Review

Role of Heat Shock Protein 27 in Cardiovascular Disease

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Abstract: Small heat shock proteins are ubiquitous ATP-independent chaperones whose expression is induced by a wide variety of physiological and environmental stress. Heat shock protein 27 (Hsp27 or HspB1) is one of the best characterized members of the sHsp family. In addition to its chaperone function, several positive biological effects exerted by this protein have gained attention, such as anti-oxidant, anti-inflammatory and anti-apoptotic. This review article provides a comprehensive overview of structure, expression and functional aspects of Hsp27 in relation to cardiovascular disease (CVD). Moreover, this review also presents overwhelming evidence in support of the role of Hsp27 in the pathophysiology of cardiovascular disease (CVD), as well as its therapeutic and clinical implications.

Keywords: small heat shock proteins (sHsps), heat shock protein 27 (Hsp27 or HspB1), chaperone, cardiovascular disease

1. Introduction

Small heat shock proteins (sHsp), varying in size from 15–30 kDa for their monomeric forms, are ATP-independent chaperones that are widely expressed in most organisms from bacteria to humans [1-2]. sHsps are induced during stress response and are involved in protecting cells from various unfavorable conditions such as high temperature, hypoxia, ischemia, endotoxins, heavy metals, and organic solvents [3-4]. As highly conserved chaperone proteins, sHsps interact with other proteins to facilitate normal cellular functions including regulating translation, maintaining cytoskeletal structure and normal redox conditions [5]. Hsps are normally intracellular proteins but when expressed outside the cell they may trigger an autoimmune response [6-7]. Heat shock protein 27 (Hsp27 or HspB1), with the molecular weight of 27 kD in humans, is one of the best characterized members of the sHsp family. Hsp27 has been demonstrated to play protective roles in TNF-α-mediated cell death, in attenuating doxorubicin-induced cardiac dysfunction and endotoxin-induced myocardium injury via stabilizing cytoskeletal, in promoting muscle contraction and regulating intracellular redox homeostasis and anti-apoptosis pathways [8-11]. In this review, we provide a comprehensive overview of structure, expression and functional aspects of Hsp27. Furthermore, we also delineate its significant role in the pathophysiology of cardiovascular disease (CVD). Additionally, we also present evidence that is in support of the role of Hsp27 in therapeutic and clinical implications.

2. Structure and expression of Hsp27

As one of the most widely distributed small heat hock proteins, Hsp27 is a protein of 205 amino acids and is composed of the conserved WDPF domain, N-terminal region, α-crystallin domain and flexible domain in the C-terminal (Fig. 1). The α-crystallin domain, a highly conserved region at residues Glu-87–Pro-168, mediates dimerization, while C-terminal amino acids are thought to form a flexible structure that is involved in oligomerization and important in chaperone function [12-13]. N-terminal domain contains a WDPF motif at Trp-16–Phe-19, which is essential for multimer formation and chaperone activity. The WDPF motif is adjacent to a mitogen-activated protein kinases associated protein (MAPKAP) kinases (MK2) phosphorylation site at Ser-15 and is linked to the α-crystallin domain by a region that varies in length depending on species [14]. The linker between WDPF motif and α-crystallin domain contains two other MK2 phosphorylation sites at Ser-78 and Ser-82, which regulates polymer assembly and actin binding. Hsp27 acts as ATP-independent molecular chaperone and the regulation of

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Hsp27 protein binding occurs at the level of phosphorylation and oligomerization. Studies have shown that oligomerization of Hsp27 is regulated by phosphorylation of Ser-78 and/or Ser-82 and the WDPF motif, while phosphorylation of Ser-15 seems to induce only a small effect on oligomerization [15-17]. Hsp27 phosphorylation, modulated by heat shock, oxidative stress, growth factors or tumor necrosis factor, promotes dissociation of the large oligomers to the smaller ones, which is catalyzed by MAPKAP kinases 2/3 and protein kinases C and D [18-19]. As 5′ untranslated region of the coding sequence in the heat-inducible proteins, heat shock response elements (HSE) can bind heat shock factors (HSF) via inverted repeats of nGAAn and increase the expression of Hsp27 [20]. It is proposed that pro-inflammatory mediators and cytokines (interleukin-6, tumor necrosis factor) may upregulate the expression of Hsp27. Moreover, expression can also be increased in response to physical and chemical stressors including heat, mechanical strain and oxidative stress [21-23].

3. Functions of Hsp27

Hsp27 has been found in cardiovascular, airway, intestinal tract, bladder and uterus and can be detected in a variety of cells, although certain cells express undetectable or relatively low levels, other cells express abundantly. In vivo, Hsp27 is produced in response to various types of stress in cardiac, skeletal muscles and the brain, which suggests that it may act as molecular chaperones suppressing the aggregation of specific client polypeptides. For instance, transgenic mice overexpressing Hsp27 are strongly protected against myocardial infarction and cerebral ischemia [24-25]. Moreover, Hsp27 is a multifunctional protein that participates in several processes in the cells. Large aggregates of Hsp27 are able to confer protection against reactive oxygen species (ROS) and TNF-α-induced injury, while phosphorylated Hsp27 has been shown to result in complex dissociation and the subsequent loss of chaperoning [1, 26-28]. Hsp27 is phosphorylated at serines 15, 78, and 82 by mitogen-activated protein kinases associated protein kinases (MAPKK) and Ser-78 and Ser-82 are the major sites of phosphorylation that is stimulated by p38 MAP kinase [15-16]. Ser-82 phosphorylation is also induced by VEGF stimulated protein kinase D, which in turn is activated by protein kinase C [29]. Hsp27 prevents the aggregation, denaturation and precipitation of target proteins under stress via affecting the slow, off-folding protein pathway. It has been suggested that subunit exchange may be facilitating the refolding of partially denatured proteins into active conformations. In addition to its chaperone function, Hsp27 also seems to be an important regulator of structural integrity and membrane stability, actin polymerization and intermediate filament cytoskeleton formation, epithelial cell-cell adhesion, cell migration, cell cycle progression, differentiation and apoptosis [30-31]. There are numerous reports that sustain a role for Hsp27 in human pathogenesis. Aberrant expression of Hsp27 can be associated with cardiovascular disease, neurodegenerative disorders, atherosclerosis, platelets and cancer [32-34].

4. Hsp27 and cardiac development

Studies have shown that Hsp27 is essential in mediating the cellular response to a wide variety of stressors, but it is not known what role Hsp27 may play in cardiogenesis under unstressed physiological conditions. A relatively high level of endogenous Hsp27 expression has been shown in developing cardiac muscle tissues in several organisms, including zebrafish [35-36], mouse [37], pig [38-39], and human [40-41]. A role for Hsp27 in heart development has been implied from an experimental model of Xenopus laevis studies showing that abrogation of Hsp27 function in Xenopus laevis embryos results in improper fusion of the cardiac progenitors, disorganization of actin filament and myofibrillar, as well as

Fig. 1. Domain structure of human Hsp27. A: Schematic diagram of Hsp27 indicating the WDPF domain, N-terminal domain, α-crystallin domain and the flexible C-terminal domain. The numbers indicate the amino acid residues. B: Alignment of Hsp27 amino acid sequences.
cardiac bifa, which in turn leads to defects in heart formation and myofibril architecture in cardiac muscle of developing frog embryos. Collectively, accumulating evidence supports an important role for Hsp27 in actin assembly, cell polarization and migration in developing cardiac muscle [42].

On the contrary, normal development of Drosophila embryos lacking Hsp27 as a result of gene disruption has also been reported. Furthermore, loss of Hsp27 does not alter the resistance of flies to heat shock or oxidative injury [43]. Mice lacking a homolog of human Hsp27 also develop normally [44]. Therefore, there are currently conflicting data regarding the role of Hsp27 during embryogenesis.

The regulation of Hsp27 expression during development has been suggested to be at least partially dependent on some transcription factors such as heat shock factor-2 (HSF-2) [45] and Brn-3 [46]. However, the exact requirement for Hsp27 during normal cardiac development remains unknown.

5. Hsp27 and cardiovascular disease

5.1. Hsp27 and ischemic heart disease

Hsp27 plays a key role in the ischaemic conditioning of the myocardium and limits the progress of oxidative stress caused by reperfusion [47-50]. Increased levels of Hsp27 are associated with cardioprotection by maintaining the integrity of microtubules and actin cytoskeleton, and may protect endothelium from ischemia. Phosphorylated Hsp27, which interacts with tropomyosin in smooth muscle cells (SMCs), may play a role in both stabilizing the cytoskeleton and protecting against ischemic injury [51]. Several studies have reported that over expressed Hsp27 in the heart protects against ischemia/reperfusion (I/R) injury and myocardial infarction, which suggests the cardioprotective role of Hsp27 [52, 55]. Patients with acute coronary syndrome (ACS) tended to have higher serum level of Hsp27 in comparison to controls, while stable coronary artery disease (CAD) did not have a significantly higher concentration of Hsp27, which indicates that serum level of Hsp27 may be a potential marker of myocardial ischemia [53]. M. Ghayour-Mobarhan also reported that in patients with ACS, Hsp27 antibody titers are high during the first 12h, then fall to near normal levels during the second 12h [54]. In a recent study, phospho-Hsp27 showed a significant decrease in ischemic vessels and the expression of cytoskeletal proteins, namely vimentin was also markedly reduced, while transgelin, tropomyosin and G-actin showed a significant increase in vessels with ischemic heart disease (IHD). This suggests that phospho-Hsp27 exerts a protective effect by reorganizing the cytoskeletal [55]. Mourouzisa et al. reported that morphine administration at reperfusion does not affect cardiac function but limits the extent of myocardial injury, possibly via increased Hsp27 phosphorylation [56]. Kwon et al. also reported that protein transduction domain (PTD)-mediated delivery of Hsp27 confers cardiac protection against ischemic insult via improving rat LV contractile and cells apoptosis associated with caspase-3 activation [57].

5.2. Hsp27 and congestive heart failure

Congestive heart failure (CHF) is a complex syndrome that consists of not only hemodynamic abnormalities, but also cellular and molecular alternations in cardiac tissue [58-59]. Overexpressing a wild-type human Hsp27 in a transgenic mouse model preserves contractile function of the Langendorff-perfusing heart with global I/R injury [55]. We have demonstrated that transgenic mice with cardiac-specific over-expression of Hsp27 were more resistant to Dox-induced heart failure in vivo and mortality was also significantly decreased [9]. The mechanisms of Dox-induced left ventricular dysfunction may involve mitochondrial dysfunction, free radical production and myocyte death [60–62]; and some of these are also features of heart failure caused by ischemia/reperfusion or cardiomyopathy. Dohke et al. used a proteomic approach to investigate global alternations in protein expression in tachycardia induced CHF dogs and their results showed that Hsp27 was highly expressed after the induction of CHF. Moreover, phosphoprotein staining and Western blotting demonstrated that the phosphorylated Hsp27 of Ser-78 and Ser-82 increased, but Ser-15 was not altered in CHF [63]. Lu et al. also reported that over-expression of Hsp27 by gene transfer in vivo protected against I/R-induced cardiac dysfunction via stabilization of troponin I and T. Such protection may result in restored post-ischaemic myofilament response to Ca²⁺ with an improved post-ischaemic contractile function [64]. We have recently observed that transgenic mice with cardiac-specific expression of Hsp27 exhibited a significant attenuation of cardiac dysfunction and improvement of survival in lipopolysaccharides-induced sepsis. Moreover, we also demonstrated that activation of the PI3K/Akt pathway and down-regulation of NF-κB activation by Hsp27 could be the mechanisms by which Hsp27 attenuated cardiac dysfunction in septic mice [10].

5.3. Hsp27 and atherosclerosis

Several reports have described the beneficial effects of Hsp27 in atherogenesis. The risk of atherothrombosis is one of the largest health care burdens worldwide. Both cellular and humoral components of the immune system have been implicated in atherogenesis [65]. Mechanical forces play an important role in the remodeling associated with the pathogenesis of atherosclerosis. As a physiological response to hemodynamic or biomechanical stress, increasing expression of Hsp27 colocalizes with vascular smooth muscle cells (VSMCs) in human atherosclerotic plaques and mammary arteries in its phosphorylated form, thereby binding and stabilizing actin microfilaments [66-67]. Furthermore, the phosphorylated form of Hsp27 is also involved in regulating apoptosis, proliferation and migration of human vascular endothelial cells (ECs) and VSMCs; which in turn prevents plaque rupture [68-70].

Nuclear factor-κB activation is thought to play an important role in the inflammatory response and therefore in determining plaque stability [71-72]. Based on the growing evidence, Hsp27 could interact with IKK protein and prevent the atherosclerotic inflammatory response by inhibiting NF-κB activation [73]. On the other hand, innate and adaptive immune responses appear to
be related to atherogenic mechanisms [74]. Wick et al. have hypothesized that the autoimmune responses to Hsps could be crucial in the initiation of atherosclerosis [75]. It has been reported that Hsp27 stimulates monocyte production of anti-inflammatory cytokines such as IL-10 as well as inhibits their expression of toll like receptor-4 (TLR-4) and their differentiation into dendritic cells [76]. Hsp27 is also identified as an estrogen receptor beta (ERβ)-associated protein in atherosclerosis [77-78]. ERβ is mainly expressed in the ECs and VSMCs of coronary arteries and is correlated with coronary calcification [79]. Stimulation of ERβ in vivo can reproduce the predicted Hsp27 atheroprotective effects and Hsp27 shows attenuated expression with coronary atherosclerosis and modulates estrogen signaling [80].

Some investigators found a significantly decreased Hsp27 expression in the complicated atherosclerotic lesion core region when compared with areas adjacent to plaques [22]. In addition, Hsp27 plasma levels are decreased in atherosclerotic patients compared with those in healthy subjects. Therefore plasma Hsp27 levels could be a potential index of atherosclerosis, although further validation is needed with large patient cohorts. Ventura et al. hypothesized that the lower levels of Hsp27 expressed by atherosclerotic plaques could be a result of its degradation by enzymes that were released from atherosclerotic plaques. Down regulation of Hsp27 was also related to the complexity of the plaque, which decreases VSMCs resistance to proteolytically-induced apoptosis, suggesting that Hsp27 might be critical in the prevention of plaque instability and rupture [81-82].

5.4. Hsp27 and atrial fibrillation

Atrial fibrillation (AF) is the most commonly sustained clinical tachyarrhythmia. Cellular stress of AF contributes to atrial remodeling; involving reduction in the expression of L-type Ca\(^{2+}\) channels and structural changes (myolysis) that eventually result in contractile dysfunction.

The evidence for the protective role of Hsp27 in AF is not only supported by experimental findings in cell culture and animal models, but also by clinical studies in humans [83-87]. Using HL-1 atrial myocytes derived from mouse atria, Brundel et al. [84-85] reported that induction of heat shock responses (including induction Hsp27 by hyperthermia or GGA) protects the heart against tachypacing-induced myolysis, as well as reduction in duration of L-type Ca\(^{2+}\) current and action potential. Furthermore, Hsp27 transient transfection was sufficient for protection against tachypacing-induced myolysis. The authors also demonstrated in dogs in vivo that GGA pretreatment attenuated tachypacing-induced electrical remodeling of atria, including reduced atrial ERP and rate adaptation of ERP, as well as AF duration by burst pacing and AF vulnerability [85].

Clinical studies indicate that there was a significant increase of Hsp27 in patients with paroxysmal AF when compared with those in non-AF control patients and patients with persistent AF. The expression level of Hsp27 was also correlated inversely with myolysis in AF patients. It is suggested that the increased level of atrial expression of Hsp27 may reflect their ability to prevent the progression of paroxysmal AF to persistent AF. In interpreting the inverse correlation between duration of persistent AF and Hsp27 expression, investigators have speculated that although Hsp27 has beneficial effects in AF patients, the persistence of AF could exhaust the heat shock response, leading to a diminished protective effect, thereby promoting progression to persistent AF [84].

These observations indicate that Hsp27 induction may be an effective approach to preventing progression of clinical AF by preventing AF electrical remodeling and structural remodeling. Detailed mechanisms regarding the protective activity of HSP27 against AF still remain to be elucidated.

5.5. Hsp27 and cardiomyopathy

The role of oxidative stress in the pathogenesis of cardiovascular diseases has been extensively investigated. Hsp27 has been classically demonstrated to play important roles in regulating intracellular redox homeostasis by its antioxidant effect, which may mostly rely on modulation of the glutathione system [5, 9, 88]. The elevated level of reduced glutathione could be the result of Hsp27-induced increase in the activity of glucose-6-phosphate dehydrogenase, glutathione reductase, glutathione transferase, and glutathione peroxidase, all of which are vital for the maintenance of intracellular redox potential [89-90].

However, perhaps the most significant findings came from our own studies [91]. We observed that mice expressing high levels of Hsp27 incur reductive stress, resulting in cardiomyopathy, cardiac dysfunction, and a reduced lifespan. Reductive stress is defined as imbalance of the redox status with an abnormal increase of reducing equivalents, such as an elevated ratio of reduced glutathione (GSH) vs. oxidized glutathione (GSSG). Rajasekaran et al [92] pointed out that reductive stress can cause cardiomyopathy in R120G αβ-crystalline transgenic (Tg) mice, which mimics clinical desmin-related myopathy. Why high levels of Hsp27 expression contribute to reductive stress is still elusive. However, we demonstrated that high expression of Hsp27 increased the glutathione pool through upregulation of glutathione peroxidase (GPx) [90, 93]. GPx is an inducible antioxidant enzyme that catalyzes the disposition of H\(_2\)O\(_2\) into H\(_2\)O and O\(_2\). Hsp27-induced cardiomyopathy is at least in part mediated by elevated GPx activity. Furthermore, cardiac-specific expression of Hsp27 at high levels leads to iron deficiency, which decreases ferritin and increases transferrin receptor 1 (TFR1) by feedback regulation in myopathic hearts. As to the underlying mechanisms, Chen et al [94] reported in CCL39 cells that Hsp27 down-regulates TFR1-mediated iron uptake via stabilization of the cortical actin cytoskeleton.

In contrast to the current concept of Hsp27 induction as a protective mechanism against oxidative stress, we have recently demonstrated that high levels of Hsp27 play an important role in the development of cardiomyopathy by increasing GPx activity and decreasing levels of reactive oxygen species, which suggests that Hsp27 is a potential therapeutic target for patients with reductive stress related cardiomyopathy.
5.6. Hsp27 and cardiac allograft rejection

Schinke et al [95] quantified Hsp27 by western blotting in cardiac allograft biopsies and in nonfailing myocardium. They provide evidence for the first time that an induction of Hsp27 expression is involved in rejecting human cardiac allografts.

Under conditions of cellular stress such as a surgical procedure, episodes of ischemia, subclinical rejection, and infection, HSPs expression in cardiac allografts can be up-regulated. These HSPs appear to be cytoprotective agents by binding to misfolded proteins and protecting them from denaturation [96], by maintaining cell and tissue integrity, and by improving organ viability and function in a number of experimental models [97-99]. Therefore, the induction of Hsp27 in the peri- and immediate posttransplantation periods is thought to be a protective response during acute cardiac rejection.

In addition to their direct cytoprotective effects, intracellular Hsp27 is also regarded as an anti-inflammatory agent that can prevent the inflammatory response by inhibiting NF-κB activation [73]. Furthermore, Hsp27 is a significant element of the innate and adaptive immune response system [76]. A lot of studies provide evidence for the proposition that self-Hsp immune reactivity is a physiological mechanism underlying down-regulation of inflammatory disease processes [100]. It would, therefore, appear that the expression of Hsp27 is not necessarily indicative of deleterious response. Indeed it might reflect an anti-inflammatory protective response.

In view of evidence described above, the Hsp27 expression in the cardiac allograft during acute rejection might be crucial in self-protection of the transplanted heart. Nonetheless, the mechanism of increased Hsp27 expression during cardiac allograft rejection remains to be elucidated.

6. Summary

Hsp27 is a ubiquitous molecular chaperone. It functions as a protein chaperone and an antioxidant. It plays an important role in apoptosis inhibition and actin cytoskeletal remodeling. All these roles may have an impact on its myocardial protection in cardiovascular disease. Most recently, a high level of Hsp27 expression has been suggested to contribute to reductive stress and cardiomyopathy. Albeit, further investigations of Hsp27 will not only improve our understanding of pathophysiology of cardiovascular diseases, but will also identify novel therapeutic strategies.

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References

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