Intracellular or extracellular heat shock protein 70 differentially regulates cardiac remodelling in pressure overload mice

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Aims

Innate and adaptive immune responses are associated with the development of hypertension-induced myocardial hypertrophy and fibrosis. As a result, we investigated whether heat shock protein (HSP) 70, which is a molecule of damage-associated molecular patterns, could induce inflammation in the myocardium and promote the development of hypertension-induced cardiac hypertrophy and fibrosis.

Methods and results

We found that HSP70 serum levels, as well as the amount of HSP70 translocation to the cardiomyocyte membranes and the interstitial space, were elevated in the hypertensive mice caused by abdominal aortic constriction (AAC). Transcriptional inhibition of HSP70 expression by a specific heat shock transcript factor inhibitor, KNK437, reduced the serum level, and the re-distribution of HSP70. It promoted myocardial hypertrophy and cardiac dysfunctions although it protected animals from AAC-induced cardiac fibrosis. On the other hand, the functional antagonism of HSP70 by an anti-HSP70 antibody attenuated AAC-induced cardiac hypertrophy and fibrosis without adverse haemodynamic effects. The cardioprotective effect of the anti-HSP70 antibody was largely attributed to its ability to block AAC-activated immune response in the heart, as was indicated by suppressing the hypertension-enhanced conjugation of HSP70 with toll-like receptor 4, reducing heart-infiltrating macrophages, decreasing the expression of pro-inflammatory factor monocyte chemoattractant protein-1 and profibrotic factor transforming growth factor beta 1, and attenuating pro-hypertrophy signal MAPK P38 and ERK.

Conclusion

These results indicate that intracellular and extracellular HSP70 have different roles in the regulation of cardiac remodelling and function in response to hypertension. Extracellular HSP70 is a potential therapeutic target against cardiac hypertrophy and fibrosis.

Keywords

Hypertension • Angiotensin • HSP70 • Cardiac Hypertrophy • Fibrosis

1. Introduction

Abnormal cardiac remodelling, which includes cardiac hypertrophy and fibrosis, plays a fundamental role in the pathogenesis of cardiovascular diseases such as hypertensive heart disease and chronic heart failure.1,2 Numerous studies have indicated that blood pressure lowering agents alone cannot improve the mortality and morbidity of hypertensive heart disease. However, agents that blunt the activity of the renin–angiotensin–aldosterone system can inhibit the progression of cardiac remodelling and reduce the mortality rate associated with hypertensive heart failure.1 Recent studies demonstrate that inhibiting inflammatory cell infiltration and pro-inflammatory cytokine expression in the myocardium markedly decreases pressure-overload-induced myocardial hypertrophy and fibrosis, as well as cardiac diastolic dysfunction,3 suggesting that inflammation is critical in the regulation of pressure overload-induced cardiac remodelling. Importantly, activating pattern recognition
receptors (PRRs), a group of receptors that regulate innate immune responses, mediates the hypertension-induced inflammatory response in the myocardium. Knocking-out toll-like receptor 4 (TLR4) or blocking MyDB88, an adaptor protein of TLRs, leads to a reduction in cardiac hypertrophy following pressure overload. Application of the BCG vaccine or TLR4 agonist protects animals from pressure overload-induced cardiac hypertrophy and fibrosis without unfavourable haemodynamic effects. Indeed, PRRs guide the polarization of adaptive immune response to determine the development of cardiac hypertrophy and fibrosis. A Th2 immune microenvironment facilitates myocardial collagen synthesis and crosslinking, and increases ventricular stiffness in diabetic rats. 

Factors that function as PRR inducers to initiate the inflammatory responses and mediate hypertension-induced myocardial hypertrophy and fibrosis are unknown. Recently, endogenous molecules termed the damage-associated molecular patterns (DAMPs), which include heat shock proteins (HSPs), high-mobility group box 1, 5,10 S100 family of proteins, uric acid, and adenosine, have been found to function as PRR ligands. These molecules are located in the cytoplasm to maintain normal cellular function. Under adverse conditions, such as ischaemia, hypoxia, or microbial infection, these DAMP molecules are released from the cells and activate the immune response by interacting with PRRs on immune and resident cells. 

Accumulating evidence indicates that DAMPs participate in the pathogenesis of arthritis, multiple sclerosis, cardiovascular diseases, tumour progression, and metastasis. For instance, S100A4 is released from macrophages in injured myocardium and plays a critical role in the growth and survival of cardiac myocytes. The expression of HSPs is induced by a number of stressors, including hyperthermia, pressure overload, oxidative stress, and radiation. HSPs are released into the extracellular space and function as DAMPs. They can induce cardiovascular disorders via stimulating DCs or macrophages to express and secrete inflammatory cytokines, such as TNF-α, and mediating the maturation of T regulatory cells to release immunosuppressive cytokines involved in the pathogenesis of atherosclerosis. 

Indeed, there is a positive correlation between the serum level of HSP70 and the severity of hypertensive heart disease. However, the precise role and mechanism of HSP70 in the development of hypertension-induced myocardial hypertrophy and cardiac fibrosis is largely unknown.

In this study, we investigated whether HSP70 could induce an immune response in the myocardium that regulates hypertension-induced cardiac hypertrophy and fibrosis. By comparing the transcriptional inhibition of HSP70 expression or the functional antagonism of extracellular HSP70, we found that changes in the levels of intracellular and extracellular HSP70 produced different responses in the pressure overload-induced cardiac hypertrophy and fibrosis in a mouse model of hypertension caused by abdominal aortic constriction (AAC). Our studies indicated that extracellular HSP70 is a potential target for the discovery and development of therapeutic agents against cardiac hypertrophy and fibrosis in cardiovascular diseases, since targeting extracellular HSP70 reversed the pressure overload-induced cardiac remodelling.

2. Methods

2.1 Animal model

All mice were purchased from Vital River (Beijing, China). The present study was in accordance with the Guide for the Care and Use of Laboratory Animals published by US National Institutes of Health, and was approved by the Institutional Committee for the Ethics of Animal Care and Treatment. AAC was performed via aortic banding as previously reported. Briefly, the mice were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.), and the aorta was dissected above the two renal arteries. A blunted needle was laid alongside the exposed aorta. A silk ligature was passed under the aorta and needle and tied securely. After the tie had been completed, the needle was withdrawn. The methods used for supplementary experiments are available online in the Supplementary material.

In experiment 1, male ICR mice were divided into four groups: sham-operated group, AAC group, and two groups that received KNK437 i.p. (10 or 25 mg/kg; kind gift from KANEKA Corporation of Japan) 2 h prior to AAC and every other day for 7 days after the procedure. Five weeks post-operatively, the animals were sacrificed and their hearts were harvested for the indicated studies. In experiment 2, male ICR mice were divided into four groups: sham-operated group, AAC group, and two groups that received either anti-HSP70 or IgG (200 µg/kg, Santa Cruz Biotech., CA, USA) i.v. 1 day prior to AAC and every 3 days after AAC. Five weeks post-operatively, the animals were sacrificed and their hearts were harvested for the indicated studies.

2.2 Echocardiography and haemodynamics

On day 34 of AAC, echocardiography was performed with the Visual Sonics Vevo 770 system (VisualSonics, Canada) using a 30 MHz image transducer. Mice were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.). After a good quality two-dimensional image was obtained, M-mode images of the left ventricle (LV) were recorded. End-diastolic left intraventricular septum (IVST) and posterior wall thickness (PWTH) were measured. Per cent fractional shortening (FS %) and left ventricular ejection fraction (EF %) were automatically calculated on a cardiac ultrasound machine. On day 35 of AAC, haemodynamic parameters were measured with MPA2000 (Alcott Biotech) as previously described.

2.3 Histological analysis

The hearts were fixed in 4% paraformaldehyde, embedded in paraffin, sectioned (4 µm thickness), and stained with haematoxylin—eosin or Masson’s trichrome blue for the analysis of hypertrophy and fibrosis. To determine the cardiomyocyte size, the shortest transverse diameter was measured in the nucleated transverse section of the myocyte (n ≥ 6 per group, eight regions per heart, at least 50 cells per heart for size estimates). To determine collagen deposition, sections stained with Masson’s trichrome were scanned and analysed with a digital image analyser. Collagen fractions were calculated as the ratio of the collagen area to the total ventricular area in the section (n ≥ 6 per group, 10 regions per heart). Immunohistochemical staining with 3, 3-diaminobenzidine was performed on paraffin-embedded slices. The sections were scanned at ×200 magnification. The images were then digitalized, and the integrated ODs of IFN-γ, TGF-β1, and MCP-1 were calculated by software Image-Pro plus 5.1.

2.4 Quantitative RT–PCR

Total RNA was extracted from the frozen LV using a Trizol kit (Invitrogen), following the manufacturer’s instructions. The RNA was reverse-transcribed and amplified. PCR was performed with Mxycler thermal cycler and analysed by agarose gel electrophoresis using the following specific primer sequences: mouse pro-collagen (5′-CCA-GAGTG GGAACAGCGATTAC-3′; (A) 5′-CAAGGGCAGATGGTTATTTT-3′; mouse β-actin (5′-T GTGAAAATCTGTGGATCCATGAAA-3′; and (A) 5′-TAAACGAGTCAGTAACAG-3′). The value was normalized to the values obtained with the β-actin.

2.5 ELISA for the detection of serum HSP70

HSP70 in serum was detected as described previously. The monoclonal primary antibody (SPA-810), which was used as a coating antibody, the
rabbit polyclonal detection antibody (SPA-812), and the HSP70 protein (NSP555) were purchased from Stressgen Biotechnologies (Victoria, Canada). Anti-rabbit immunoglobulin peroxidase, bovine serum albumin, and o-phenylenediamine dihydrochloride substrate were purchased from Sigma-Aldrich (St Louis, MO, USA).

2.6 Immunolabelling and confocal microscopy
Frozen sections (10 μm) were fixed for 10 min with acetone, followed by staining with primary and secondary antibodies. Specific binding of primary Abs was detected using corresponding secondary FITC- or Rhodamine-conjugated goat anti-rabbit, FITC-conjugated goat anti-mouse/rabbit, or Rhodamine-conjugated goat anti-mouse/rat (Santa Cruz Biotech). F-actin in cardiomyocyte was stained directly with anti-conjugated phalloidin. The distribution of HSP70 in cardiomyocyte and the binding of HSP70 to TLR4 were examined using an E2000U confocal microscope and evaluated using Leica TCS SP2 software. Heart-infiltrating macrophages were evaluated using upright microscopes at ×400 magnification.

2.7 Angiotensin converting enzyme activity
Five days after AAC, the mice were sacrificed and the LV was isolated, frozen in liquid N2, and stored at −80°C. Frozen tissue was homogenized with 0.9% NaCl, and angiotensin converting enzyme (ACE) activity in the homogenates was detected by UV-spectrophotometry as described previously.19

2.8 Membrane HSP70 analysis
To detect HSP70 on the surface of cardiomyocytes, cardiomyocytes were isolated from sham, model, and candesartan-treated mice. Membrane HSP70 on surface of cardiomyocytes was analysed by flow cytometry and western blot as described previously.20

Figure 1 Pressure-overload-induced HSP70 expression in the myocardium, deposition on the cardiomyocyte membrane, and elevation of serum levels. (A) AAC induced a time-dependent increase in the systolic arterial pressure. Treatment with HSF-1 inhibitor KNK437 or HSP70 antibody did not decrease blood pressure. (B) Hypertension enhanced the mRNA expression of HSPs. The hearts were harvested 8 h after AAC. Total RNA were prepared from the hearts of sham operated, AAC, and KNK437 treated mice. Expression of HSP mRNAs was detected by RT−PCR. With the exception of HSP60, the expression of HSP mRNAs was increased after pressure overload. Only HSP70 expression was inhibited by KNK437. (C) KNK437 suppressed HSP70 expression by inhibiting phosphorylation of HSF-1. Total proteins were prepared from the hearts of sham operated (S), AAC (M), and KNK437 (KNK) treated mice. The expressions of HSF-1, phosphorylated HSF-1, and HSP70 were detected by western blot. (D) Hypertension-induced time-dependent increase in the serum concentration of HSP70 detected by ELISA analysis. (E) Translocation of HSP70 to the cardiomyocyte membrane stimulated by hypertension was detected by confocal microscopy on days 3 and 7. (F) Anti-HSP70 antibody decreased the interaction between HSP70 and TLR4. The left ventricular sections were stained with antibodies against TLR4, HSP70, and nuclei and detected by three-colour confocal microscopy. Images are representative sections from six mice per group (green, HSP70; red, TLR4; blue, nuclei). Scale bar, 30 μm. Data are mean ± SEM (n ≥ 6). *P < 0.05, **P < 0.01 vs. the sham group; #P < 0.05, ##P < 0.01 vs. the model group.
2.9 Western blot analysis

Cytoplasmic fractions were prepared as described previously. Western blots were performed as described. Specific antibody binding was visualized by ECL (Amersham Biosciences).

2.10 Statistical analysis

All results are expressed as mean ± SEM. Multiple comparisons among three or more groups were performed by one-way ANOVA, and LSD’s exact test was conducted for post hoc analyses. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1 Hypertension increased the serum level of HSP70 and resulted in accumulation of HSP70 on the cardiomyocyte membrane

We found that the AAC procedure led to a 30 mmHg elevation in blood pressure from the beginning to the end of experiment (Figure 1A), which significantly enhanced mRNA expressions of HSP27, HSP32, HSP70, and HSP90 in the myocardium at 8 h.
3.2 Angiotensin II mediated the AAC-induced accumulation of HSP70 on the cardiomyocyte membrane and its release into the intercellular space

ACE, a key enzyme converting angiotensin I into II, was activated in myocardium at the fifth day after AAC (Figure 2A and B). Moreover, a sub-depressurization of angiotensin II (Ang II) receptor antagonist candesartan significantly inhibited the accumulation of HSP70 on the membrane of cardiomyocytes (Figure 2C and D), which resulted in a reduction in the release of HSP70 into the intercellular space (Figure 2E). These results indicate that Ang II receptor mediated, at least partially, the AAC-induced accumulation of HSP70 on the cardiomyocyte membrane and the release of HSP70 into the intercellular space.

3.3 Transcriptional inhibition of HSP70 expression enhanced myocardial hypertrophy but suppressed cardiac fibrosis induced by AAC

KNK437 is a specific inhibitor of HSF-1, which inhibits the hyperthermia-induced expression of HSP in tumour cells. Treatment of AAC mice with KNK437 resulted in suppression of the phosphorylation of HSF-1 (Figure 1C). It selectively decreased the serum level and myocardium expression of HSP70, but did not change the expression of HSP32, 60, and 90 (Figure 1B). It also reduced the AAC-induced abnormal distribution of HSP70 on the cardiomyocyte membrane (see Supplementary material online, Figure S1). Although KNK437 did not induce cardiac hypertrophy in untreated mice (see Supplementary material online, Figure S2), the treatment of animals with KNK437 (10 or 25 mg/kg) 1 week after AAC significantly enhanced hypertension-induced cardiac hypertrophy, as indicated by a significant increase in the IVST and PWTH at end-diastole, as well as an increase in myocyte diameter in AAC mice (Figure 3A and B). However, transcriptional inhibition of HSP70 expression remarkably blunted the AAC-induced interstitial and perivascular fibrosis (Figure 3C and D), and decreased collagen accumulation (Figure 3E). Cardiac fibroblasts are integral to the pathogenesis of cardiac fibrosis because they secrete pro-collagen I and numerous cytokines to promote its development. Inhibition of HSP70 expression decreased the expression of α-SMA, a marker of activated cardiac fibroblasts (Figure 3G), and reduced the expression of pro-collagen I mRNA (Figure 3F). In addition, transcriptional inhibition of HSP70 expression enhanced advanced myocardial apoptosis induced by AAC (Supplementary material online, Figure S3A and C). Although KNK437 did not suppress the elevation of blood pressure induced by AAC, it significantly decreased the left ventricular EF and FS (Figure 3A), which suggest that intracellular HSP70 has a critical role in the regulation of cardiac function.

3.4 Functional antagonism of HSP70 attenuated pressure overload-induced cardiac hypertrophy and fibrosis

In contrast to the HSF inhibitor KNK437, the functional antagonism of extracellular HSP70 by an anti-HSP70 antibody significantly attenuated AAC-induced cardiac hypertrophy without inhibiting the HSP70 expression in myocardium (Figure 4H), as indicated by the increase in IVST and PWTH (Figure 4A), as well as myocyte diameter (Figure 4B). Moreover, inhibition of extracellular HSP70 significantly attenuated both perivascular and interstitial fibrosis induced by pressure overload (Figure 4C and D), as indicated by a reduction of α-SMA and pro-collagen I mRNA expression when compared with isotype IgG treatment (Figure 4E and F). Additionally, functional blockade of AAC-induced myocardial apoptosis induced by AAC (see Supplementary material online, Figure S3B and D). Importantly, functional antagonism of extracellular HSP70 did not inhibit cardiac contractility in the hypertensive animals (Figure 4A).

3.5 Transcriptional inhibition of HSP70 expression or functional inhibition of the HSP70 suppressed pressure overload-induced macrophage infiltration and inflammation in the myocardium

The macrophage is a major cell type involved in chronic inflammation, and MCP-1 is a major chemokine that recruits circulating monocytes into the myocardium and promotes their differentiation into macrophages. AAC-induced pressure overload stimulated a high level of MCP-1 expression in the myocardium, resulting in more pronounced macrophage infiltration in the myocardium, especially around the vasculature (Figure 5A and B). The transcriptional inhibition of HSP70 expression or the functional blockade of extracellular HSP70 decreased the pressure overload-induced expression of MCP-1, thereby reducing the amount of heart-infiltrating macrophages (Figure 5E). Pressure overload did not change the expression of a pivotal TH1 cytokine IFN-γ (data not shown) but increased the expression of a typical TH2 cytokine TGF-β1 in the myocardium (Figure 5C and D). However, the inhibition of HSP70 expression or the functional blockade of HSP70 decreased the expression of TGF-β1 in the myocardium (Figure 5C and D).

3.6 Inhibition of HSP70 expression enhanced the AAC-activated MAPK while functional blockade of HSP70 inhibited MAPK activation

We examined whether the inhibition of HSP70 expression or the blockade of the HSP70 protein could modulate myocardial hypertrophy signalling. GSK-3β, a negative mediator of hypertrophy, is negatively regulated by AKT. In comparison to the untreated AAC heart, inhibition of HSP70 expression or blockade of HSP70 inhibited
Figure 3  Transcriptional inhibition of HSP70 expression enhanced myocardial hypertrophy but suppressed cardiac fibrosis. The mice were subjected to AAC or a sham surgical operation and treated with or without the HSF inhibitor, KNK437 for 5 weeks. (A) Treatment with KNK437 increased intraventricular septum thickness (IVST) and posterior wall thickness (PWTH) but significantly weakened fraction shortening (FS) and ejection fraction (EF) as indicated by heart echocardiography. (B) Transcriptional inhibition of HSP70 enhanced cardiomyocyte diameters as measured with H&E staining. Scale bar, 50 μm. (C and D) Inhibition of HSP70 expression significantly decreased the pressure-overload-induced myocardial interstitial fibrosis and perivascular fibrosis, as measured with Masson’s Trichrome’s staining. Scale bar, 100 μm. (E) Myocardial fibrosis was assessed as percentage of heart sections with collagen deposition, which was stained bright blue. (F) The level of pro-collagen I mRNA in the hearts was detected by RT-PCR. (G) Expression of α-SMA was evaluated by immunohistochemistry. Data are mean ± SEM (n ≥ 6). *P < 0.05, **P < 0.01 vs. the sham group; #P < 0.05, ##P < 0.01 vs. the model group.
Figure 4 Functional antagonism of HSP70 attenuated pressure-overload-induced cardiac hypertrophy and fibrosis. The mice were subjected to AAC and administered anti-HSP70 or isotype-matched IgG antibody intravenously 1 day before and every 3 days after AAC for 5 weeks. (A) Anti-HSP70 antibody significantly attenuated pressure-overload-induced thickening of intraventricular septum thickness (IVST) and posterior wall thickness (PWTH), and did not change fraction shortening (FS) and ejection fraction (EF). (B) Functional blockage of HSP70 blunted AAC-induced increase in myocyte size (H&E staining). Scale bar, 50 μm. (C and D) Blocking HSP70 significantly attenuated pressure-overload-induced myocardial interstitial fibrosis and perivascular fibrosis. Scale bar, 100 μm. (E) Myocardial fibrosis was evaluated by Masson Trichrome’s staining and was assessed as percentage of heart sections with collagen deposition, which was stained bright blue. (F and G) HSP70 inhibition decreased the mRNA expression of pro-collagen I and the expression of α-SMA. (H) Blocking HSP70 did not inhibit the expression of intracellular HSP70 in the hypertensive hearts. Data are mean ± SEM (n ≥ 6). *P < 0.05, **P < 0.01 vs. the sham group; #P < 0.05, ##P < 0.01 vs. the model group.
AKT phosphorylation in the pressure-overloaded hearts. However, the inhibition of HSP70 expression decreased GSK-3β activity in the myocardium (Figure 6A and C). MAPK is another important signal modulating myocardial hypertrophy. Expression of p-P38 and p-ERK in mice treated with KNK437 was significantly increased in comparison to vehicle-treated mice (Figure 6B). However, inhibition of extracellular HSP70 activity decreased the hypertension-enhanced p-P38 and p-ERK in the myocardium (Figure 6D). Although anti-IgG attenuated AKT/GSK-3β to the same level as anti-HSP70 (Figure 6C), IgG did not suppress the p-P38 and p-ERK. Thus, the inhibition of P38 and ERK, but not AKT/GSK3β, was responsible for the anti-hypertrophic role of HSP70 antibody.

4. Discussion

Despite a link between serum levels of HSP70 or anti-HSP70 autoantibody and hypertension-induced cardiovascular disease has been established, it is unclear how HSP70 contributes to the pathogenesis of cardiovascular diseases, especially the development of hypertension-induced cardiac hypertrophy and fibrosis. In this study, we found that sustained pressure overload elevates serum HSP70 levels and enhances the translocation of HSP70 to the membrane of cardiomyocytes and the myocardial interstitial space. Moreover, the accumulation of HSP70 on the membrane of cardiomyocytes and its release into the intercellular space is likely mediated by the activation of Ang II AT1 receptor, because a sub-therapeutic dose of AT1 blocker, candesartan, inhibits the pressure overload-induced expression, and liberation of HSP70 in AAC mice. Importantly, although myocardial hypertrophy and cardiac contractile dysfunctions are promoted, transcriptional inhibition of HSP70 expression with a HSF inhibitor attenuates pressure overload-induced cardiac fibrosis by suppressing the liberation of HSP70 into the cardiac interstitial space and decreasing serum levels of HSP70, which would act as a DAMPs. In contrast, functional antagonism of extracellular HSP70 significantly protects the heart from pressure overload-induced cardiac hypertrophy and fibrosis without adverse haemodynamic effects.

Recent studies have shown that HSP70 overexpression protects against simulated ischaemia and metabolic stress. Kim et al. demonstrated that HSP70 gene deficiency led to myocardial hypertrophy, dysfunctional cardiomyocytes, and impaired stress responses of HSP70-KO hearts against ischaemia/reperfusion.
Although mechanisms for cardiac hypertrophy induced by decreased intracellular HSP70 are still unknown, accumulated evidence has demonstrated a strong correlation between myocardial calcium handling and the function of HSP70. Kim et al. found that HSP70 deficiency-induced cardiac hypertrophy is associated with impaired cardiac contractile function and calcium handling. Moreover, intracellular HSP70 modulates the components of calcium-handling, such as PKA, PKC, PLC2, SERCA2a, and phosphorylation of the ryanodine receptor in cardiomyocytes. A very recent study revealed that HSP70 has a crucial role in the stress-induced cell death via HSP70-lipid interaction to stabilize the membranes of lysosomes and protect these intracellular vesicles from releasing of degradative enzymes. Although we currently do not know whether the lysosome-stabilizing action of HSP70 is responsible for the cardiac dysfunction promoted by the absence of HSP70 as indicated in this study, it is worth investigating whether this role of HSP70 participates in the regulation of cardiac function and remodelling in response to hypertensive stress.

Numerous studies have demonstrated that pressure overload-induced cardiac hypertrophy and fibrosis are the consequences of inflammatory responses. In this study, we observe that functional antagonism of extracellular HSP70 attenuates the conjugation of HSP70 with TLR4, and decreases the macrophage infiltration and the inflammatory cytokines expression, which attenuates the pressure overload-induced cardiac hypertrophy and fibrosis. Our results indicate that HSP70 serves as a DAMP molecule and participates in the pathogenesis of pressure overload-induced cardiac remodelling, similar to its action in the pathogenesis of arthritis and atherosclerosis by inducing or regulating the immune response. Indeed, HSP60, 70, and 27 can be actively or passively released from tumour cells, macrophages, neurites, and synoviocytes via the necrotic or non-classical pathway (e.g. exosomes). These extracellular HSPs have pro-inflammatory properties when they interact with PRRs, resulting in the activation of immune and resident cells, and the rapid release of cytokines. Indeed, Zou et al. recently reported that extracellular HSP70 plays a critical role in regulating the myocardial innate immune response and cardiac function after ischaemia–reperfusion through its activation of TLR4.

AKT and MAPK are two important signalling pathways associated in the cardiac hypertrophy, one of which is sufficient to induce cardiomyocyte hypertrophy in vitro. Activation of AKT/GSK-3β signal cascade is involved in the pathogenesis of cardiac hypertrophy. Pharmacological inhibition of ERK and P38 phosphorylation attenuates ET-1- and PE-induced myocardial hypertrophy. Kee et al. recently reported that overexpression of HSP70 induced by isopreterenol or aortic banding will trigger cardiac hypertrophy in an AKT-dependent manner by activating Histone deacetylase2. However, Kim et al. demonstrated that the increased phosphorylation of ERK and P38 is responsible for the cardiac hypertrophy in the hsp70 knockout model.

**Figure 6** Transcriptional or functional inhibiting HSP70 differentially regulated cardiac hypertrophy signalling. The left ventricular homogenates were obtained from the animals 35 days after the AAC procedure. (A and C) Inhibiting HSP70 expression or functionally blocking HSP70 suppressed the activation of AKT induced by hypertension. Inhibition of HSP70 expression significantly inhibited pressure-overload-induced phosphorylation of GSK3β, whereas anti-HSP70 antibody did not. (B and D) Phosphorylation of P38 kinase and ERK was up-regulated after the synthesis of HSP70 has been inhibited, whereas the functional blockage of HSP70 significantly attenuated the activation of P38 and ERK. Data are expressed as folds of the sham group in four independent experiments. *P < 0.05, **P < 0.01 vs. the sham group; #P < 0.05, ##P < 0.01 vs. the model group.
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Hearts. Coincidentally, our studies indicate that intracellular and extracellular HSP70 play a different role in the regulation of pressure overload-induced myocardial remodelling and cardiac contractile function. The extracellular HSP70 is a potential therapeutic target for agents against cardiac hypertrophy and fibrosis in cardiovascular diseases such as hypertension and chronic heart failure. Our studies also suggest a potential cardiovascular risk with the use of anti-cancer drugs that are currently developed by targeting the transcription expression of HSPs.

Supplementary material

Supplementary material is available at Cardiovascular Research online.

Conflict of interest: none declared.

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