Heat Shock Proteins in Cardiovascular Diseases: From Bench to Bedside

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Abstract Heat shock proteins (HSP) are stress proteins induced in response to a wide variety of physiological and environmental insults. HSP function as molecular chaperones and they are required to maintain the proteome in a folded and functional state, allowing the cells to survive stress conditions. These key proteins, which may be located intracellularly or extracellularly, have multiple functions that range from the regulation of essential cells function to the renaturation of misfolded proteins. In the last decades, the HSP involvement in both normal cell function and disease pathogenesis is widely studied, especially in the context of cardiovascular diseases (CVDs). This chapter covers the current knowledge on the function HSP in the cardiovascular system and particular in the relationship between these proteins and CVDs. Initially, the roles of HSP in cardiovascular health are outlined, followed by an evaluation of the role of HSP in CVDs key processes, such as atherosclerosis, vascular hypertrophy and heart failure. Finally, the therapeutic potential of roles HSP are examined in a CVDs context, considering how the knowledge actually gained may be capitalized in future clinical studies.

Keywords Cardiovascular diseases · Heat shock proteins · Hsp40 · Hsp60 · Hsp70 · Hsp90 · Small heat shock proteins · Therapeutics

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Abbreviations

AIF	apoptosis inducing factor
$ApoE^{-/-}$	apolipoproteinE knock out mice
Ca^{2+}	calcium
CEL	celastrol
CVDs	cardiovascular diseases
GGA	geranylgeranylacetone
HSEs	heat shock elements
Hsf1	heat transcription factor 1
HSP	heat shock protein
LDL-C	low-density lipoprotein-cholesterol
RIPC	remote ischemic preconditioning
ROS	reactive oxygen species
sHSP	small heat shock protein
sHSP60	soluble heat shock protein60
SMCs	smooth muscle cells
TLR4s	toll-like receptors 4
VSMCs	vascular smooth muscle cells

Introduction

Nowadays, despite a variety of therapeutic advances, cardiovascular diseases (CVDs) remain a leading cause of mortality worldwide (Kessing et al. 2016). CVDs include various diseases that affect heart and/or blood vessels, such as coronary artery diseases like angina and myocardial infarction. Heat shock proteins (HSP) are stress proteins, also called chaperones that are massively produced by almost all prokaryotic and eukaryotic species when exposed to elevated temperatures. These proteins were discovered in 1962 by Ritossa in the *Drosophila melanogaster* as a set of highly conserved proteins whose expression was induced in salivary gland chromosome in response to transient exposures to elevated temperatures (Kliková et al. 2016; Ritossa 1962). The increased expression of HSP in cells during the heat shock response was demonstrated to inhibit stress-mediated cellular death as shown also by recent experiments indicating a highly versatile role for these proteins (Garrido et al. 2006). These functions are summarized in Fig. 1. HSP play an important role in cell-cycle control, folding, defolding and assembling of protein complexes (Nakai and Ishikawa 2001) and they have been classified into six families on the basis of their approximate molecular weight: small HSP, HSP40, HSP60, HSP70, HSP90 and HSP100 (Khalil et al. 2011). These proteins constitute the 5–10% of the total protein content in cells in physiological conditions and play important roles in cellular homeostasis. They control maturation and turnover of intracellular proteins and play significant roles in the maintenance of cellular integrity.

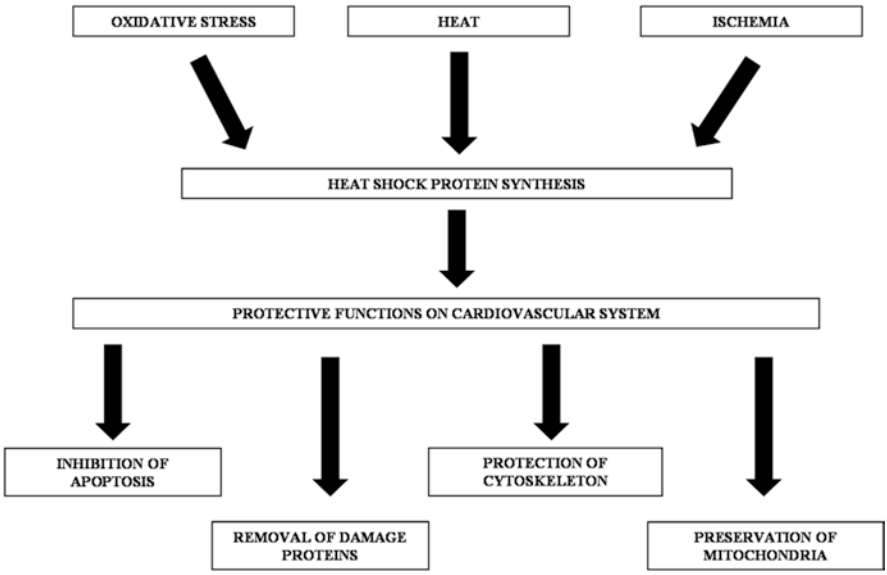

Fig. 1 Main heat shock proteins protective functions at cardiovascular level

Interestingly, the synthesis of these proteins can be markedly induced by various cellular insults ranging from elevation in temperature to exposure to different stimuli which are potentially damaging to the cell (Pockley 2002). These latter conditions include several known risk factors for CVDs onset and development. It is clear, however, from the extensive studies which have been carried out in a variety of cell types that the HSP are induced in response to cellular stress and they confer protection against nonthermal cytotoxic stimuli. A recent in vitro experiment demonstrated that induction of the stress response protects endothelial cells against superoxide generation (Chen et al. 2017). Other examples in vivo include protection of cardiomyocytes against apoptosis, induced by ischemia/reperfusion injury or by prolonged beta-agonist stimulation (Fan et al. 2004, 2005). Even if new functions continue to be discovered, the stress proteins are generally thought to maintain cellular homeostasis by acting as molecular chaperones, facilitating the proper folding and assembly of nascent polypeptides, as well as assisting in the refolding and stabilization of damaged peptides (Bond and Schlesinger 1985). HSP act as intracellular chaperones, protecting protein structure and folding under stress condition, but are also able to assist with numerous reparative processes including the refolding of denatured proteins and removal of irreparably damaged proteins (Kampinga and Craig 2010). Under particular conditions HSP could be released from cells into the extracellular space, where they exhibit a range of immunoregulatory activities (Chebotareva et al. 2017).

To date numerous studies suggest that the upregulation of HSP could be associated with cardioprotection (Jimenez et al. 2014). In fact, before myocardial infarction, an increase in HSP70 and HSPB1 levels induced through an infection with

Table 1 Heat shock proteins involvement in cardiovascular diseases

Superfamily	Protein names	Alternative names	Low protein expression levels or mutations
sHSPs	HSPB1	HSP27,HSP25, HMN2B	Coronary artery disease
	HSPB2	MKBP	Altered mitochondria functions
	HSPB3	HSPL27	Deregulation of myoblast viability
	HSPB5	alphaB-crystallin	Desmin-related myopathy
	HSPB6	HSP20	Decreased contractile function
	HSPB7	CvHSP	No protection against age-related dysfuction
	HSPB8	H11, HSP22	Lack of protection after myocardial ischemia
HSP40	DNAJA3	Tid-1; Tid11	Respiratory chain deficiency
HSP60	HSP60	Cpn60, Hsp60	Protection from atherosclerosis and proliferation of vascular smooth muscle cells
HSP70	HSPA1A	HSP72; HSPA1	Plaque rupture and thrombotic complications
	HSPA8	HSC70; HSC71	
HSP90	HSPC4	GP96, endoplasmin	Increased in plaque stability
			Cardiomyocyte apoptosis

replication deficient adenovirus encoding for these two HSP has been shown to protect heart (Wei et al. 2006). Moreover, Chen et al. (2005) showed that along with the rise in reactive oxygen species (ROS) levels, there was a significant increase in the heart levels of HSP (HSP70, HSPB1) in response to remote ischemic preconditioning (RIPC). Moreover, treatment with antioxidants reduced the levels of HSP and also attenuated cardioprotection (Chen et al. 2005). These results suggest that HSP production may be induced in condition characterized by an increase in free radical production and this enhancement, in turn, may confer resistance to myocardium against sustained ischemia reperfusion injury. Furthermore, Madamanchi et al. (2001) in an in vitro study showed that upregulation of HSP may be secondary to increase in ROS in vascular smooth muscle cells. In contrast, other studies showed that HSP may stimulate the production of ROS (Baruah et al. 2014; Multhoff et al. 2015). So, more studies are required to better clarify the possible interaction between heat shock proteins HSP in cardioprotection (Singh et al. 2017). Table 1 summarizes the main involvement of H HSP in CVDs. In the following sections, we present an overview of the HSP roles and functions in cardiovascular health conditions and their implication in the onset and development of cardiovascular diseases, firstly we presented the small HSP, characterised by a low molecular weight (15–43 kDa), and then the HSP with a higher molecular weight (HSP40, HSP60, HSP70, HSP90). Finally, we provided a brief summary about potentially preventive and therapeutic approaches that involved HSP modulation.

Emerging Roles of Heat Shock Proteins in Cardiovascular Diseases

Small Heat Shock Proteins

Small HSP (sHSP), also called HSPβ (HSPBs), have molecular weight which varied from 15 to 43 kDa and are mainly known for their chaperoning function in the process of embryonic development (Jee 2016). This family of HSP consists of ten members (HSPB1-HSPB10), which are characterized by different functions and display different expression profiles. Some members of this family are widely expressed, like HSPB1, HSPB5 and HSPB8, while some other members show exclusively a restricted expression pattern. In particular, HSPB2 and HSPB3 are only found in cardiac and muscle cells, HSPB4 is lens specific, while HSPB9 and HSPB10 are specifically expressed in testis (Clark et al. 2012; Fontaine et al. 2003; Kappe et al. 2003; Sugiyama et al. 2000; Suzuki et al. 1998; Verschuure et al. 2003). Some members of this family, such as HSPB1 and HSPB4, exert mainly refolding activities, while other members display only anti-aggregation function (HSPB6, HSPB7 and HSPB8). Furthermore, other members exert very specialized activities, for example HSPB2 and HSPB3 seem to have an essential role in muscle differentiation, while HSPB1, HSPB5 and HSPB7 show very specific chaperone functions at the levels of nuclear structures forming during stress (Boncoraglio et al. 2012).

sHSP present a highly conserved sequence of 80–100 amino acids called the α-crystallin domain. This structural domain is responsible for many intra- and intermolecular interactions leading to the formation of dimers, which are considered as sHSP basic unit (Kim et al. 1998; Van Montfort et al. 2001a, b;). These dimers can interact with each other forming higher molecular weight oligomers. Besides the α-crystallin domain, both the C-terminal and the N-terminal regions are involved in the stabilization of the oligomers (Kim et al. 1998; Lambert et al. 1999; Van Montfort et al. 2001a, b). However, besides these different aspects, ten members of the HSPB family share the same properties and so alteration of these properties represent a key mechanism that can lead to diseases onset, also at cardiovascular level.

Cardiac and skeletal muscle cells express the largest variety of HSPBs: HSPB1, HSPB2, HSPB3, HSPB5, HSPB6, HSPB7 and HSPB8. These family members are commonly considered the main focus of interest of CVDs researches and new scientific evidences emphasize the functions of these molecules in cardioprotection (Carra et al. 2017; Charmpilas et al. 2017; Edwards et al. 2011; Fan et al. 2005; Martin et al. 2014; Weintraub and Rubinstein 2013).

HSPB1 is a ubiquitously expressed, multifunctional protein chaperone. A decrease in HSPB1 secretion was shown in human atherosclerotic plaques respect to control vessels and also plasmatic HSPB1 levels are reduced in atherosclerotic patients compared to healthy subjects (Martin-Ventura et al. 2004). Moreover,

Seibert et al. (2013) showed that in human low serum HSPB1 levels are associated with coronary artery disease and prognostic of adverse clinical events; while in mouse models of atherosclerosis the increase in HSPB1 level in aorta induce a reduction of atherosclerotic lesion progression and promote plaque stability. It was also shown that HSPB1 overexpression in atherosclerotic-prone mouse models (apolipoproteinE knock out mice – $ApoE^{-/-}$) modulates plaque formation preventing inflammation, foam cells formation and reducing atherosclerotic plaque area (Rayner et al. 2008). In heart tissue HSPB1 expression was associated with sarcomeres and so it was found to be cardioprotective (Brundel et al. 2006a, b). Moreover, Ghayour-Mobarhan et al. (2012) reported that increased expression of myocardial HSPB1 is a prognostic marker of myocardial ischemia. HSPB1 has been demonstrated to play also an important role in regulating intracellular redox homeostasis and anti-apoptotic pathway (Arrigo et al. 2005; Zhang et al. 2010). Panneerselvam et al. (2017) observed that differential expression of HSP in the rat myocardium could serve as a balance between pro-survival and death signal during acute fluoride-induced heart failure. In detail, the Authors suggested that increased expression of myocardial HSPB1 serves as a prognostic marker for myocardial ischemia and this increase is correlated with myocardial necrosis, impaired contractile function and regulation of intracellular redox homeostasis.

HSPB2 has shown to be highly expressed in heart and it has protective effects against heart diseases such as cardiac hypertrophy and ischemia (Ishiwata et al. 2012; Nakagawa et al. 2001; Sugiyama et al. 2000). Moreover, HSPB2 was associated with the outer membrane of mitochondria and involved in mitochondria permeability transition and calcium uptake. Overexpression of this sHSP was found to conserve ATP synthesis during ischemic/reperfusion injury in mice (Nakagawa et al. 2001). Moreover, mice knockout for HSPB2 with ischemic stress show altered mitochondria respiration rates and reduced ATP production as well as modification in expression of several metabolic and mitochondrial regulators (Ishiwata et al. 2012). These findings suggest that HSPB2 has cardioprotective effects maintaining mitochondrial function and metabolic activity during cardiac stress.

Golenhofen et al. (2006) showed that the cytosolic calcium increase due to the lack of HSPB2 in knockout animals may modify the calcium sensitivity of myofibrils altering the cardiac contractility, suggesting an involvement of HSPB2 in maintaining muscular elasticity during ischemic insult. Interestingly, mice with HSPB2 overexpression in heart revealed lower levels of cardiac injury biomarker troponin I in the blood after ischemia/reperfusion stress, confirming the involvement of HSPB2 in preserving contractile function of the heart (Grose et al. 2015).

HSPB3 was considered not expressed in the heart (Vos et al. 2009), but recently Carra et al. (2017) reported in patients with myopathy two novel mutations in the HSPB3 gene that lead respectively to protein aggregation or truncation and destabilization. Remarkably, these mutations could abolish HSPB2-HSPB3 complex formation, leading to free HSPB2 proteins that tend to mislocalize inside the cells. Moreover, the same authors reported that HSPB2-HSPB3 interaction would lead to deregulation of HSPB2, with potential consequences on myoblast function and viability. These studies suggested that alterations in expression and solubility of

specific HSPBs due to aggregation propensity and deregulated association with other HSPBs could be the basis of complex diseases, such as myopathies.

HSPB5 was found in cardiomyocytes on the I-band and M-line region of sarcomeres (van de Klundert et al. 1998). It is known to bind and stabilize intermediate filaments, actin microfilaments, and sarcomeric proteins, including actin, desmin and titin in physiological conditions (Bullard et al. 2004; Ghosh et al. 2007; Perng et al. 1999). HSPB5 has shown to be involved also in stabilization of the cytoskeleton (Vicart et al. 1998). Interestingly, mutations in HSPB5 are associated with cardiac and muscular disorders. In fact, HSPB5 mutations result in an irregular protein structure and defective chaperone-like function (Bova et al. 1999), which leading to desmin-related myopathy and also early onset of cardiomyopathy (Selcen and Engel 2003; Vicart et al. 1998).

HSPB6 is abundantly expressed in skeletal muscle and heart in two complex formations: 43 kDa dimers and 470 kDa multimers and that this protein is able to bind itself and other HSPBs, like HBPB1, HSPB5 and HSPB8 (Pipkin et al. 2003). Recently, in a more recent study, the overexpression of HSPB6 resulted in enhanced cardiac function by interaction with protein phosphatase 1 and in turn inducing calcium (Ca^{2+}) cycling and sarcoplasmic reticulum Ca^{2+} load (Qian et al. 2011). In addition, this stress protein induces the Ca^{2+} cycling in the sarcoplasmic reticulum and increases the contractile function of the cardio myocyte (Qian et al. 2011). Further, in an experimental study in mice, the phosphorylation of HSPB6 at serine 16 level was shown to be needed for modulating cell injury during ischemia/reperfusion (Qian et al. 2009). So, HSPB6 acts maintaining the heart integrity in mice with ischemia/reperfusion injury (Fan et al. 2005).

HSPB7 is expressed in heart and skeletal muscle. It was shown that HSPB7 is significantly expressed in aged muscle like to HSPB5 (Doran et al. 2007). HSPB7 was also shown to be upregulated in the muscular dystrophy-affected diaphragm, suggesting its induction under stress conditions. Additionally, HSPB7 is involved in protections of cells from protein aggregation, probably by facilitating cargo delivery to autophagosomes (Vos et al. 2010). To note, HSPB4, HSPB6 or HSPB7 could not promote the cellular capacity to chaperone heat-denatured luciferase as shown by HSPB1, indicating different functions and properties of HSPB members (Vos et al. 2010, 2011). Additionally, in cardiomyocytes was observed the co-localization of HSPB7 on myofibrils, suggesting a protective role maintaining the sarcomeric structure (Golenhofen et al. 2004).

HSPB8 is showed in striated and smooth muscles, brain, and keratinocytes level (Vos et al. 2008). This protein can be phosphorylated in vitro, but, respect to HPB1 and HSPB5, HSB8 phosphorylation influences only marginally its tertiary and quaternary structure. HSPB8 exists, both in its wild type and phosphorylated form, as low molecular mass oligomers. Respect to HSPB1 and HSPB5 that showed reduced oligomeric size and increased chaperone activity after phosphorylation, the phosphorylation of HSPB8 results in larger oligomeric structures and decreased chaperone activity (Basha et al. 2006). In in vitro experiments, it has been shown that HSPB8 interacts with several proteins and forms stoichiometric complexes (Carra et al. 2008a). This complexes was found to induce autophagy, which may be benefi-

cial in response to irreparable protein damage (Carra et al. 2008b, 2009). In addition, HSPB8 has been shown to be cardioprotective in experimental models of myocardial ischemia. In detail, Depre et al. 2006 showed that HSPB8 overexpression promotes cardiomyocyte survival after ischemia in mice, while Chen et al. 2011 indicated that this sHSP attenuates the myocardial damage and contractile dysfunction in experimental animals. On the other hand, it was demonstrated that depletion of HSPB8 in mice with pressure overload supports cardiac dysfunction and promotes transition to heart failure (Qiu et al. 2011). Other studies showed that HSPB8 maintains mitochondrial function and energy production that results in attenuation of oxidative stress in infarcted hearts (Marunouchi et al. 2014). In contrast these beneficial HSPB8 effects on cardiomyocyte function, HSPB8 overexpression, both in in vitro and in vivo model systems, was found to induce cardiac hypertrophy (Depre et al. 2002; Hedhli et al. 2008). Therefore, the function of HSPB8 seems to have two faces in heart diseases: HSPB8 acts as a cardioprotective protein during myocardial ischemia by conserving the mitochondrial function and energy production but, at the same time, HSPB8 is a mediator of cardiac hypertrophy and thereby results in heart failure.

HSP60

HSP60 protein is structurally highly conserved and abundantly expressed by prokaryotic and eukaryotic cells under stress conditions. It is considered a constitutively cytosolic protein that translocated to the mitochondria (Gething and Sambrook 1992), but it is also stress inducible, indeed ischemia has been demonstrated to be a potent inducer (Marber et al. 1993). During heart failure this protein is translocated to the plasma membrane and released into the plasma; in fact was shown that HSP60 levels were doubled in end-stage heart failure (Knowlton et al. 1998; Lin et al. 2007). Some studies reported that high titers of anti-HSP60 were correlated with coronary atherosclerosis (Prohászka et al. 2001; Zhu et al. 2001), moreover high levels of anti-HSP60 in children increase the probability to develop CVDs (Cohen and Young 1991). Other studies suggested that plasmatic levels of HSP60 was increased early in heart failure (Brundel et al. 2006a, b). During heart failure, HSP60 localizes to the plasma membrane and the cell surface, in addition to its normal distribution to the mitochondria and cytosol (Wang et al. 2010) and acts as a "danger signal". This localization of HSP60 could be linked with an increase in apoptosis of the affected cell, because the cell-surface expression of HSP60 may be able to interact with other cells to trigger the innate immune response, resulting in the release of pro-inflammatory cytokines which could induce myocyte loss and contribute to heart disease. Previous studies (Malik et al. 2013; Gupta and Knowlton 2007) demonstrated that HSP60, via exosomal pathway, was released by cardiomyocytes and this stress protein was increased by exposure of the cardiomyocytes to ethanol. Li et al. (2011) reported that, HSP60 induced inflammation in the heart, via activation

of the TLR4- MyD88-IRAK-1 pathway, and led to cardiomyocyte apoptosis, promoting progression of heart failure.

Some studies in human associated HSP60 expression with atherosclerosis and its severity and it was detected on endothelium, smooth muscle cells (SMCs) and mononuclear cells (Kleindienst et al. 1993; Lamb et al. 2002). Other studies reported a positive association between titers of antibody to HSP60 and the extent of atherosclerosis (Burian et al. 2001; Veres et al. 2002; Xu et al. 2000; Zhao et al. 2015; Zhu et al. 2001). Xiao et al. (2005) reported that soluble HSP60 (sHSP60) is probably involved in activating proinflammatory processes associated with early vessel pathology providing the first prospective data confirming an association between elevated levels of sHSP60 and early carotid atherosclerosis. Moreover, studies in cholesterol-fed rabbits showed that plasma titers of anti-HSP60 are consistent with atherosclerotic formation and increased expression of HSP60 on the endothelium during atherogenesis (Ghayour-Mobarhan et al. 2007; Khan et al. 1998; Lamb et al. 2002; Pfister et al. 2005; Zhao et al. 2015). Recently, in vitro experiments have shown that HSP60 can activate the proliferation of vascular SMCs (VSMCs) (de Graaf et al. 2006; Fukuoka et al. 2004; Sasu et al. 2001; Zhao et al. 2015). Moreover, Zhao et al. (2015), in an experimental study, suggested that HSP60 is implicated in the VSMC migration during atherosclerosis. The authors reported that activation of HSP60 could be considered one of the most powerful methods of sending a 'danger signal' to the immune system to generate chemokine, such as interleukin-8, which is involved in stimulation of VSMC migration.

HSP70

HSP70 is one major member of HSP family, which is able to bind its substrate through the substrate binding domain, dephosphorylate substrate and in turn maintain its "normal" functions (Connarn et al. 2014). This HSP have some function in the cells including binding to partially synthesized peptide sequences, keeping translocation competent structures of endoplasmic reticulum and mitochondrial precursors in the cytosol and simplify translocation from inside the target compartment (Craig et al. 1994; Mamipour et al. 2017). Previous studies have provided evidences that during myocardial ischemia/reperfusion HSP70 had protective effects and it was able to reduce the deleterious effects characteristics of this pathological condition (Feng et al. 2014; Garrido et al. 2006; Vicencio et al. 2015). In particular, during myocardial ischemia/reperfusion HSP70 accelerates the dephosphorylation of the stress kinase JNK inducing cardiac protection (Gabai et al. 2000). Moreover, in this pathological condition HSP70 is able to prevent the nucleus translocation of apoptosis inducing factor (AIF), acting as a molecule chaperone, so it stabilizes nucleus and antagonizes cardiomyocyte apoptosis (Choudhury et al. 2011). Other data confirming this action showed that the deletion of the domain of AIF involved in interaction with HSP70 enhanced AIF translocation to nucleus inducing apoptosis (Gurbuxani et al. 2003; Zhang et al. 2017). Recently, Vicencio et al. (2015) showed that plasma exosomes,

which carry HSP70 on their surfaces, attenuated myocardial reperfusion injury in rats. In in vitro studies, exosomes derived from plasma, containing HSP70, bound to toll-like receptors 4 (TLR4s) on cardiomyocytes and activated various kinases leading to cardioprotection and suggesting the therapeutic use of exosomes for reperfusion injury. Mayr et al. (1999) showed that, in human atherosclerotic aorta, HSP70 is expressed with a heterogeneous pattern respect to control vessels. This HSP70 altered pattern could be due to its release from damaged cells into the plaque. Mazzaferro et al. (2003) hypothesized that insufficient HSP70 in SMC of the aorta leads to their death and favors plaque rupture and thrombotic complications. Moreover atherosclerotic plaque thickness was correlated with the HSP70 expression to the center of the lesion (Pockley et al. 2003). Other studies attributed to HSP70 a protective role in atherosclerosis due to its pro-survival effect on VSMCs. It was demonstrated that HSP70 localization changed during plaque development and progression and was positively related to atherosclerosis severity (Dybdahl et al. 2005; Johnson et al. 1993; Pockley et al. 2003). Furthermore Zhu et al. (2003) have reported that there is an inverse relation between HSP70 expression and severity of coronary disease.

HSP90

The 90 kDa heat shock protein, HSP90, is one of the most abundantly expressed protein in eukaryotic cells, comprising 1–2% of cellular proteins under physiological conditions. It contributes to various cellular processes as signal transduction, protein folding and degradation and morphological evolution (Csermely et al. 1998; Pearl and Prodromou 2001; Picard 2002; Pratt and Toft 2003; Richter and Buchner 2001; Sreedhar et al. 2004). The activity of HSP90 is in cooperation with other co-chaperones, playing a key role in the folding of newly synthesized proteins and stabilization and refolding of denatured proteins during stress conditions (Sreedhar et al. 2004). Some studies indicated that HSP90 plays an important protective role in ischemic pre-conditioning and post-conditioning, acting as a cardioprotective and antiapoptotic protein (Amour et al. 2009; Zhong et al. 2014). Moreover, targeted overexpression of HSP90 protects myocardial reperfusion injury reducing infarct size and myocardial dysfunction (Kupatt et al. 2004). On the other hand, Wang et al. (2009) showed that HSP90 inhibition increases cardiomyocyte apoptosis. It has also been shown that, in isolated hearts, HSP90 attenuates ischemia/reperfusion-induced cardiomyocyte apoptosis and necrosis (Budas et al. 2010). Moreover, recent reports demonstrated that HSP90 is anti-apoptotic in cardiomyocytes acting as antioxidant and preserving mitochondrial function under stress conditions (Wu et al. 2012).

Previously, a human study demonstrated augmented systemic HSP90-specific cellular and humoral responses in carotid atherosclerosis patients (Mu et al. 2017; Rigano et al. 2007). Furthermore, other authors showed that HSP90 is highly expressed in plaque and serum from patients with atherosclerosis, in which immune responses are stimulated, suggesting that HSP90 is a potential target in the pathogenesis of carotid atherosclerosis (Businaro et al. 2009; Mu et al. 2017). Recently, Mu et al. (2017) showed

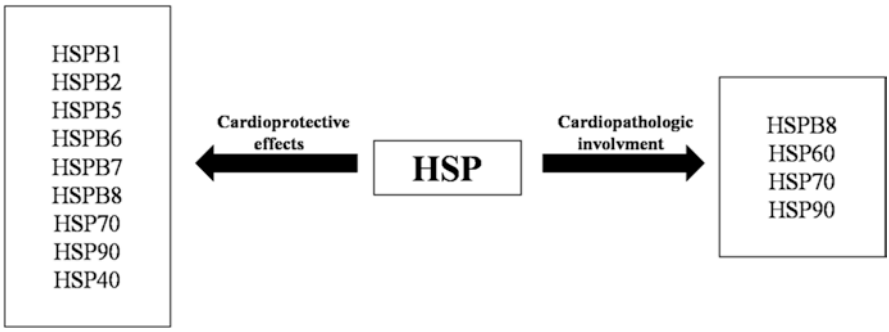

Fig. 2 Schematic representation of heat shock proteins involvement in cardioprotective and/or cardiopathological processes

that in a mouse model of atherosclerosis HSP90 overexpression in vulnerable carotid plaque induced a decrease in plaque stability, along with increased accumulation of lipids, macrophages, inflammation, as well as a decrease in VSMCs and collagen, while HSP90 inhibition exerted opposing effects. So, these authors suggested that regulation of the local HSP90 expression could be a new route for atheroprotection.

HSP40

HSP40 is the co-chaperone of HSP70. This heat shock protein has also been shown to be involved in the pathology of dilated cardiomyopathy that is an important cause of sudden cardiac death and heart failure (Hayashi et al. 2006; Henderson and Pockley 2012). In fact, inactivation of the gene encoding mitochondrial HSP40 in mice resulted in the development of severe cardiomyopathy which induced death probably due to the role played by HSP40 in mitochondrial biogenesis (Hayashi et al. 2006). Hayashi et al. (2006) showed that in cardiomyocytes lacking HSP40 there were progressive respiratory chain deficiency and reduced copy number of mitochondrial DNA, underlining the key role of this protein in CVDs. Figure 2 summarizes the HSP involvement in cardioprotective/cardiopathological processes.

Heat Shock Proteins Modulation as a Potential Therapeutic Target

The broad function on cardiovascular system discussed above strongly sustains the notion that strategies aimed at modulating HSP expression may be important for preserving CVDs onset and related symptoms. In fact, some experimental studies have tested the hypothesis that HSP modulation improves the outcome of CVDs,

particularly in the context of ischemia/reperfusion injury. The modulations of HSP can be performed in different ways such as by the use of small molecules able to modulate HSP expression or through their activation by phosphorylation. The induction of chaperones in response to different stress conditions are mediated at the transcriptional level by the heat transcription factor 1 (Hsf1) that binds to its target sites (heat shock response elements) in the promoter regions of stress-induced genes and thus, following Hsf1 phosphorylation, induces HSP gene expression (Westerheide et al. 2012). In physiological conditions, without stress, Hsf1 is retained in an inactive state in the cytoplasm binded to HSP90. Stress conditions induce dissociation of HSP90 from Hsf1 that, in turn translocate to the nucleus where it up-regulates transcription of chaperone genes. Although the HSP were identified on the basis of their induction by stressful procedures, from a clinical standpoint there are several drugs and natural compounds that modulates HSP and could be beneficial in the treatment of CVDs.

Geranylgeranylacetone (GGA) is a cyclic polyisoprenoid used as a gastromucoprotective drug. In experimental conditions, it has been showed that this pharmacological compound can induce transcriptional activation of HSP70 (Chang et al. 2013; Hirakawa et al. 1996) and that ischemia/reperfusion-induced damage of myocardial cells was prevented in GGA-treated myocytes (Chang et al. 2013; Ooie et al. 2001). Moreover, GGA upregulates HSP expression in heart and attenuates ischemia/reperfusion injury, degradation of myofibrils, and atrial fibrillation genesis caused by rapid atrial pacing or atrial ischemia (Brundel et al. 2006a, b; Chang et al. 2013; Ooie et al. 2001). Recently, Chang et al. (2013) showed that HSP induced by GGA can regulate the atrial arrhythmogenesis in heart failure by modulation of sodium and potassium channels, as well as calcium homeostasis (Chang et al. 2013).

HSP co-inducer arimoclomol has been tested in a number of clinical trials (Cudkowicz et al. 2008; Lanka et al. 2009; Kirkegaard et al. 2016). Arimoclomol belongs to a group of HSP-modulating drugs that act as inducers of HSP70, whose mechanism of action involves stabilization of the interaction of HSF1 with heat shock elements (HSEs), the transcriptional elements controlling HSP production (Anckar and Sistonen 2011; Crul et al. 2013; Kieran et al. 2004; Kirkegaard et al. 2016; Neef et al. 2011; Parfitt et al. 2014; Vígh et al. 1997).

Celastrol (CEL), a quinone methide triterpene, is a derived from the Celastraceae family of plants, that is used in traditional Chinese medicine. This compound has been shown to induce HSF1 and HSP70 expression in skeletal muscle cells (Gwag et al. 2013; Trott et al. 2008; Westerheide et al. 2004). Furthermore, this compound has been demonstrated to exhibit a broad range of functions like antioxidant, anti-inflammatory (Gwag et al. 2013; Lee et al. 2006; Sassa et al. 1990; Trott et al. 2008) and neuroprotective activity (Franklin et al. 2005; Gwag et al. 2013). All of these activities could be related to its action s HSP inducer.

Moreover, Deane and Brown (2016) in a recent in vitro research on neuronal cells showed that co-application of celastrol and arimoclomol induced higher HSP levels compared to heat shock paired with arimoclomol. This co-application targets multiple alterations including protein misfolding, protein aggregation, inflammation and oxidative stress. Several studies demonstrated the beneficial effects of

Table 2 Pharmacological compounds that modulate heat shock proteins and relative beneficial effects

Compounds	Heat shock proteins modulation	Beneficial effects
GGA	↑HPS70	Prevents ischemia/reperfusion-induced damage at myocardial level
		Regulate the atrial arrhythmogenesis in heart failure
ARIMOCLOMOL	↑HSP70	Neuroprotective activity
CELASTROL	↑HSP70	Antioxidant and anti-inflammatory activities
		Neuroprotective activity
STATIN	↑HSP70	Contributes to plaque stability
	↑HSPB1	Reduces both mortality and the incidence of acute coronary syndrome
	↑HSP90	

statins in reducing both mortality and incidence of acute coronary syndrome (Noguchi et al. 2015; Smith et al. 2011 Weintraub et al. 2011). In addition, to reducing levels of serum low-density lipoprotein-cholesterol (LDL-C), statins also may contribute to plaque stability by reducing inflammation (Noguchi et al. 2015; Puato et al. 2010), improving endothelial function (Noguchi et al. 2015; Schönbeck and Libby 2004) and reinforcing the fibrous cap (Hattori et al. 2012, Komukai et al. 2014; Noguchi et al. 2015); these effects play crucial roles in the protection from CVDs (Fuster et al. 1992a, b; Noguchi et al. 2015). Remarkably, Frostegård et al. (2016) showed that statin, in particular atorvastatin, restores the proatherogenic HSP profile, characterized by an increase of pro-atherogenic HSP60 and HSP70 and a decrease of HSPB1, suggesting that statins could modulate immune reactions pivotal to atherosclerosis and CVDs pathogenesis. Simvastatin induces also vascular endothelial cells HSF1 translocate in nucleus and so cause the transcription of HSP70 and HSP90 (Uchiyama et al. 2007; Willis and Patterson 2010). All the above reported studies suggest that several drugs, have the potential to be cardioprotective because of their ability to modulate HSP in cardiovascular system. The effects of pharmacological compounds are summarized in Table 2.

In addition to the pharmacological induction of HSP, physical exercise was also discovered to induce HSPB levels and consequently has important cardioprotective effects. In fact, various studies demonstrated that gene and protein levels of HSPB1 and HSPB6 in heart are increased after physical exercise in animal models. In these studies, HSPB1 and HSPB6 were phosphorylated inducing myofilaments' stabilization, contractile damaged proteins restoration that resulted in improved contractile function of the heart (Boluyt et al. 2006; Burniston 2009; Campos et al. 2012; de Moraes et al. 2015; Rinaldi et al. 2006; Sakamoto et al. 2006; Hu et al. 2017). Moreover, it has been showed that physical exercise induced elevation of myocardial HSP expression (such as HSP70, HSP90 and HSPB5) and that regular endurance exercise protected the heart against ischemia/reperfusion and infarction (Harris and Starnes 2001; Powers et al. 2002). Therefore, physical exercise may represent a promising preventive therapeutic therapy against CVDs by also its activity in HSP

modulation. Furthermore, gene therapy can also be considered as a possible option to modulate HSP levels and function. In particular, adeno-associated viral vectors, which have been successfully utilized in clinical trials for the treatment of cardiomyopathy with SERCA2 overexpression, are currently the most effective in vivo delivery system (Jessup et al. 2011).

Conclusions

During the development of CVDs there is a modulation of chaperones and co-chaperones. Molecular chaperones are members of a large family of proteins that provide physiological control of proteostasis. Lack of regulation of the physiological balance between protein synthesis, folding, and degradation results in accumulation of misfolded proteins, so inducing several CVDs. In particular, we reported in this chapter that different cellular stress proteins have opposed role in promotion or inhibition of CVDs or related symptoms. The data discussed in this manuscript suggest that modulation of molecular chaperone expression could have a protective function in CVDs. Indeed, molecules capable of regulating chaperone expression are currently used in clinical trials, principally for the treatment of neurodegenerative diseases. Therefore, the discovery and the development of novel strategies to modulate chaperone expression and functions could be considered a crucial step for expanding drugs against CVDs.

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