Attenuation of cardiac dysfunction by HSPA12B in endotoxin-induced sepsis in mice through a PI3K-dependent mechanism

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Aims
Cardiac dysfunction is a critical manifestation of severe sepsis/septic shock and is responsible for high mortality due to sepsis. Recent evidence suggests that angiogenic factors have a protective effect on sepsis-induced organ damage. Heat shock protein A12B (HSPA12B) is a newly discovered gene that is essential for angiogenesis. We hypothesized that overexpression of HSPA12B would induce protection against endotoxin-induced cardiac dysfunction.

Methods and results
To evaluate this hypothesis, we generated transgenic mice overexpressing the human hspa12b gene (Tg). Wild-type (WT) littermates served as controls. Tg and WT mice were treated with lipopolysaccharide (LPS) and cardiac function was measured after 6 h. LPS treatment caused cardiac dysfunction in WT mice. In contrast, cardiac function was significantly preserved in Tg mice following LPS administration. LPS increased the expression of vascular cell adhesion molecule-1 (VCAM-1)/intercellular adhesion molecule-1 (ICAM-1) and leucocyte infiltration into the myocardium of WT mice. In Tg mice, LPS-increased VCAM-1/ICAM-1 expression and leucocyte infiltration were significantly attenuated. Overexpression of HSPA12B also prevented the decrement in the activation of phosphatidlyinositide 3-kinase (PI3K)/protein kinase B (Akt) signalling in the myocardium. Importantly, PI3K inhibition with Wortmannin abolished the protection of HSPA12B against LPS-induced cardiac dysfunction.

Conclusion
These results suggest that HSPA12B plays an important role in the attenuation of endotoxin-induced cardiac dysfunction and that the mechanisms involve the preserved activation of PI3K/Akt signalling, resulting in attenuation of LPS-increased expression of VCAM-1/ICAM-1 and leucocyte infiltration into the myocardium.

Keywords
Cardiac dysfunction • Endotoxaemia • Heat shock protein A12B • Phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) signalling pathway • Angiopoietin-1

1. Introduction
Sepsis is a systemic inflammatory response syndrome and is the leading cause of mortality in hospitalized patients who are critically ill. Cardiac dysfunction is a critical manifestation of severe sepsis/septic shock and is responsible for high mortality due to sepsis.1,2 Current evidence suggests that attenuation of cardiac dysfunction could significantly improve survival of septic patients.3 Therefore, developing effective approaches for preventing septic cardiac dysfunction has become of great interest in clinical and basic research.2,3

Recent studies suggest that angiogenic factors play critical roles in the development of sepsis-induced injury. For example, lipopolysaccharide (LPS) administration decreases the expression of angiopoietin-1 (Ang-1) which is positively correlated with the enhanced vascular leakage seen in severe sepsis/septic shock animals.4,5 Consistent with this observation, administration of Ang-1 significantly increases the survival, improves cardiac haemodynamics and reduces lung injury in endotoxemic mice.6–10 Similar protective effects against endotoxin challenge were observed in transgenic mice overexpressing endothelial nitric oxide synthase (eNOS).11
In contrast, plasma vascular endothelial growth factor was increased during the first 48 h of human septic shock and correlated with vascular permeability.

Heat shock protein A12B (HSPA12B) was discovered by Han et al. in 2003. Subsequently, HSPA12B was identified as the newest member of the HSP70 family and is mainly expressed in endothelial cells. Recently, HSPA12B has been demonstrated to play an essential role in the induction of angiogenesis in vitro, partially attributable to activation of Akt. Akt is a critical kinase in the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) signalling pathway, which has been shown to play an important role in limiting proinflammatory responses during sepsis/septic shock both in vitro and in vivo. Moreover, we reported recently that overexpression of heat shock protein 27 (HSP27) attenuated LPS-induced cardiac dysfunction through activation of the PI3K/Akt signalling pathway. It is possible, therefore, that overexpression of HSPA12B may activate the PI3K/Akt signalling pathway, resulting in attenuation of endotoxin-induced cardiac dysfunction.

To test this possibility, we generated transgenic mice overexpressing the human hspa12b gene and examined the effects of HSPA12B on cardiac dysfunction during endotoxaemia in mice. We observed for the first time, to the best of our knowledge, that overexpression of HSPA12B significantly attenuated cardiac dysfunction in endotoxin-septic mice compared with wild type (WT) controls. The mechanisms by which HSPA12B attenuates cardiac dysfunction in endotoxaemia involve the preserved activation of the PI3K/Akt signalling pathway, resulting in the limitation of LPS-induced expression of vascular cell adhesion molecule-1 (VCAM-1) and inducible nitric oxide synthase (iNOS), and leucocyte infiltration into the myocardium.

2. Methods

2.1 Antibodies and chemicals

Escherichia coli LPS (0111:B4) and primary antibody for α-Tubulin were purchased from Sigma-Aldrich (St Louis, MO, USA). Primary antibody for HSPA12B was a generous gift from Han. Primary antibodies against IκBα, Akt and phospho-Akt (p-Akt), GSK-3β, and phospho-GSK-3β (p-GSK-3β) were obtained from Cell Signaling Technology (Beverly, MA, USA). Primary antibody for Ang-1 was from Abcam (Cambridge, UK); CD31 from BD Pharmingen (San Jose, CA, USA); and iNOS and eNOS from BD Biosciences (San Jose, CA, USA). Primary antibody for Ang-1 was from Abcam (Cambridge, UK). For examination of PI3K/Akt activation and IκBα degradation, hearts were cut and subjected to Haematoxylin–Eosin staining (H&E). Histological changes in erythrocyte leakage and leucocyte infiltration into cardiac interstitium were examined under a light-microscope. Myocardial leucocytes were counted according to the methods described previously. Briefly, myocardial leucocytes were counted on five random fields on each slide. The infiltration of myocardial leucocytes was expressed as the average number of leucocytes per field (mean ± SD).

2.2 Generation of transgenic mice

The red recombination system was employed to construct the human hspa12b transgene which consists of 20.4 kb of coding sequence (gene ID: ENSG number 0000132622), 7.3 kb of 5′ flanking sequence and promoter, and 3.6 kb of 3′ flanking sequence. To construct a retrieval vector, a 570 bp of 5′ homology and a 618 bp of 3′ homology to the transgene were amplified by PCR and subcloned into the PL253 plasmid. A PAC clone (from BAC/PAC resources centre) containing the human hspa12b gene was electrophoresed into EL350 E. coli, in which the expression of red recombination protein was induced at 42 °C for 15 min. The HindIII-linearized retrieval vector was then electrophoresed into PAC harbouring EL350. The recombination between the linearized retrieval vector and PAC led to subcloning of 3.1 kb of the human hspa12b gene into PL253 plasmid. The transgene was introduced into the pronuclei of C57BL/6 mouse fertilized eggs by microinjection. PCR of tail genomic DNA with specific primers for the transgene was used to identify the transgenic founders. The founders and all subsequent generations were crossed with C57BL/6 mice. All experiments were conducted with F6 and later generations. The schematic construction of the HSPA12B transgene is shown in Figure 1A. The overexpression of HSPA12B in the hearts of HSPA12B mice was confirmed by immunoblot (Figure 1C, n = 6/group).

2.3 Experimental animals and induction of endotoxaemia

Transgenic mice overexpressing the human hspa12b gene (Tg) and gender-matched WT littermates at 2-months of age were employed. Endotoxaemia was induced by intraperitoneal (i.p.) injection of LPS (10 mg/kg body weight) as described previously. Saline-treated mice served as controls. All experiments were performed with the guidelines for the ‘Principles of Laboratory Animal Care’ and the ‘Guide for the care and use of laboratory animals’ published by NIH (NIH Publication No. 85-23, revised 1996). Animal care and experimental protocols were approved by the Nanjing University Committee on Animal Care.

2.4 Echocardiographic analysis

WT and Tg mice were injected with LPS. Saline-treated WT and Tg mice served as controls. In a separate experiment, WT and HSPA12B Tg mice were pre-treated with Wortmannin (WM, 1 mg/kg body weight) 1 h before LPS administration (n = 8–15/group).

Six hours after LPS administration, cardiac function was examined by echocardiography as described previously. Mice were anaesthetised with chloral hydrate injection (360 mg/kg, i.p.). Two-D guide M-mode transthoracic echocardiographic examinations were performed by using the Vevo770 system equipped with a 35-MHz transducer (Visualsonics, Toronto, Canada). Parameters of cardiac function were measured digitally on the M-mode tracings and averaged from three to five cardiac cycles. The individual who performed the echocardiographic analysis was blinded to the treatment.

2.5 Blood pressure measurement

Blood pressure (BP) was measured before and 6 h after LPS administration using a non-invasive tail cuff computerized system (ACL-NIBP, Alcott Biotech, China). The BP value measured before LPS administration served as baseline controls (n = 8/group).

2.6 Histological examination

Six hours after LPS administration, hearts were harvested and cut into transverse blocks (2 mm thick) at the level of papillary muscles. The tissues were immersion-fixed in 4% buffered paraformaldehyde and embedded in paraffin. Serial sections of 4 μm were cut and subjected to Haematoxylin–Eosin staining (H&E). Histological changes in erythrocyte leakage and leucocyte infiltration into cardiac interstitium were examined under a light-microscope. Myocardial leucocytes were counted according to the methods described previously. Briefly, myocardial leucocytes were counted on five random fields on each slide with a magnification of 200 ×. The individual who analysed the histologic samples was blinded to the treatment. The infiltration of myocardial leucocytes was expressed as the average number of leucocytes per field (n = 6/group).

2.7 Western blot

For examination of PI3K/Akt activation and IκBα degradation, hearts were collected 1 h after LPS injection. In a separate experiment, hearts were collected 6 h after LPS injection for the analysis of the levels of iNOS, eNOS, and Ang-1.

Western blot was performed as described previously. Briefly, cellular proteins were prepared from heart samples, separated on 10% SDS–PAGE, and transferred onto Immobilon-P membranes (Millipore).
The membranes were probed with appropriate primary antibodies followed by incubation with peroxidase-conjugated secondary antibodies. The signals were detected by enhanced Pierce chemiluminescence. For loading control, the same membranes were probed with anti-α-tubulin. The signals were quantified by scanning densitometry and the results from each experimental group were expressed as relative integrated intensity compared with that of controls ($n = 6$ /group).

### 2.8 Immunohistofluorescence analysis

Two-month-old mice were anaesthetised and the hearts were perfused with saline for 5 min before the hearts were harvested, fixed in 4% paraformaldehyde for 2 h, and incubated with 30% sucrose overnight. The hearts were frozen in OCT and sectioned at 5 μm. After blocking with 3.5% normal goat serum for 30 min, the cryosections were incubated with rabbit anti-mouse/human HSPA12B antibody (1:100) and rat anti-mouse CD31 antibody (1:250) overnight. Subsequently, the sections were washed and incubated with FITC-labelled goat anti rabbit IgG (1:100) and Cy3-labelled goat anti-rat IgG (1:100) for 60 min. The sections were observed under a fluorescent microscope (Axiovert 200, Zeiss Ltd., Germany) at a magnification of 200 ×.

### 2.9 Statistical analysis

Results are expressed as means ± standard deviation (X ± SD). Comparison data between groups was performed using two-way analysis of variance. $P < 0.05$ was considered to be significant.

### 3. Results

#### 3.1 Generation of HSPA12B transgenic mice

Two independent transgenic lines (lines 2 and 6) were obtained. The successful transmission of transgene to offspring was genotyped by PCR (Figure 1B). The protein levels of HSPA12B in Tg hearts were significantly increased by 13-fold in line 2 and nine-fold in line 6, respectively, compared with WT mice (Figure 1C). The results of immunohistochemistry showed that HSPA12B (green) was colocalized with CD31 (red), a marker of endothelial cells (Figure 1D), suggesting that HSPA12B was expressed in the endothelial cells of cardiac capillaries. Both transgenic lines showed normal reproductive...
patterns and longevity. There was no apparent cardiac morphological or pathological abnormality in both transgenic lines.

### 3.2 Overexpression of HSPA12B attenuated cardiac dysfunction following LPS administration

As shown in Figure 2A and Table 1, there was no significant difference in left ventricular function between saline-treated WT and Tg mice. LPS administration significantly decreased cardiac function in WT mice as evidenced by the reduction of ejection fraction (EF) by 53.3%, fractional shortening (FS) by 61.1%, and stroke volume (SV) by 63.0% compared with the saline-treated WT group (P < 0.01). However, cardiac dysfunction induced by LPS was attenuated in Tg mice. EF, %FS, and SV in LPS-treated Tg mice were significantly improved by 56.0, 72.5, and 49.5%, respectively, compared with LPS-treated WT mice (P < 0.01 or P < 0.05).

We examined BP in the mice 6 h after LPS administration. There was no significant difference in the baseline BP between WT and Tg mice. Following LPS administration, systolic BP (sBP) was significantly decreased in both WT and Tg mice compared with their respective controls that did not receive LPS. There was no significant difference of sBP between LPS-treated WT and LPS-treated Tg mice. The diastolic BP was undetectable in both WT and Tg mice after LPS treatment (Figure 2B).

### 3.3 Overexpression of HSPA12B protected against LPS-induced heart damage

There was no apparent difference of cardiac morphology between saline-treated WT and Tg mice (Figure 3A and B). However, LPS administration significantly increased erythrocyte leakage and leucocyte infiltration into the cardiac interstitium of WT mice (Figure 3C). In contrast, LPS-induced myocardial morphological changes were obviously attenuated in Tg mice (Figure 3D). As shown in Figure 3E, LPS-increased cardiac leucocyte infiltration by 6.5-fold in WT mice and by 3.6-fold in Tg mice, when compared with their respective saline controls (P < 0.01). However, LPS-induced cardiac leucocyte infiltration was significantly decreased by 45.1% in Tg mice compared with LPS-treated WT mice (P < 0.01).

### 3.4 Overexpression of HSPA12B attenuated LPS-induced increase in myocardial VCAM-1 and ICAM-1

Figure 4A shows that there was no significant difference in the levels of both VCAM-1 and ICAM-1 between saline-treated WT and Tg mice. However, LPS administration significantly upregulated the expression of VCAM-1 by 103.7% and ICAM-1 by 185.6%, respectively, in WT mice compared with saline-treated WT controls (P < 0.01). LPS also increased the levels of VCAM-1 and ICAM-1 in Tg mice compared with saline-treated Tg mice (P < 0.01). However, the levels of both VCAM-1 and ICAM-1 in LPS-treated Tg mice were significantly lower than that in LPS-treated WT mice (P < 0.01 or 0.05).

### 3.5 Overexpression of HSPA12B attenuated LPS-induced decrease in myocardial Ang-1 and I-κBα

As shown in Figure 4B, there was no significant difference in the cardiac levels of Ang-1 and I-κBα between saline-treated WT and Tg mice. LPS administration significantly decreased the levels of...
Ang-1 by 64.3% in WT and by 23.6% in Tg mice, respectively, compared with their respective saline-treated controls (P < 0.01). However, LPS-decreased Ang-1 levels were significantly attenuated by 63.4% in Tg mice compared with LPS-treated WT mice (P < 0.01).

LPS administration also significantly decreased the levels of i-kBx by 46.9% in WT and by 24.3% in Tg mice, respectively, compared with their respective saline-treated Tg controls (P < 0.01). However, the levels of i-kBx in Tg mice were significantly greater than that of LPS-treated WT mice (P < 0.01).

### 3.6 Overexpression of HSPA12B attenuated LPS-induced increase in iNOS and decrease in eNOS

Figure 4C shows that LPS administration significantly decreased the levels of eNOS in WT mice by 38.0% compared with saline-treated WT controls. In contrast, LPS administration did not significantly decrease the levels of eNOS in Tg mice. The eNOS levels in LPS-treated Tg mice were maintained at the level of saline-treated Tg control group.

The levels of iNOS were undetectable in saline-treated WT and Tg mice (Figure 4C). LPS treatment increased the levels of iNOS significantly in both WT and Tg mice. However, LPS-induced increase in iNOS was significantly attenuated by 76.4% in Tg mice compared with WT mice (P < 0.01).

### 3.7 Overexpression of HSPA12B attenuated LPS-induced decrease in myocardial p-Akt and p-GSK3β

As shown in Figure 5A, there is no significant difference in the levels of p-Akt and p-GSK-3β between WT and HSPA12B Tg mice that were treated with saline. LPS administration significantly decreased the levels of p-Akt by 47.5% and p-GSK-3β by 43.2% in WT mice, respectively, compared with saline-treated WT controls (P < 0.01).

LPS challenge also reduced the levels of p-Akt by 21.7% and p-GSK-3β by 31.4%, respectively, in Tg mice compared with saline-treated Tg controls (P < 0.01). However, the LPS-induced decrease in the levels of p-Akt and p-GSK-3β were significantly attenuated by 54.3 and 27.4% in Tg mice, respectively, compared with LPS-treated WT mice (P < 0.01 or < 0.05).

### 3.8 PI3K inhibition abolished the protective effect of HSPA12B on LPS-induced cardiac dysfunction

Figure 5B shows that WM administration significantly decreased the levels of p-Akt and p-GSK-3β either in the presence or in the absence of LPS in both WT and Tg mice. Furthermore, WM administration abrogated the HSPA12B-induced preservation of p-Akt and p-GSK-3β levels following LPS treatment.

Figure 5C and Table 1 show that inhibition of PI3K with WM in LPS-treated WT mice significantly decreased EF by 33.7%, %FS by 36.9%, and SV by 47.4%, respectively, compared with LPS-treated WT mice that did not receive WM (P < 0.01). In WM-treated Tg mice that received LPS challenge, EF was significantly reduced by 59.1%, %FS reduced by 64.2%, and SV reduced by 63.6% compared with LPS-treated Tg mice that did not receive WM (P < 0.01). Importantly, WM abolished the beneficial effect of HSPA12B on attenuation of cardiac dysfunction in LPS-induced sepsis. There was no significant difference in the parameters of cardiac function between LPS-treated WT and LPS-treated Tg mice in the presence of WM.

### 4. Discussion

The significant finding in the present study is that transgenic mice with overexpression of HSPA12B exhibited significant attenuation of cardiac dysfunction in endotoxin-induced sepsis. We also observed that overexpression of HSPA12B significantly attenuated the decrease of phospho-Akt and phospho-GSK3-β levels following endotoxin challenge. Inhibition of PI3K abolished the protective effect of HSPA12B on LPS-induced cardiac dysfunction. These data suggest that overexpression of HSPA12B will result in a beneficial effect on cardiac dysfunction during sepsis/septic shock and the mechanisms involve the preserved activation of the PI3K/Akt signalling pathway.
resulting in the limitation of LPS-induced expression of VCAM-1/ICAM-1 and iNOS, and leucocyte infiltration into the myocardium.

It has been demonstrated that neutrophil/leucocyte infiltration contributes to cardiac dysfunction during sepsis/septic shock. Increased expression of adhesion molecules, such as VCAM-1 and ICAM-1, will be a key step for the infiltration of neutrophils and leucocytes into the myocardium. We observed that LPS administration significantly increased leucocyte infiltration and upregulation of VCAM-1 and ICAM-1 in the myocardium. However, the induction of ICAM-1 and VCAM-1 by LPS was suppressed by HSPA12B overexpression. Numerous studies have shown that LPS increased the cell surface expression of ICAM-1 and VCAM-1 on both coronary endothelial cells and cardiac myocytes. Binding of leucocyte to ICAM-1/VCAM-1 on the surface of endothelial cells will mediate its transmigration and infiltration into the myocardium, while binding of ICAM-1/VCAM-1 on the surface of cardiomyocytes will mediate myocardium depression via variant signalling, such as the cortical actin cytoskeleton, leading to increased heterogeneity of intracellular Ca²⁺ release and decreased cardiomyocyte contractility. In addition to ICAM-1/VCAM-1, increased matrix metalloproteinase-9 (MMP-9) activity has been reported to be positively correlated with the recruitment of inflammatory cells and leakage of erythrocytes. Therefore, blockade of either ICAM-1/VCAM-1 production or MMP-9 activity will reduce myocardial leucocyte accumulation and abrogate LPS-induced cardiac dysfunction. Indeed, we observed in the present study that the LPS-induced myocardial infiltration of leucocytes was attenuated in HSPA12B Tg mice. Thus, overexpression of HSPA12B attenuated cardiac dysfunction in endotoxic mice is related to the inhibition of induction of ICAM-1/VCAM-1.

The role of eNOS and iNOS in sepsis/septic shock has been well-demonstrated. For example, sepsis-induced vascular inflammation and endothelial dysfunction are associated with a loss of eNOS and overproduction of iNOS, which causes vasodilation and acts as one of the key inflammatory mediators. Indeed, we observed that LPS

Figure 3  Histological examination of heart tissues from WT and Tg mice. WT and Tg mice were treated with LPS for 6 h and hearts were harvested and sectioned for HE counterstaining and leucocyte infiltration examination. Original magnification 200×. *P < 0.01, n = 6/group.
administration significantly decreased the levels of eNOS and increased iNOS production in the myocardium of WT mice, suggesting that the homeostatic balance of iNOS and eNOS was altered following LPS-induced progression of sepsis. Imbalance of iNOS and eNOS could contribute to hypotension, dysfunction of multiple organs, and mortality. Importantly, LPS-decreased eNOS and increased iNOS were significantly attenuated in HSPA12B Tg mice, suggesting that HSPA12B will have a potential ability to maintain the balance of eNOS and iNOS during the development of sepsis/septic shock. Indeed, transgenic mice with overexpression of eNOS in endothelial cells showed a reduction in LPS-induced mortality.

Whereas increased NF-κB binding activity promoted the production of ICAM, VCAM, and iNOS, activation of the PI3K/Akt pathway has been demonstrated to negatively regulate NF-κB binding activity (Figure 6). Recent studies have shown that overexpression of HSPA12B will activate Akt in endothelial cells in vitro. We observed that the levels of phospho-Akt and phospho-GSK-3β were significantly preserved in HSPA12B Tg mice following LPS administration compared with LPS-treated WT mice. It is possible, therefore, that the cardiac protection against LPS challenge by HSPA12B could be mediated, at least in part, by activation of the PI3K/Akt pathway. To evaluate our hypothesis, we administered WM, a PI3K inhibitor, to the mice 1 h prior to LPS administration. We observed that pharmacologic inhibition of PI3K by WM abolished the protection of HSPA12B against LPS-induced cardiac dysfunction. PI3K inhibition also abrogated HSPA12B-preserved phospho-Akt and phospho-GSK-3β following LPS administration. The data suggests that HSPA12B-induced cardioprotection against endotoxin-induced cardiac dysfunction is mediated, at least in part, by a PI3K/Akt-dependent mechanism. The dosage of WM selected in the present study has been employed in our previous study and did not induce detrimental effects on cardiac function or animal survival. The differential effects of WM on mice treated with saline or LPS.
Figure 5 Effect of HSPA12B on activation of PI3K/Akt signalling. (A) Overexpression of HSPA12B-preserved the levels of phospho-Akt and phospho-GSK-3β in the myocardium following LPS challenge. Hearts were collected from the mice treated with LPS for 1 h. Cellular proteins were prepared and subjected to western blot analysis. *P < 0.01, **P < 0.05, n = 6/group. (B) PI3K inhibition by WM abolished the HSPA12B-preserved levels of phospho-Akt and phospho-GSK-3β in the myocardium following LPS administration. WM was administered to mice 1 h prior to LPS injection. *P < 0.01, n = 6/group. (C) PI3K inhibition by WM abrogated the cardioprotection of HSPA12B from LPS-induced cardiac dysfunction. Both WT mice and Tg mice were treated with WM 1 h prior to LPS administration. Cardiac function was examined by echocardiography 6 h after LPS injection. Representative M-mode images of echocardiography are shown. n = 8–15/group.
may be explained by different responses to the two treatments. In saline-treated mice, there was no endotoxin-increased NF-κB binding activity (decreased IkBa), ICAM-1/VCAM-1 production and infiltration of inflammatory cells in the myocardium. However, in LPS-treated mice, we observed inactivation of the PI3K/Akt signalling pathway as evidenced by decreased levels of p-Akt and p-GSK-3b which were positively correlated with the cardiac dysfunction. Our observation suggests that cardiac function will be determined by modulation of NF-κB activation pathway and PI3K/Akt signalling.

Ang-1 has been demonstrated to have a protective effect on sepsis/septic shock.\textsuperscript{6-8} More specifically, Ang-1 will activate PI3K/Akt pathway after binding to the Ang-1 receptor tie2 and activation of the PI3K/Akt signalling pathway will negatively regulate the LPS-induced, NF-κB-dependent inflammatory responses (Figure 6).\textsuperscript{37,38} We observed that LPS treatment significantly decreased the expression of Ang-1 in the myocardium. Interestingly, the levels of Ang-1 in the myocardium of LPS-treated Tg mice were significantly greater than that of LPS-treated WT mice. The data suggests that HSPA12B will promote Ang-1 production via an unknown mechanism, resulting in activation of the PI3K/Akt signalling pathway. Collectively, activation of the PI3K/Akt signalling pathway may play a role in the limitation of LPS-induced expression of inflammatory factors, such as ICAM, VCAM, and iNOS, and subsequently suppressing the development of septic cardiac dysfunction.

Induction of HSPs (e.g. 70 kDa Hsp70, Hsp27, αB-crystallin) has been reported to play a protective role in various diseases, such as ischaemic heart disease, diabetes, and neurodegeneration. We have previously reported that cardiac-specific overexpression of Hsp27 protects the myocardium from LPS-induced cardiac dysfunction.\textsuperscript{19} In the present study, we demonstrated that HSPA12B, a novel heat shock protein which is predominantly expressed in endothelial cells, attenuated endotoxin-induced cardiac dysfunction. Our studies suggest that increased expression of HSPs either in cardiac myocytes or endothelial cells will induce cardioprotection against endotoxin-induced cardiac dysfunction. Our findings strengthen the viewpoint that HSPs are new therapeutic targets in cardiovascular disease.\textsuperscript{39}

In summary, our results showed that overexpression of HSPA12B attenuated LPS-induced cardiac dysfunction. The mechanisms by which HSPA12B attenuated cardiac dysfunction involve the preserved activation of the PI3K/Akt signalling pathway, resulting in the limitation of LPS-induced expression of VCAM-1/ICAM-1 and iNOS, and leucocyte infiltration in the myocardium.

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