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Small Heat Shock Protein 20 (HspB6) in Cardiac Hypertrophy and Failure

Guo-Chang Fan* and **Evangelia G. Kranias**

Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, OH 45267-0575

Abstract

Hsp20, referred to as HspB6, is constitutively expressed in various tissues. Specifically, HspB6 is most highly expressed in different types of muscle including vascular, airway, colonic, bladder, and uterine smooth muscle; cardiac muscle; and skeletal muscle. It can be phosphorylated at Ser-16 by both cAMP- and cGMP-dependent protein kinases (PKA/PKG). Recently, Hsp20 and its phosphorylation have been implicated in multiple physiological and pathophysiological processes including smooth muscle relaxation, platelet aggregation, exercise training, myocardial infarction, atherosclerosis, insulin resistance and Alzheimer's disease. In the heart, key advances have been made in elucidating the significance of Hsp20 in contractile function and cardioprotection over the last decade. This mini-review highlights exciting findings in animal models and human patients, with special emphasis on the potential salutary effects of Hsp20 in heart disease.

Keywords

small heat shock protein; HspB6; cardioprotection; heart failure; apoptosis

1) Introduction

Since the first heat-shock response was described in 1964 by Ritossa [1], a vast array of heat shock proteins (Hsps) has been widely reported in various organisms ranging from prokaryotic *E.coli* to eukaryotic mammals [2,3]. The Hsps are categorized into three major classes according to their molecular weight and/or the stimuli, which induce their expression. The first group consists of the high molecular weight Hsps, with Hsp110, Hsp90, Hsp70 and Hsp60, being the best-characterized members. The second group includes those Hsps induced under conditions of glucose deprivation and are referred to as the minor Hsps, including glucose-regulated proteins (GRP) 34, 47, 56, 75, 78, 94 and 174 [4]. Finally, the third group consists of the small Hsps (sHsps), which includes at least ten members (HspB1-B10), whose molecular weight ranges from 12 to 43 kDa [5]. These sHsps contain a unique N-terminus and a conserved α -crystallin domain at their C-terminus, which facilitates their chaperone activity [5]. Of particular interest in the sHsp sub-family is a protein of ~17

*Correspondence to Guo-Chang Fan, Ph.D, Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, 231 Albert Sabin Way, Cincinnati, OH 45267-0575, Phone: (513) 558-2340, Fax: (513) 558-2269, fangg@ucmail.uc.edu.

Conflict of Interest Disclosures:

None

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kDa, namely Hsp20 or HspB6, which contains a domain inhibiting platelet aggregation and a homology sequence of troponin I [6,7] (Figure 1). This is the only small heat shock protein that has the consensus motif (RRAS) for PKA/PKG-dependent phosphorylation at its Ser16 site [7]. This suggests that Hsp20 may be subject to neurohormonal control via the β -adrenergic cascade in the heart. Therefore, several studies have focused on elucidating its role in the heart, as a putative mediator of the β -adrenergic effects [7].

2) Expression and regulation of Hsp20 in animal models

Hsp20 was first identified in rat and human skeletal muscle by Kato et al. [8]. In particular, these authors reported that, contrary to other heat shock proteins, Hsp20 was not induced by hyperthermia, a finding which was later contradicted by a study by O'Connor and colleagues [9]. These investigators reported that Hsp20 levels were increased upon heat pre-treatment in swine carotid artery [9]. Nevertheless, the study by Kato and co-workers showed that Hsp20 was redistributed from the cytoplasm to the insoluble fraction during heat shock in rat diaphragm, suggesting that Hsp20 was a stress-related protein, similar to other proteins in the same family [8]. Furthermore, Bukach and colleagues [10] showed that human Hsp20 exhibits high chaperone activity, comparable to that of the *bona fide* chaperone α -crystallin, contradicting an earlier report, which concluded that rat Hsp20 is a poor chaperone [11]. Despite these discrepancies, it is well accepted that Hsp20 is ubiquitously expressed and forms oligomeric subunits, which may undergo heat-induced dissociation accompanied by increased chaperone activity [6,7].

Even though Hsp20 is detectable in multiple tissues, it is most highly expressed in skeletal, cardiac and smooth muscles [6,7]. Similar to other Hsps, expression of Hsp20 can be induced by a wide range of stimuli, such as oxidative stress [12,13], exercise training [14], exposure to endotoxin [15] and chemotherapy drugs [16]. Intriguingly, Chu et al. found that Hsp20 expression levels and phosphorylation at Ser16 were increased upon prolonged stimulation with the β -adrenergic agonist, isoproterenol [17]. This is of particular importance since β -adrenergic neurohormonal stimulation is of the utmost importance in mediating augmented contractility in the heart under flight-or-fight scenarios. Notably, Hsp20 levels and phosphorylation were strongly upregulated in tachycardia-induced canine failing hearts, which may be attributed to the increased plasma norepinephrine levels [18]. Similarly, Hsp20 and its phosphorylation were enhanced in post-infarcted animal hearts [19,20]. Taken together, these studies indicate that Hsp20 and its phosphorylation may play a compensatory role in the pathophysiology of heart disease. More interestingly, exercise training elevated the abundance of detergent soluble Hsp20 in the rat heart by 2.5-fold [14]. Further studies indicated that chronic exercise training was necessary to increase the levels of Hsp20, whereas a single brief bout of exercise was not sufficient to achieve a detectable increase in levels of Hsp20 protein [14]. These observations suggest that increasing the abundance of Hsp20 protein may be a mechanism by which exercise yields cardiovascular benefits.

It is important to note that the levels of Hsp20 in plasma of cardiomyopathic hamsters were much higher than controls [21]. This study suggested that Hsp20 may be secreted from dysfunctional cardiomyocytes. In addition, the levels of Hsp20 in injured arteries were markedly lower than those of non-injured arteries *in vivo* [21]. This observation indicates that Hsp20 in normal blood vessel walls immediately responds to mechanical stress (i.e. endothelial injury) and subsequently, released from the injured arterial wall into the circulation. Of interest, circulating Hsp20 possesses strong inhibitory properties to platelet aggregation via the PAR-1 receptor and GPIb/V/IX-vWF axis, which may be beneficial in myocardial infarction [21,22].

3) Hsp20 enhances cardiac contractile function and protects hearts against stress conditions

In the heart, recent studies have begun to unravel the role of Hsp20 in contractile function and cardioprotection. Pipkin et al. observed that Hsp20 became phosphorylated upon treatment with the nitric oxide donor, sodium nitroprusside [23]. Using the phospho-peptide analogues of Hsp20, these authors found that phosphorylated Hsp20 increased shortening and re-lengthening rates in rat cardiomyocytes [23]. Importantly, this study demonstrated that Hsp20 was present in transverse bands, where actin is localized, supporting earlier data from this group in the vasculature [6]. A subsequent study by Chu et al. reported that adenoviral-mediated Hsp20 overexpression in adult rat cardiomyocytes was associated with significant increases in contractility and Ca transients [17], similarly to the aforementioned study by Pipkin et al. [23]. Additionally, cardiac-specific overexpression of Hsp20 in a transgenic mouse model resulted in enhanced contractility [15,19]. Conversely, knockdown of endogenous Hsp20 by antisense RNA reduced cardiomyocyte contractility *ex vivo* [15]. However, the mechanisms responsible for these effects are not well-understood. Further studies will be needed to yield novel mechanistic insights into Hsp20's mode of action, which confers enhanced cardiac function.

On the other hand, accumulating evidence has implicated Hsp20 as a key mediator of cardioprotection in the heart [7]. Initial studies using adult rat cardiomyocytes revealed that adenoviral-mediated overexpression of Hsp20 was protective against β -agonist-induced apoptosis [24]. Interestingly, these beneficial effects were enhanced by the constitutively phosphorylated mutant S16D-Hsp20, while the constitutively dephosphorylated mutant S16A-Hsp20 conferred no protection [24]. Furthermore, transgenic mice with cardiac-specific overexpression of Hsp20 were protected against β -agonist-induced remodeling, associated with attenuation of the pro-apoptotic ASK1-JNK/p38 (apoptosis-signal-regulating kinase 1/c-Jun NH2-terminal kinase/p38) signaling pathway [25]. In addition, Hsp20 transgenic mice exhibited improved cardiac function and prolonged survival after chronic administration of Doxorubicin [16]. The mechanisms underlying these salutary effects were associated with preserved Akt phosphorylation/activity and attenuation of Doxorubicin-induced oxidative stress [16]. Similar beneficial effects of Hsp20 were also observed in protection against endotoxin-triggered myocardial injury [15].

Importantly, Hsp20 transgenic hearts displayed better functional recovery and decreased cellular injury upon ischemia/reperfusion (I/R) *in vivo* and *ex vivo* [19], whereas knockdown of Hsp20 by microRNA-320 resulted in increased cardiac apoptosis and infarct size upon I/R relative to the wild-type controls [26]. Further mechanistic studies unveiled that Hsp20 interacted directly with the pro-apoptotic protein Bax, which may prevent its translocation to the mitochondria and thus block the initiation of apoptosis via inhibition of the caspase-3-mediated apoptotic pathway [19]. Similarly, Zhu et al. reported that Hsp20-overexpressing H9c2 cells were resistant to simulated I/R conditions [27]. The same group further observed that gene transfer of Hsp20 in rat hearts *in vivo* successfully protected against I/R-induced injury [28]. A subsequent study by Islamovik et al. observed that the C-terminal extension of Hsp20 is essential for cardioprotection but is dispensable for its contractile function [29]. Collectively, these studies demonstrate that Hsp20 and its phosphorylation at Ser16 can affect the heart at multiple levels by modulating cardiac contractility and apoptosis.

4) Dys-regulation and mutation of Hsp20 in human patients with cardiomyopathy

In human patients with dilated and ischemic cardiomyopathy, the levels of Hsp60, Hsp27 and Hsp20 have been shown to increase, whereas Hsp70, Hsp72, and Hsp90 were not changed, compared with healthy hearts [20,30]. Specifically, both total Hsp20 and its phosphorylation at Ser-16 were upregulated in human failing hearts by ~2-fold over healthy controls [20]. These observations suggest that Hsp20 and its phosphorylation at Ser16 may function as an innate protector during heart failure. Recently, Nicolaou et al. identified a substitution of C59T in the human Hsp20 gene [31]. Analysis of C59T in a total of 1347 DCM patients and 744 non-cardiomyopathic individuals from three different populations (Greek, German and American) revealed the C59T substitution at a low frequency in the Greek (0.48%) and German (0.82%) populations, in individuals with no heart disease as well as in DCM patients, suggesting that the C59T transversion is a rare human mutation [31]. Of particular interest, the C59T genetic variant results in substitution of a highly conserved proline at position 20 to leucine (P20L), just 4 amino acids downstream of the PKA/PKG-dependent Ser 16 phosphorylation site (Figure 1). Indeed, P20L-Hsp20 failed to become phosphorylated to the same extent as WT-Hsp20 upon simulated I/R in cardiac myocytes or by protein kinase A *in vitro* [31]. Importantly, this diminished phosphorylation was associated with a complete loss of Hsp20's cytoprotective properties, pointing to an instrumental role of phosphorylated Hsp20 in cardioprotection [31]. In agreement with this notion, blockade of Ser16-Hsp20 phosphorylation *in vivo* exacerbated cardiac ischemia/reperfusion injury by suppressed autophagy and increased cell death [20]. While the C59T mutation is identified at low frequencies in both the cardiomyopathic and the non-cardiomyopathic populations, its relevant contribution to cardiac pathogenesis remains unclear at this point. Therefore, generation of an animal model with cardiac-specific knock in for the C59T mutation would yield significant insights into its functional role *in vivo*.

5) Therapeutic potential of Hsp20 in heart disease

Hsp20 has recently garnered considerable attention as a therapeutic target, because it has been implicated in the regulation of diverse processes such as relaxation of vascular muscle, myocardial contraction, myometrium functioning, platelet aggregation, and apoptosis [6,7,32]. Further, Hsp20 appears to participate in a variety of pathological processes such as Alzheimer's disease, atherosclerosis, asthma, intimal hyperplasia, insulin resistance, heart failure, and septic shock [15,32–34]. In addition, increased levels of Hsp20 and its phosphorylation have been correlated with cytoprotection in many experimental injury models such as mesenchymal stem cell transplantation [12], sepsis-triggered myocardial dysfunction [15], chronic doxorubicin-induced cardiomyopathy [16], and cardiac ischemia/reperfusion injury [19]. However, the induction of Hsp20 to improve outcome in human disease has not been exploited. While stress conditions can effectively upregulate Hsp20 expression, this approach would be poorly tolerated by patients and would have detrimental effects on many cellular functions. Of interesting, two pharmacological agents: 1) vasoactive intestinal peptide (VIP), a naturally occurring neurotransmitter; and 2) sildenafil, an oral phosphodiesterase type-5 inhibitor, have been shown to induce substantial Hsp20 phosphorylation in rabbit colonic circular smooth muscle cells [35] and pig coronary artery [36], respectively. Although there is accumulating evidence for a cardioprotective role of VIP and sildenafil [37–40], it is currently unknown whether their beneficial effects are associated with Hsp20 and its phosphorylation. Thus, future studies will be needed to investigate whether sildenafil and VIP activate Hsp20 in the heart. Additionally, transgenic model of Hsp20 showing cardioprotection exhibited that 16±1% of total Hsp20 was phosphorylated in mouse hearts [19]. Therefore, it would be also needed to evaluate the degree of Hsp20 phosphorylation achieved by sildenafil and VIP in cardioprotection.

While genetic manipulation by viral vectors or permeabilization to increase intracellular levels of Hsp20 has displayed cardioprotective effects [7,23,28], these approaches have significant limitations in clinical application. Excitingly, recent studies have discovered a series of small protein domains which can cross biological membranes efficiently independent of transporters or specific receptors, thereby promoting the delivery of peptides and proteins into cells [41,42]. Among these cell-penetrating protein s/peptides (CPPs) identified thus far, TAT protein from HIV is well defined to deliver biologically active proteins *in vivo* and has been shown to be of considerable interest for protein therapeutics [41,42]. Using this technique, Brophy's team observed that Hsp20 peptide analogs covalently linked to the TAT protein transduction domain prevent smooth muscle contraction in various animal species [32]. However, recombinant TAT-Hsp20 is more efficacious than TAT-Hsp20 peptide in the regulation of smooth muscle contraction [32]. This suggests that the functional effectiveness of Hsp20 may be dependent on its inherent stability of conformation.

6) Most important questions and problems for future studies on Hsp20

While increasing evidence has demonstrated the potential importance of Hsp20 in human disease, there are many questions remaining about this molecule. Recent published crystal structure of the excised α -crystallin domain from rat Hsp20 indicates that it forms homodimers with two pockets and a shared groove at the interface by C-terminal extensions [43]. Importantly, Hsp20 has a more extended interface than α B-crystallin, which results in Hsp20 dimers being less compact and more assessable to client proteins than α B-crystallin [43]. This unique structure of Hsp20 may partly explain its unusual versatility. Actually, multiple cellular proteins have been identified to interact with Hsp20, including Hsp22, Hsp27, α B-crystallin, 14-3-3, type-1 protein phosphatase (PP1), ASK1, Akt, Bax, Bag3, Beclin-1, and actin [7,16,19,20,44–46]. However, sites for subunit interaction, target substrate protein binding, phosphorylation and interaction with cytoskeletal elements have not been identified. Furthermore, we have observed that increased Hsp20 levels were associated with activation of cardiac autophagy [20], but the functional role of Hsp20-mediated autophagy in the heart remains unclear. Further work is also needed to confirm the experimental results described in this review and identify other possible mechanisms of action using inducible overexpression and cardiac-specific knockout approaches. In addition, based on the effects of the Hsp20 fused to cell-penetration peptide (CPP) in relaxation of a variety of smooth muscles from multiple sources [32], it becomes important to test the CPP-Hsp20 efficacy in the treatment of heart disease. However, several problems associated with protein/peptide-based therapy must be taken into consideration, such as synthesis of bulk quantities of protein/peptide, maintenance of protein/peptide activity, protection against degrading enzymes and/or detrimental biological environments, as well as the specificity of targeting selective tissues. Nevertheless, efforts by several dedicated laboratories may lead to the rapid development of novel therapies for cardiovascular diseases based on the function of Hsp20.

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Figure 1.

Human Hsp20 contains 160 amino acids including the highly conservative α -crystallin domain, variable N-terminal and C-terminal extensions. A motif of WLRRASAPL can inhibit platelet aggregation. A motif similar to the minimal inhibitory region of cardiac troponin I is present at residues Gly111–Leu123. Serine 16 is an important site for phosphorylation by cyclic nucleotide-dependent protein kinases (PKA and PKG). Proline 20 is found to be mutated in humans.