, p38 kinases, and extracellular signal-related kinase 1/2 (ERK1/2), have been implicated in the pro- and antiapoptotic effects of the enhanced β-adrenergic signaling pathway.10,11 However, it is not currently clear whether increased sympathetic tone directly activates the MAPKs or whether regulation occurs upstream of these molecules.

Interestingly, sustained β-adrenergic stimulation has been shown to induce expression of a small heat-shock protein, Hsp20, in the perfused mouse heart and in adult rat cardio-
myocytes. Overexpression of Hsp20 was associated with cardiac protection from ischemia/reperfusion-induced injury and reduced myocardial infarction–mediated apoptosis, consistent with the protective effects of Hsps in the adaptive responses of the heart to physiological/pathophysiologica

Cell Culture and Apoptosis Assays

For transient transfection, H9c2 cells were seeded onto a 6-well plate or 96-well plate and cultured in DMEM supplemented with 10% FBS. Adult ventricular cardiomyocytes were isolated from 3-month-old male Sprague–Dawley rats (Harlan Laboratory), as previously described. An adenosinergic vector Ad.as.Hsp20 was generated by reverse insertion of Hsp20 cDNA into the AdEasy-1/Shuttle backbone, similar to our Ad.Hsp20 generation. Ad.ASK1 was generated as described previously. Cell morphology as well as apoptosis were studied after transfection/infection for 48 hours. Cell viability assessment was performed with the CellTitre 96 AQueous One Solution Cell Proliferation Assay Kit (Promega). Apoptosis analysis was performed as described above.

Statistical Analysis

All data are presented as mean±SEM. Statistical significance was determined with 1-way ANOVA, followed by Duncan multiple range comparison test using SuperANOVA (Abacus Concepts Inc). Differences were considered statistically significant at a value of \( P<0.05 \).

Results

Attenuation of Isoproterenol-Induced Hypertrophy by Hsp20 Overexpression

The function of Hsps, a set of highly conserved cytoprotective proteins, has not been defined in the regulation of \( \beta \)-adrenergic receptor (\( \beta \)-AR)–triggered cardiac injury. To determine the role of a small heat-shock protein, Hsp20, in \( \beta \)-agonist–induced cardiac hypertrophy and cardiomyopathy, we chronically infused Hsp20 TG and littermate WT mice with isoproterenol (ISO). WT mice displayed a marked increase in heart size and heart to body weight ratio by 1.5-fold (Figure 1A and 1B); however, in ISO-infused Hsp20 TG mice, the heart to body weight ratio was increased by only 1.2-fold, indicating that Hsp20 attenuates the ISO-mediated cardiac hypertrophic response (Figure 1A). Importantly, measurements of cardiomyocyte size in cross-sections further confirmed attenuated cardiomyocyte hypertrophy in Hsp20 TG mice (Figure 1B). In accordance, there were no significant increases in the mRNA levels of hypertrophy markers: ANF and BNP in Hsp20 TG hearts, subjected to chronic ISO infusion (Figure 1C).

Reduced ISO-Induced Interstitial Fibrosis and Apoptosis by Hsp20

It has been previously shown that prolonged stimulation with ISO induces cardiac apoptosis and fibrosis in mice, leading to decompensation of the hypertrophic myocardium and the progression to cardiomyopathy. Therefore, we performed histological analysis of our TG mice subjected to chronic ISO infusion to determine the degree of cardiac remodeling. In control hearts (WT), treatment with ISO resulted in disorganization of the myocytes and a marked increase in cardiac fibrosis (Figure 2A), consistent with previous reports. By contrast, Hsp20 TG mice displayed no obvious cardiac fibrosis or alteration in myocyte organization (Figure 2A). Importantly, the number of apoptotic cardiomyocytes in ISO-treated WT hearts was 4-fold higher, compared with Hsp20 TG hearts (Figure 2B). Concomitantly, there was a marked increase in DNA fragmentation assayed by cell death ELISA in WT compared with TG hearts (Figure 2C). In addition, after ISO infusion for 14 days, the

Materials and Methods

Animal Preparation and Osmotic Minipump Infusion

Generation of cardiac-specific overexpressed Hsp20 mice has been described previously. The protein level of Hsp20 was 10-fold overexpressed in the heart. Adult WT and TG mice, inbred on a FVB/N background, were studied at 8 to 10 weeks. All procedures were in accordance with institutional guidelines for animal research. Osmotic minipumps (model 2002, Alzet) were implanted into 10-week-old male mice over a period of 14 days, as described previously.

Cardiac Remodeling Assay and Cardiac Contraction Measurements

Heart sections were stained with a fluorescence Oregon Green 488–labeled wheat germ agglutinin (1:100 dilution; Invitrogen), and cardiac interstitial fibrosis was determined by Masson’s Trichrome staining. Myocardial apoptosis assay was performed using the DeadEnd Fluorometric TUNEL system (Promega), followed by staining with an anti–DNA fragmentation was assessed using the Cell Death Detection ELISAplus kit (Roche). In situ DNA fragmentation was assessed using the DeadEnd Fluorometric TUNEL system (Promega), followed by staining with an anti–α-sarcosomic actin antibody (Sigma-Aldrich) and 4’,6-diamidino-2-phenylindole (DAPI) (Invitrogen). Dot blot analysis of total RNA from cardiac ventricles was performed to examine of the atrial natriuretic factor (ANF) and brain natriuretic peptide (BNP) mRNA levels, as described previously. Cardiac function was assessed by Langendorff preparations ex vivo and left ventricular pressure/volume relationships in vivo, as previously described.

Western Blot Analyses, In Vitro ASK1 Kinase Assay, and Cardiac Lipid Peroxidation Level Measurements

Frozen hearts were homogenized in Cell Lysis Buffer (Cell signaling Inc) supplemented with 1 mmol/L phenylmethylsulfonyl fluoride (PMSF) and protease inhibitor cocktail. Then equal amounts of protein (100 μg) from each heart were analyzed by 10% SDS-PAGE. The activity of ASK1 was measured by an immune complex kinase assay, as described previously. The effect of ISO administration on oxidative stress in the heart was examined by measurements of the content of cardiac thiobarbituric acid reactive substances (T-BARS), as previously described.

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ratio of lung weight to body weight increased by \( \approx 30\% \) in WT mice, whereas there was no significant difference in Hsp20 TG mice (Figure 2D).

**Preserved Cardiac Function in ISO-Treated Hsp20 TG Mice**

Given the marked differences in cardiac remodeling and degree of apoptosis, we investigated the functional alteration in mice infused with ISO. As previously shown, Hsp20 overexpression was associated with enhanced basal cardiac function,\(^{13,15}\) and this was maintained on chronic infusion of ISO as revealed by hemodynamic measurements using in vivo cardiac catheterization. The left ventricular ejection fraction, maximal derivative of LV pressure (+dP/dt\(_{\text{max}}\)), and minimal derivative of LV pressure (−dP/dt\(_{\text{min}}\)) were significantly depressed on prolonged \( \beta \)-agonist stimulation in WT animals (Figure 3A), indicating severe impairment of heart function. However, Hsp20 TG mice exhibited a significant reduction in only +dP/dt\(_{\text{max}}\) (Figure 3A). Importantly, the treated Hsp20 TG hearts retained enhanced cardiac contractility relative to control or nontreated WT hearts. To avoid the in vivo confounding effects, such as systemic circulation and a host of peripheral complications, we further assessed cardiac function ex vivo, using the Langendorff preparation. Chronic infusion of ISO was associated with a significant reduction in left ventricular developed pressure (LVDP) (by 33%) and rates of contraction (+dP/dt: by 36%) and relaxation (−dP/dt: by 26%) in WT mice (Figure 3B). However, the reduction of these contractile parameters was attenuated in ISO-treated TG mice (LVDP was decreased by 14%; +dP/dt by 17%; and −dP/dt by 12%; Figure 3B), consistent with the in vivo findings.

**Downregulation of ISO-Mediated Activation of Stress Kinases by Hsp20**

MAPK signal transduction pathways have been shown to be activated in isolated cardiac myocytes and heart tissue by a variety of hypertrophic stresses, including proinflammatory cytokines, neurohormonal stimuli, ischemia/reperfusion, and hemodynamic overload.\(^{24}\) Recent studies demonstrated that acute administration of ISO in rat hearts significantly increased ERK1/2, p38, and JNK phosphorylation via \( \beta \)-AR.\(^{10,22}\) Thus, we investigated activation of the MAPK pathway in Hsp20 TG or WT hearts treated with or without ISO for 14 days. Western blotting analysis indicated that there were no increases in the activity of ERK1/2, p38, and JNK kinases (data not shown), consistent with previous reports.\(^{10,22}\) We then acutely treated hearts with intraperito-
neal injections of different doses of ISO (10, 15, 30, 50 mg/kg body weight) for 30 minutes, and heart tissues were rapidly harvested for analysis of MAPK signaling. We found that the phosphorylated levels of p38, SAPK/JNK, and ERK1/2 were significantly increased in both WT and Hsp20 TG hearts treated with various ISO doses; however, the degree of p38 and SAPK/JNK activation was significantly greater in WT hearts, whereas ERK was similarly activated in the 2 groups (Figure 4). Although a wealth of information indicates that Akt signaling plays a crucial role in regulation of cardiac remodeling and apoptosis, and recent findings showed that JNK mediates reactivation of Akt in cardiomyocytes, we did not observe any differences in Akt activation triggered by ISO between WTs and TGs, as determined by immunoblotting for phosphorylation of either Thr308 or Ser473 (Figure 4). Intriguingly, the endogenous Hsp20 levels were decreased by 36±3% in these acutely ISO-treated WT hearts (Figure 4), whereas prolonged ISO-perfusion of hearts ex vivo resulted in increased expression of Hsp20. These alteration of Hsp20 levels may be attributable to the adaptive responses of the heart to the stress. Collectively, these data suggest that Hsp20 overexpression downregulates the activation of p38 and SAPK/JNK, although it has no effects on ERK or Akt activation in hearts subjected to β-adrenergic stimulation.

**β-Agonist–Induced Activation of ASK1 and Attenuation by Hsp20**

The increases in both p38 and JNK in WT mice suggest that their upstream regulators ASK1 or mixed-lineage kinase (MLK) 3/7 may be possibly activated by ISO stimulation. It has been previously shown that activation of JNK/p38 is responsible for apoptosis and is mainly mediated by ASK1 in neonatal myocytes. ASK1 usually exists as an inactive form in resting cells, and on stress, it becomes activated by subsequent modifications, including oligomerization as well as auto- and/or cross-phosphorylation of Thr845 or -dephosphorylation of Ser967, which are both located within the activation loop of ASK1. Therefore, we first explored the possibility of cardiac ASK1 activation, as determined by immunoblotting for phosphorylation of either Thr845 or Ser967 (Figure 5A). Although the immunoreactive signals, associated with ASK1 protein levels or its degree of phosphorylation, were rather weak in the mouse heart, quantitative immunoblotting of at least 10 hearts per group indicated that ISO stimulation resulted in higher increases in the levels of phosphorylated Thr845-ASK1 in WT (1.8-fold) than TG (1.2-fold) hearts. In contrast, the phosphorylation levels of Ser967-ASK1 were significantly decreased only in WT hearts, whereas there were no alterations in TGs, on ISO treatment. In vitro kinase activity assays further indicated that ASK1 activity was significantly reduced in ISO-treated TG hearts.
hearts (Figure 5B), suggesting that Hsp20 overexpression may suppress ISO-mediated ASK1 activation.

It has been previously reported that β-AR stimulation evokes cardiac oxidative stress, which possibly induces dephosphorylation of Ser967 and phosphorylation of Thr845, both of which result in increased ASK1 activity. To clarify whether the difference of ASK1 activation between WT hearts and TG samples is generated by the difference of ISO-induced oxidative stress, the left ventricle levels of T-BARS were measured. The concentration of T-BARS significantly increased and its levels were similar in WT and TG hearts, indicating that the overexpressed Hsp20 did not suppress the production of T-BARS levels, evoked by ISO (Figure 5C). These data suggest that downregulation of ISO-triggered ASK1 activation by Hsp20 is not derived from the alteration of oxidative stress generated by ISO.

Interestingly, coimmunostaining of adult WT mouse cardiomyocytes with ASK1 (Figure 5D) and Hsp20 antibodies (Figure 5E) demonstrated their colocalization (Figure 5F). However, coimmunoprecipitation studies with either ASK1 or Hsp20 antibodies did not reveal physical interaction between these proteins, although Hsp20 did complex with 14-3-3, and 14-3-3 did coimmunoprecipitate with ASK1 in WT mouse cardiomyocytes (Figure 5G), consistent with previous reports. Thus, it is currently unclear whether Hsp20 enhances 14-3-3 inhibition of ASK1 or Hsp20 weakly binds with ASK1.

**Hsp20 Inhibits Enforced ASK1-p38/JNK Signaling Pathway In Vitro**

To further examine whether the in vivo findings on downregulation of ASK1 activation involves direct or indirect effects by Hsp20, we overexpressed ASK1 in H9c2 cardiomyoblasts, which were also cotransfected with Hsp20. Immunobots revealed that ASK1 was coexpressed with Hsp20; however, the levels of phosphorylated T845-ASK1 were significantly decreased, whereas the levels of Ser967-ASK1 phosphorylation were greatly increased by Hsp20 overexpression, compared with the controls (Figure 6A). Consequently, endogenous JNK and p38, the downstream molecules of the ASK1 signaling cascade, were inactivated, consistent with the suppressed ASK1 activity by Hsp20 (Figure 6A).

To investigate the effects of ASK1-p38/JNK signaling on cellular growth or viability, we measured MTS-based cell incorporation. The decreased absorbance values (490 nm) of the formazan product, resulting from reduction of the MTS tetrazolium compound, were observed in the cells transfected with ASK1 alone or cotransfected ASK1 with empty vector phospho–enhanced green fluorescent protein (p-EGFP). However, cotransfection ASK1 with Hsp20 did not result in any alteration of the absorbance values, similar to control transfections with Hsp20 or p-EGFP alone (Figure 6B). These data suggest that Hsp20 inhibits ASK1-induced cell death. In parallel studies, overexpression of ASK1 indicated a significant increase in the number of apoptotic cells, exhibiting cytoplasmic shrinkage and nuclear condensation, typical morphological properties of apoptotic cells, which were observed by Hoechst staining. However, the number of apoptotic cells was greatly reduced by cotransfection with Hsp20, but not with the empty vector p-EGFP (Figure 6C). Our observation that Hsp20 inhibited ASK1-mediated apoptosis was further confirmed by an in situ staining of cells.
Figure 5. Inhibition of ASK1 activation in response to ISO stimulation without alteration of T-BARS levels in Hsp20 TG hearts. A, Western blot analysis of ASK1 activation in ISO-injected WT or TG hearts (n=10 per group). *P<0.05 vs saline injected, #P<0.05 vs WT hearts injected with ISO. B, ASK1 activity was measured by immune complex assay, using glutathione S-transferase/MKK6 (GST-MKK6) as a substrate (n=7). *P<0.01. C, Measurement of T-BARS content to examine the effects of acute ISO administrations on oxidative stress in WT or Hsp20 TG hearts (n=7 per group). *P<0.05 vs saline injected. D through F, Colocalization of ASK1 with Hsp20 in adult WT mouse cardiomyocytes, assessed by confocal microscopy. Staining was performed with: rabbit anti-ASK1 (1:25 dilution; sc-7931, Santa Cruz Biotechnology Inc), followed by Alexa Fluor 488 goat anti-rabbit IgG (1:500 dilution) and mouse anti-Hsp20 (1:1000 dilution, RDI-TRK4Hsp20), followed by Alexa Fluor 594 goat anti-mouse IgG (1:500 dilution). G, WT mouse heart was homogenized with 1× Cell Lysis Buffer (Cell signaling Inc) and incubated with agarose-conjugated ASK1 antibody or 14-3-3β antibody (Santa Cruz Biotechnology Inc) overnight. The immune complex was washed 5 times with 1× Cell Lysis Buffer, then boiled in sodium dodecyl sulfate–loading buffer and subjected to immunoblot analysis. Controls included mouse cardiac homogenates (+) or agarose beads alone (−).
with the terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) method (data not shown). Taken together, these observations indicate for the first time that overexpression of Hsp20 directly blocks ASK1-induced cell death and apoptosis.

**Downregulation of Hsp20 in Adult Rat Cardiomyocytes Is Sensitive to ASK1-Mediated Apoptosis**

To further examine the antiapoptotic role of Hsp20, we downregulated its expression levels in adult rat ventricular cardiomyocytes by antisense mRNA of Hsp20. Immunoblots indicated that ASK1 was coexpressed with Hsp20, and endogenous Hsp20 was decreased by 40% in cardiomyocytes infected or coinfected with Ad.asHsp20 or Ad.asHsp20 + Ad.ASK1, respectively. The ASK1 signaling cascade activation (phospho-p38 [p-p38] and phospho-JNK [p-JNK]) was attenuated by Hsp20 overexpression, consistent with the data generated in H9c2 cells (Figure 6A). In accordance, downregulation of Hsp20 was associated with increased activation of the ASK1 signaling pathway (Figure 7A). Consequently, ASK1-induced cell death, determined by MTS, was significantly inhibited in cardiomyocytes coinfected with Ad.Hsp20. Importantly, decreased Hsp20 expression led to higher sensitivity to ASK1-triggered cell death, compared with cardiomyocytes infected with Ad.ASK1 or Ad.ASK1 + Ad.GFP (Figure 7B). Similarly, the number of pyknotic nuclei induced by ASK1 overexpression was increased by 18% in cardiomyocytes coinfected with Ad.asHsp20, whereas this number was reduced by 50% in cardiomyocytes coinfected with Ad.Hsp20, compared with coinfection with Ad.GFP (Figure 7C). These observations were further confirmed in DNA fragmentation assays, as determined by a cell death ELISA kit (Figure 7D). Collectively, these data further demonstrate that increased Hsp20 expression protects adult rat cardiomyocytes against ASK1-induced cell death and apoptosis.

**Discussion**

Chronic enhancement of sympathetic tone most commonly derives from primary decreases in cardiac output, which elicit graded physiological increases in adrenergic drive to preserve function.7,8 However, chronic stimulation of β-ARs has been shown to exert adverse effects on cardiac myocytes, including left ventricular hypertrophy, oxidative stress, calcium overload, fibrosis, and continuous loss of viable myocytes.10,11,22 The underlying mechanisms have been suggested to involve the reactive oxygen species (ROS)/JNK-dependent mitochondrial pathway11 and the Ca2+–activated calmodulin kinase II pathway.28,29 Recent studies have also shown that β-adrenergic stimulation activates p38 MAPK, which provides a negative feedback to ISO-induced contractile response in cardiomyocytes; and cardiac overexpression of p38 MAPK results in cardiac remodeling and severe cardiomyopathy.30,31 The current study provides additional insights and identifies ASK1, upstream of JNK/p38, as a mediator of the detrimental effects elicited by enhanced β-agonist stimulation. In addition, Hsp20 is identified as an important inhibitor of ASK1 activation, leading to protection from chronic β-agonist–induced cardiac remodeling.

ASK1 activity has been reported to be inhibited by several cellular factors and some large Hsps. A reduction/oxidation (redox)-regulatory protein, thioredoxin (Trx), was identified...
as an interacting partner of ASK1 in epithelial cells.26 Under normal conditions, Trx in the reduced state binds to and inhibits ASK1, whereas on oxidative stress, oxidation of Trx triggers its dissociation from ASK1, allowing the activation of ASK1 and the subsequent activation of downstream kinases. 14-3-3, a phosphoserine-binding molecule, was also shown to bind ASK1 specifically via Ser967 and to inhibit ASK1-induced apoptosis in HeLa cells and COS7 cells.32 Furthermore, Hsp72 physically associates with ASK1, thereby inhibiting H2O2-induced activation of ASK1 in NIH 3T3 cells.33 Hsp90 also forms a complex with ASK1 to retain ASK1 in an inactive state and protects endothelial cells from H2O2-induced apoptosis.34 Thus, various regulators may act via different mechanisms to block the activity of ASK1. In the present study, we show that overexpression of Hsp20 inhibits ASK1 activation triggered by ISO stimulation, which may result in decreased cardiac necrosis, consequently leading to attenuated fibrosis and hypertrophy. Moreover, our data demonstrate that Hsp20 overexpression greatly decreases ASK1-mediated apoptosis in vivo and in vitro, which could be a key modulator in the transition from compensatory hypertrophy to heart failure. Although it is currently unclear whether Hsp20 binds with ASK1, coimmunostaining results demonstrated that Hsp20 colocalized with ASK1 in mouse cardiomyocytes. Therefore, it is plausible that overexpressed Hsp20 renders ASK1 inaccessible to activation, resulting in reduced activity of downstream signaling cascade kinases JNK/p38 (Figure 8).

The ASK1-JNK/p38 signaling pathway has been reported to be crucial in cardiac apoptosis and remodeling,13,35 and upstream activators for JNK and p38 elicit characteristic hypertrophic responses in cardiomyocytes.18,35 Our findings are consistent with these reports and indicate that ASK1 is involved in ISO-mediated cardiac remodeling. Suppression of the ASK1-JNK/p38 signaling pathway by Hsp20 may underlie its cardioprotective effects (Figure 8). Although there is general agreement that ASK1 inactivation is cytoprotective, there are contradictory reports as to whether activation of the JNK and p38 MAPK pathway is protective or detrimental. Downregulation of JNK or p38 MAPK signaling by either genetic or pharmacological approaches has demonstrated cardioprotective effects.24,36,37 In contrast, other studies suggest that JNK or p38 MAPK is capable of transducing antiapoptotic signals.24,38 Some studies have also shown that p38 MAPK is not required, but the Raf/MKK/ERK pathway is essential for hypertrophy.39 More recently, a study further indicates that dual JNK/p38 inhibition leads to increased ischemia-reperfusion-induced cardiomyocyte apoptosis; however, this report also shows that the proapoptotic effects of the dual JNK/p38 inhibitor V-150 are possibly attributable to suppression of JNK as opposed to p38 MAPK.25 Nevertheless, these previous studies collectively suggest that although inhibition/activation of either p38MAPK or JNK signaling pathways produces the same cardiac phenotype, the temporal manifestation of the disease possibly depends on the overall extent of cellular signaling inhibition/activation, and especially the upstream molecules of JNK/p38MAPK. This view is further supported by a recent study on MLK7, another member of the MAPKKK family. MLK7 was reported to overexpress MLK7-induced cardiac hypertrophy,18 Interestingly, the rapid progression to decompensation and death observed in ISO-treated MLK7 TG mice indicated that dual activation (p38 and JNK) leads to cardiac apoptosis and fibrosis, which in turn promotes the progression of hypertrophy to heart failure. Hsp20 overexpression suppresses the activation of ASK1-JNK/p38 signaling, which results in attenuation of ISO-mediated hypertrophy and cardiac remodeling.
tissue- and time-specific manner and further clarify the beneficial effects of this protein under stress insults.

In summary, the present work demonstrates that Hsp20 may attenuate cardiac remodeling and heart failure, in response to prolonged activation of the β-receptor signaling pathway. The mechanism underlying the protective effects of Hsp20 appears to involve the downregulation of the ASK1-p38/JNK cascade, leading to inhibition of cardiac hypertrophy, apoptosis, and fibrosis, consequently hindering the progression of β-agonist–mediated heart failure (Figure 8). Thus, Hsp20 may represent a potential therapeutic target for heart disease.

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Disclosures

None.

References


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