Hold Me Tight

Role of the Heat Shock Protein Family of Chaperones in Cardiac Disease

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During the development of cardiac hypertrophy, heart failure, and ischemia reperfusion challenge, the heart accumulates misfolded proteins as a result of cellular stresses.[1–4] Although the compensatory increases in chaperones/cochaperones work to prevent misfolding, refold denatured proteins, and/or target them for degradation, this system can become overwhelmed, leading to worsening of cardiac function. In fact, recent studies have demonstrated experimentally that increasing the burden of misfolded proteins in the heart can contribute to the development of cardiac dysfunction.[5] In this review, we discuss the role of heat shock proteins (HSPs) in common cardiac diseases, including cardiac hypertrophy, heart failure, and ischemia/reperfusion injury. Furthermore, we delineate the many specific mechanisms by which these chaperones, cochaperones, and heat shock factor (HSF) transcription factors have been found to be cardioprotective in experimental models. Lastly, we review recent studies involving drugs that are being developed (and currently used) to increase the expression (and presumably function) of chaperone/cochaperone systems that may be applicable to the treatment of common cardiac diseases and familial cardiac diseases with a pathogenesis that includes a major component of misfolded proteins (eg, desminopathies).

Chaperones Enhance Productive Protein Folding and Refolding and Prevent Protein Aggregation

There are several general families of molecular chaperones in the cytoplasm of mammalian cells, including HSP90, HSP70, chaperonin containing TCP1 (CCT; also called TCP1-ring complex [TRiC]), and small HSP (sHSP) family proteins (the Figure, A). Members of the HSP90 family of chaperones are the most abundant chaperones located in the cytosol. They form dimers consisting of HSP90α and HSP90β subunits and are inducible with stress, although they also are quite abundant without stress.[8,9] HSP90 assists many proteins involved with signal transduction, including >40 kinases and many steroid hormone receptors, with a supporting role in conformational changes involved in ATP hydrolysis.[10,11] The HSP70 chaperone family consists of 6 member proteins that are found in the cytosol,[12] including HSP70 and the cognate of HSP70 (HSC70). Like HSP90, HSP70 proteins are inducible with stress, but they are also highly abundant without stress. HSP70 family members are functionally highly homologous, recognizing hydrophobic surfaces of unfolded proteins and partially folded intermediates. Their activity is controlled by their hydrolysis activity and by their ability to bind ATP.[13] HSP90 and HSP70 proteins both inhibit protein aggregation, thereby promoting productive folding of proteins (for comprehensive reviews, see References 14 through 16).

Other molecular chaperones present in the cytosol include TRiC (or CCT).[17,18] The central cavity of TRiC uses a lid-like structure to encapsulate substrate proteins to allow it to trap and fold target proteins. This encapsulation prevents aggregation and allows changes in conformation that ensure correct folding (in an ATP-dependent manner) before substrates are released. The family of sHSPs also maintain protein confirmation in an ATP-independent manner.[19] More than 10 sHSP chaperone proteins have been characterized, and all function by maintaining an equilibrium between the dimer and large oligomer states of their target proteins.[20] In contrast to HSP90 and HSP70, however, only a few sHSPs (including HSP27, HSP22, and alphaB-crystallin [CryAB]) are increased in response to stress.[21] HSP27 and CryAB are abundant in cardiac and skeletal muscles and increase in response to stress to protect against insults such as ischemia.[22] Both of these proteins associate with actin and are vital to muscle development and assembly.[23] CryAB also interacts with several other cytoskeletal proteins such as desmin to maintain protein folding and to prevent aggregation.[24–27]

In addition to the molecular chaperones that are found predominantly in the cytosol, there are chaperones that are known to maintain proteins in other compartments of the cell, in particular the mitochondria. HSP60 is a chaperone that was originally identified in the mitochondria[28] but is also found in the cytosol.[29] HSP60 is responsible for refolding and transportation of proteins between the mitochondrial matrix and the cytoplasm of the cell[30] and associates with a number of cytosolic proteins involved in apoptosis such as B-cell lymphoma-1–associated X protein, B-cell lymphoma-xl, and B-cell lymphoma-2 homologous antagonist/killer.[31,32] HSP60 is believed to be a homolog of the bacterial HSP groEL; thus, it is believed that HSP60 assists in folding linear amino acids

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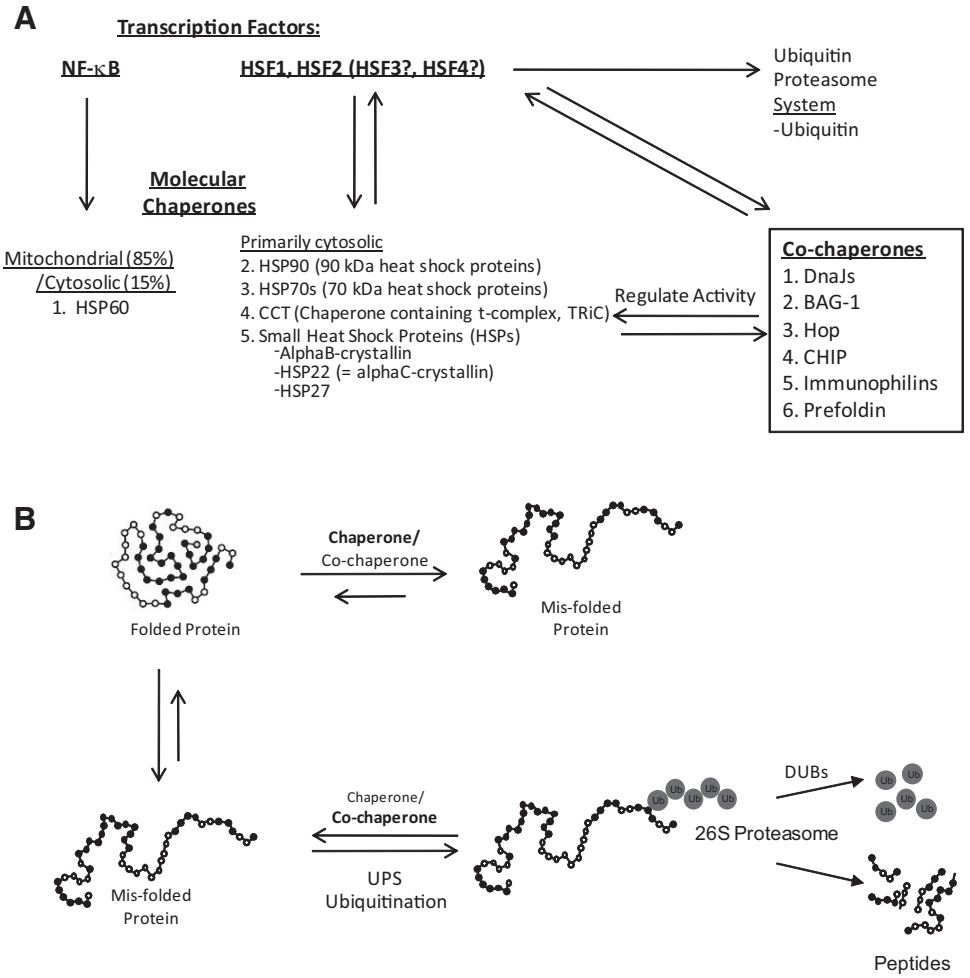

Figure. The regulation of the protein quality control and chaperone systems in the heart that protect against the toxicity of misfolded proteins. A, Both NF-κB and HSF transcription factors regulate the expression of key molecular chaperones in the mitochondria and cytosol, respectively. Stress-activated HSF1 and HSF2 also increase the expression of cochaperones and ubiquitin expression. B, The upregulation of both chaperones and ubiquitin help maintain protein quality control by either refolding the protein (chaperone) or ubiquitinating misfolded proteins (cochaperone, eg, CHIP). This dynamic process is particularly important during cardiac stress. UPS indicates ubiquitin proteasome system; DUBs, de-ubiquitinating enzymes. Adapted from Kubota[6] and includes recent data from Wang et al.[7]

into their 3-dimensional structures in ways that have been described for groEL.[33]

Cochaperones Control the Activity of Chaperones: DnaJ, Bcl2-Associated Athanogenes, Hop, Carboxy Terminus of HSP70-Binding Protein, Immunophilins, and Prefoldin

As their name implies, cochaperones are proteins that assist chaperone functions, including protein folding. There are >40 genes in the HSP40/DnaJ protein family, with the different members playing diverse roles in regulating protein folding, assembly, translocation, and even degradation. DnaJ proteins bind to the ATPase-binding domain of HSP70 proteins to enhance their ATPase activity.[34] They also bind substrate proteins to modulate folding in a substrate-specific manner. Bcl2-associated athanogene (BAG) proteins all have a conserved BAG region that also binds the ATPase domain of HSP70 proteins to affect the rate of substrate binding and release.[35] Conversely, Hop, carboxy terminus of HSP70-interacting protein (CHIP), and immunophilins all bind both HSP70 and HSP90 via tetra-tricopeptide repeat domains, which allow the transfer of substrates between them.[8,11] CHIP is critical to quality control processes and ubiquitinates misfolded proteins when correct folding cannot be achieved (see the Figure, B),[36,37] whereas immunophilins are necessary for the functions of the p23 steroid aporeceptor–associated protein.[11] Lastly, prefoldin, also known as GimC, helps TriC/CCT-dependent folding of tubulin and actin by way of its 6 tentacle-like processes that trap unfolded substrates and assist with folding in collaboration with HSP70/HSP90.[38,39]

The Transcription Factors HSF1 and HSF2 Regulate Chaperone and Cochaperone Expression in the Cell

The number of misfolded proteins increases during times of cellular stress, including oxidative stress and proteasome inhibition. Therefore, many chaperones are induced at the transcriptional level in the presence of these conditions to protect against the toxicity of misfolded proteins. At least 4

transcription factors (HSF1 through HSF4) regulate HSPs. HSF1, the primary transcription factor involved in this process, binds heat shock response elements in the promoter regions of stress-induced genes. HSF1 is found throughout the cytosol as a monomer that binds HSP90 to inhibit its chaperone activity. During stress, denatured proteins competitively bind HSP90,[40] effectively releasing HSF1, thereby allowing it to translocate to the nucleus as trimers.[41] In the nucleus, increased HSF1 upregulates the expression of chaperones, including HSP70. HSP70 then binds HSF1, which in turn attenuates the HSF response, resulting in a negative feedback mechanism.[42] Interestingly, part of the stress response induced by HSF1 includes the upregulation of ubiquitin, suggesting that it regulates the ubiquitin proteasome system to enhance the capacity of the cell to degrade proteins during stress (recently reviewed by Willis et al[43]). The roles of the remaining 3 HSFs in the stress response are less well studied. Studies have shown that HSF2 contributes to the inducible expression of HSP genes by interacting with HSF1.[44] The roles of HSF3 and HSF4 as factors that regulate the expression of nonclassic and sHSPs are just beginning to be understood.[45,46]

Cells have a diverse array of molecular chaperones available to them in the cytosol and mitochondrial compartments that are regulated by the transcription factors nuclear factor-κB (NF-κB) and HSF1 through HSF4. Although these chaperones play a role primarily in refolding proteins, some cochaperones cross-talk with the ubiquitin proteasome system to directly ubiquitinate proteins for subsequent degradation by the proteasome. Although much of the work on molecular chaperones has been in noncardiovascular systems, their role in the heart during health and disease is becoming more greatly appreciated.

Cardiac Chaperones Are Regulated in the Pathophysiology of a Number of Common Cardiac Diseases

Cardiac Hypertrophy

Cardiac chaperones such as HSP70, CryAB, and HSP22 (alphaC-crystallin) increase in expression during the development of cardiac hypertrophy (the Table). HSP70 can be induced by a variety of hypertrophic stimuli, including aortic banding, angiotensin II infusion, isoproterenol infusion, and swimming.[57] CryAB can be induced in rat cardiomyocytes in response to endothelin-1–induced cardiomyocyte hypertrophy, resulting in a 2-fold increase in CryAB expression. The DNA-binding activity of HSF1 is heightened as a result of increasing preload in heart.[71] Because cardiac hypertrophy is a response to a number of stress stimuli, this general increase in cardiac chaperone expression in cardiac hypertrophy is not surprising. However, the purpose behind this increased chaperone expression is much less well understood. Mice with transgenic overexpression of CryAB gene challenged with 2 weeks of aortic banding to induce pathological cardiac hypertrophy exhibit a significant reduction in nuclear factor of activated T cells (NFAT) transactivation and attenuated hypertrophy.[86] This contrasts to CryAB-null mice, which display an enhanced NFAT transactivation at baseline and an accelerated development of heart failure in response to pressure overload induced by aortic banding.[86] These studies demonstrate that cardiac CryAB plays a role in suppressing cardiac hypertrophic response, possibly through inhibiting NFAT signaling.

In contrast to the ability of CryAB to attenuate cardiac hypertrophy, HSP70 is necessary for the induction of cardiac hypertrophy. One potential mechanism by which HSP70 may accomplish this is through its association with the activated form of histone deacetylase 2 (HDAC2). Overexpressing a dominant-negative form of HSP70 or decreasing HDAC2 with small interfering RNA (siRNA) blunts the hypertrophic response in the heart. Furthermore, cardiac hypertrophy induced by isoproterenol infusion or aortic banding in mice lacking HSP70 results in a blunting of HDAC2 activity.[57] This suggests a role for HSP70 in the induction of cardiac hypertrophy, possibly through its stabilization of HDAC2. This HSP70 dependence of HDAC2 activity is interesting because of the importance of HDAC2 in cardiac hypertrophy signaling. The HDACs are instrumental in regulating hypertrophic gene expression in pathological settings.[49] Class II HDACs (HDAC4, HDAC6, HDAC7, HDAC9) negatively regulate hypertrophy by repressing myocyte enhancer factor/GATA/NFAT-mediated gene transcription.[87] Conversely, the class I HDAC2 has been implicated in having acetylase-dependent prohypertrophic activity, possibly by releasing repression of insulin-like growth factor-1 signaling.[49,57,88]

Another cardiac chaperone, HSP22, has been shown to increase during the development of cardiac hypertrophy. HSP22 has several names; the one used most commonly in the literature is H11 kinase, but alphaC-crystallin is sometimes used and indicates its relationship to other sHSPs (see elsewhere for a recent review[89]). Increasing HSP22 expression results in the activation of signaling pathways involved in survival and cell growth, including the PI3K/Akt pathway, AMP kinase, protein kinase Cε, nitric oxide, and mTOR.[89] These signaling pathways induce preconditioning, growth, and protection against apoptosis among other cardioprotective pathways.[89] Recently, studies have identified that HSP22 increases during the development of cardiac hypertrophy in a variety of animal models. Inducing a slowly progressive cardiac hypertrophy in puppies through the use of aortic banding (which parallels the gradual progression of human disease to a much greater extent than acute aortic banding models) causes HSP22 to increase ≈3-fold.[59] Increasing HSP22 expression in cultured cardiomyocytes and the intact mouse heart (≈7-fold) results in the development of a spontaneous hypertrophy characterized by the re-expression of the fetal gene program.[59,90] This suggests that the many cardioprotective pathways induced by HSP22[89] may be detrimental if expressed chronically.

Physiological Versus Pathological Hypertrophy

The differences in the underlying signaling between physiological and pathological cardiac hypertrophy are only superficially understood. Discovering these differences is critical to our understanding of what makes patients undergoing pathological hypertrophy so susceptible to heart failure, whereas patients undergoing physiological hypertrophy (eg,

Table. Summary of the Known Regulation of Chaperones, Cochaperones, and Transcription Factors Involved in the HSP Response in Cardiac Hypertrophy, Heart Failure, and Ischemia/Reperfusion Injury

	Example Substrate Recognition/Other	Cardiac Hypertrophy	Heart Failure	I/R Injury
Chaperones				
HSP90	Actin, tubulin, TGF-β1, CHIP[47,48]	ND	HSP90 in DCM=IHD=normal controls[60]	Rat cardiac I/R results in increased HSP90[65]
HSP70	Calcineurin, HDAC6, CHIP[49,50]	Hypertrophic stimuli (aortic banding, isoproterenol, angiotensin II, swimming) induce HSP70 expression[57]	HSP72 not increased in heart failure induced by coronary artery ligation in rats for 9–12 wk[7]	Rat cardiac I/R results in increased HSP70[65]
		HSP70 supports prohypertrophic signaling via interaction with HDAC2[57]	No difference in HSP72 expression 8 wk after coronary artery occlusion vs controls[61]	HSP72 increased at 1 wk after coronary artery occlusion[61]
			HSP70 and HSC70 in DCM=IHD=normal controls[6]	
CCT (TRiC)	TRiC binds actin[51]	ND	ND	ND
sHSPs				
CryAB (HSPB5)	CryAB binds desmin[52]	CryAB increases 2-fold in ET-1–treated neonatal rat cardiomyocytes[58]	ND	ND
HSP20 (HSPB6)	HSP20 binds actin and α-actinin[53]	ND	ND	HSP20 increased in I/R, regulated in part by miR-320[66]
HSP22 (HSPB8, H11 kinase, alphaC-crystalline)	HSP22 binds lipid membranes[54]	HSP22 expression increases $\approx$3-fold in cardiac hypertrophy induced in dogs by aortic banding[59]	ND	HSP22 expression is increased $\approx$3-fold 1 h after reperfusion following ischemia in a pig model[67]
				In human hibernating myocardium and swine model of hibernating myocardial, HSP22 is increased[68]
HSP27 (HSPB1, HSPB2)	HSP27 binds IκB[55]	ND	No change in HSP27 expression at 8 wk after coronary artery occlusion (rat)/increased HSP27 at 1 wk after coronary artery occlusion[61]	ND
			HSP27 increased in human DCM vs control subjects[60]	
Mitochondrial HSPs				
HSP60	HSP60 binds Bax and Bak[56]	HSP60 decreased 13-fold in ET-1–treated neonatal rat cardiomyocytes[58]	HSP60 increased $\geq$8 wk (but not at 1 wk) after coronary artery occlusion (rat)[7,61,62]	ND
			Increased expression of cardiac HSP60 in heart failure (coronary artery ligation in rats for 9–12 wk); may be driven by NF-κB activation[7]	
			HSP60 doubled in human DCM and IHD vs control subjects[60]	
			HSP60 moves from cytoplasm to mitochondria in DCM and IHD[63]	
			Serum HSP60 levels are associated with the severity of heart failure in patients[64]	
Transcription factors regulating chaperones				
HSF1	HSF1 binds the heat shock elements (HGAAN) of HSP72; HSP90 interacts to repress	Increased preload/mechanical stress increases HSF1 activity[71]	HSF1 levels increased without an increase in activity in heart failure (8 wk after coronary artery ligation in rats)[7]	Rat cardiac I/R results in increased HSF1 but not HSF2 activity[65]; HSF1 induction in ischemia mediated by ROS and ATP levels[72,73]
HSF2	HSF-2 binds the heat shock elements of HSP90, HSP27, c-Fos[69]; interacts with HSF1 and nucleoporin p62[70]	ND	HSF2 levels increased without an increase in activity in heart failure (8 wk after coronary artery ligation in rats)[7]	

(*Continued*)

Table. Continued

	Example Substrate Recognition/Other	Cardiac Hypertrophy	Heart Failure	I/R Injury
Cochaperones				
DnaJ	DnaJ binds ribosome-bound nascent polypeptides[74]	ND	ND	DnaJ-like pDJA1 increased 4-fold after reperfusion in a pig model of I/R[82]
BAG-1	BAG-1 binds Bcl-2, Raf1[75]	ND	ND	BAG-1 protects against I/R injury[83,84]
Hop	Hop binds HSP70, HSP90[76]	ND	ND	ND
CHIP	CHIP binds HSP70, HSP90, HIF1-α[77]	CHIP increases in response to high glucose and regulates prohypertrophic GATA4 in cardiomyocytes in vitro[81]	ND	CHIP protects against I/R injury[85]
Immunophilins	FKBP38 binds mTOR complex[78]	ND	ND	ND
Prefoldin	Prefoldin binds Nascent chain of actin and tubulin Chaperonin[79,80]	ND	ND	ND

I/R indicates ischemia/reperfusion; TGF, transforming growth factor; ND, not determined; DCM, dilated cardiomyopathy; IHD, ischemic heart disease; ET, endothelin; Bax, B-cell lymphoma-1-associated X protein; Bak, Bcl-2 homologous antagonist/killer; ROS, reactive oxygen species; and HIF, hypoxia-inducible factor.

through exercise) are not. Pathological cardiac hypertrophy is most commonly induced by persistent pressure or volume overload as a result of hypertension or valvular heart disease. Physiological hypertrophy, on the other hand, is induced by exercise. Both stimuli result in increases in cardiomyocyte size; however, pathological hypertrophy is limited in its ability to maintain cardiac function, eventually resulting in heart failure. In contrast, physiological cardiac hypertrophy maintains and improves function, as illustrated in athletes. The difference in cellular signaling between these 2 processes has been of great interest to researchers. A number of recent studies have led to the hypothesis that HSF1 may regulate some of the differences in the development of physiological and pathological cardiac hypertrophy (recently reviewed by Toko et al[91]). This is based largely on gene expression studies that have shown a differential expression of ≈100 genes.[92–95] Among these differentially expressed genes are a number of HSF1-regulated genes such as HSP70 and HSP27 and increases in HSF1 itself. HSF1 may be one of many differences regulating the differential signaling of physiological and pathological cardiac hypertrophy.

Heart Failure

To protect cardiomyocytes from injury, HSPs within the cell increase in response to externally applied stressors, including oxidative stress and inflammation. Beginning in the late 1990s, researchers have been investigating the expression of HSPs in the failing human heart (the Table). To this end, investigators have examined the expression of HSP90, HSP72, HSC70, HSP27, and HSP60 from dilated cardiomyopathy patients, ischemic cardiomyopathy patients, and normal control subjects.[60] HSP72, HSC70, and HSP90 are not significantly changed between the 3 groups.[60] In contrast, dilated cardiomyopathy patients exhibit a 2-fold increase in HSP27 expression in the heart compared with healthy control patients. This is in addition to a doubling of the HSP60 level, which also occurs in the hearts of ischemic heart disease patients.[60] The fact that HSP72 protein does not increase in heart failure (even though it is cardioprotective[96]) while levels of the HSP60 protein are doubled[60] suggests that there is differential regulation between HSP60 and either HSF1- or HSF2-regulated HSPs. Indeed, after induction of heart failure by placement of a permanent high left anterior descending coronary artery ligation in rats, no differences can be seen between HSF1 and HSF2 activity as determined by electrophoretic mobility shift assay.[7] Additionally, HSP72 messenger RNA (mRNA) levels are not increased. In contrast, HSP60 mRNA increases, apparently caused by increased binding of NF-κB to both of the NF-κB binding elements in the HSP60 gene. The fact that HSP60 contains NF-κB binding elements but HSP72 does not[7] may explain why HSP60, but not other HSPs, are increased during heart failure.

In more short-term studies in which Wistar rats undergo a permanent left anterior descending coronary ligation to induce heart failure, acute increases in HSP72 and HSP27 are seen and HSP60 expression remains unaffected.[61] However, after 8 weeks, at which time the development of heart failure is significant, a decrease in HSP72 and HSP27 expression is observed, which appears to be somewhat contradictory compared with the results seen in human heart failure patients (see above). Induction of heart failure in these rats also results in a parallel increase in cardiac HSP60 levels. Additional studies have determined that these increases in HSP60 correlate with a decrease in mitochondrial oxygen consumption rate and an increase in markers for reactive oxygen species (determined by thiobarbiturate-reacting substance[62]). The differences in HSP regulation found in these Wistar rat studies compared with the studies in human disease may be due to the relatively short-term nature of these studies compared with human studies or possibly to species- and even strain-dependent effects.

HSP60 in Heart Failure

Although HSP60 is found predominantly in the mitochondria, ≈15% of total cellular HSP60 normally resides within the cytoplasm.[29] However, in both dilated cardiomyopathy and ischemic heart disease hearts, the distribution of HSP60 changes, with cytosolic HSP60 translocating to the mitochondria.[63] In other studies of heart failure using animal models and human explanted failing hearts, HSP60 has been found

localized to the plasma membrane, where it is detectable on the cell surface by both flow cytometry and confocal microscopy. Interestingly, localization of HSP60 to the plasma membrane of a cell correlates with an increase in apoptosis of the affected cell, possibly because the cell-surface HSP60 may be able to interact with other cells to trigger the innate immune response, resulting in the release of proinflammatory cytokines such as tumor necrosis factor-α. This would make HSP60 an early signal-inducing myocyte loss and contributing to heart failure.[56] These few studies indicate a number of potentially conflicting data that might be due to differences resulting from species or strain variations or from experimental design. Considerably more work is needed to delineate the regulation of HSP60 in heart failure.

The involvement of HSP60 in heart failure is made even more complicated by the fact that it is also released from the cells and can be found circulating in plasma early in heart failure.[56] Circulating HSP60 has been hypothesized to play a role in atherosclerosis by inducing inflammation and autoimmune mechanisms (see recent reviews[97,98]). The presence of HSP60 in the blood of normal patients was first described in 1999.[99] Recent studies have investigated the relationship between chronic heart failure severity and serum HSP60 levels.[64] In 112 patients with congestive heart failure and 62 control subjects, serum HSP60 levels were higher in patients with congestive heart failure compared with control subjects.[64] Congestive heart failure patients with advancing New York Heart Association functional classes also had higher levels of HSP60.[64] Likewise, patients with cardiac events during the average 569 days of follow-up had higher serum HSP60 levels compared with event-free patients.[64] These findings demonstrate a relationship among serum HSP60 levels, the severity of congestive heart failure, and a high risk for adverse cardiac events in patients with heart failure. The role of circulating HSP60 in the underlying pathophysiology of heart failure has not been delineated.

Cardiac Ischemic Injury

Most studies investigating HSPs in cardiac ischemia/reperfusion injury have reported on their cardioprotective effects. However, a few studies have concentrated on the regulation of chaperone protein expression and activity during the course of ischemia/reperfusion injury (the Table). Reperfusion after 20 minutes of ischemia results in increases in both HSP70 and HSP90 mRNA levels, with the increase in HSP70 being much higher than that of HSP90 (≈75-fold and ≈16-fold, respectively).[65] This increase in HSP70 and HSP90 expression is most likely due to a concurrent increase in the transcription factor HSF1 (but not HSF2), which in turn appears to be driven by an accumulation of reactive oxygen species during ischemia/reperfusion injury.[72] However, other studies have identified that HSF1 activation can be modulated by ATP concentrations within the cell. Moderate decreases in intracellular ATP correlate with HSF1 activation, whereas severe ATP depletion results in an attenuated HSF1 response, which can subsequently be rescued on ATP restoration.[73] Studies investigating differential expression of genes in a pig model of ischemia/reperfusion injury reveal that HSP22 significantly increases ≈3-fold after 1 hour of reperfusion.[67] HSP22 is also significantly increased in cases of human hibernating myocardium and pig models of hibernating myocardium.[68]

HSP70/HSP72

Both HSP70 and HSP72 have proven to be beneficial to the outcome of cardiac ischemia/reperfusion injury. Knocking down HSP72 expression in isolated feline cardiomyocytes increases their susceptibility to cell death in response to hypoxia and reoxygenation.[100] In addition, increasing HSP72 in adult male rats by successive bouts of endurance exercise improves the outcomes of ischemia/reperfusion injury, illustrated by a decrease in cardiac infarct size and the amount of cardiac apoptosis in endurance-trained rats compared with sedentary controls.[96] In the case of HSP70, adenovirus-mediated gene transfer into rabbit hearts results in a reduction in injury after ischemia/reperfusion injury.[101] Furthermore, at least 4 studies have demonstrated that the transgenic overexpression of HSP70 in the heart of mice significantly protects against ischemia/reperfusion injury.[102–105] Because all of these studies increase HSP70 before the ischemia/reperfusion insult, it is not clear what the clinical utility of increased HSP70 at therapeutically plausible time points (after ischemia/reperfusion injury) would be.

Small HSPs: HSP20, HSP22, HSP27, and CryAB

Increasing the HSP20 expression (the Table) in isolated cardiomyocytes improves their function[106] and protects against apoptosis induced by β-agonist stimulation.[107] Cardiac-specific overexpression of HSP20 in mouse models (≈10-fold) protects against ischemia/reperfusion injury. When HSP20 transgenic hearts are challenged with ischemia/reperfusion injury ex vivo, they exhibit an improved contractile performance, a decrease in indexes of myocyte cell death, and a significant decrease in infarct size compared with wild-type hearts.[108] This protective effect of HSP20 appears to be due to the role of HSP20 in activating autophagy, a critical mechanism for dealing with ischemia/reperfusion injury.[109] Transgenic mice in which serine 16 on HSP20 is mutated such that it is nonphosphorylatable are more susceptible to ischemia/reperfusion injury than wild-type mice, in part because of the inability of the mutant HSP20 to activate autophagic pathways.[109] HSP20 can protect against ischemia/reperfusion injury via other mechanisms also. Not only does HSP20 protect against oxidative stress resulting from ischemia/reperfusion injury, but recent studies have found that it can also protect against other injuries caused by increased oxidative stress such as doxorubicin therapy.[110] Recent studies have demonstrated that HSP20 expression is regulated, at least in part, by the microRNA miR-320. Downregulation of miR-320 with an antagomir has been shown to be cardioprotective in ischemia reperfusion, in part by its upregulation of HSP20.[66]

In the case of HSP22, transgenic mice that have increased expression of HSP22 are protected against ischemia/reperfusion injury. After 45 minutes of coronary artery occlusion and reperfusion, HSP22 transgenic mice have an 82% reduction in infarct size compared with controls,[90] with HSP22 transgenic hearts exhibiting significant activation of a number of

survival kinases, including Akt and AMP kinase to which HSP22 binds directly.[90] The cardioprotective effect of HSP22 appears to be mediated specifically through BMP signaling via activation of the PI3K/Akt pathway.[111] The sHSP27 has also been shown to protect against ischemia/reperfusion injury using dog cardiomyocytes, with just minimal (2- to 3-fold) increases in expression.[112]

We previously described the protective nature of CryAB in inhibiting cardiac hypertrophy. However, this sHSP is also cardioprotective against ischemia/reperfusion injury when its expression is increased before insult. Transgenic mice in which CryAB is overexpressed suffer less cardiac oxidative stress, decreased extent of infarction, and attenuated apoptosis and necrosis when challenged with ischemia/reperfusion injury.[113] Likewise, mice lacking CryAB and HSP27, both of which are highly expressed in the heart, subjected to ischemia/reperfusion challenge exhibit a nearly 2-fold decrease in contractility recovery, with parallel increases in necrosis and apoptosis measures compared with controls.[114] These studies indicate that although CryAB and HSP27 are not necessary for cardiac development (CryAB/HSP27 mice develop normally and have no discernable differences in heart structure compared with wild type), they do play a key role in antioxidative mechanisms during ischemia/reperfusion injury.[114]

HSF Proteins

As the heart senses stress, it induces HSPs by a number of mechanisms. Studies have identified that many of the HSPs are regulated by the HSF family of transcription factors (the Table). In the context of cardiac ischemia/reperfusion, heat shock factor 1 (HSF1) expression can upregulate HSP expression to protect against subsequent ischemia/reperfusion injury. Mice with cardiac overexpression of HSF1 challenged with ischemia/reperfusion injury recover faster and have smaller infarcts and decreased cardiomyocyte cell death compared with wild-type mice.[115] In addition, Akt is enhanced and Jun N-terminal kinase and caspase 3 (apoptotic mediators) are less activated than in wild-type mice.[115]

The cardioprotective role of HSF1 has been studied by using experimental models known to induce HSF1. Specifically, cardiac HSF1 has been induced by whole-body hyperthermia or by transgenic overexpression of Ca^{2+}/calmodulin-dependent kinase II (CaMKII)-ΔB. On HSF1 induction, these models were then challenged with ischemia/reperfusion injury. Preconditioning mice with whole-body hyperthermia for 48 hours and then subjecting the isolated hearts to 20 minutes of normothermic ischemia and 30 minutes of reperfusion resulted in an increase in HSF1 mRNA and protein and overall cardioprotection.[116] The increase in HSF1 expression is directly related to the cardioprotection in that this effect is abolished with siRNA HSF1.[116] Inhibiting HSF1 with siRNA in the face of whole-body hyperthermia results in an inhibition of HSP32, HSP47, and HSP60 and increased thermal intolerance, resulting in a higher mortality rate.[116] CaMKII is also instrumental in protecting the heart against ischemia/reperfusion injury. CaMKII is a multifunctional kinase that regulates Ca^{2+} handling and regulates cell death in response to ischemia/reperfusion injury. Increasing CaMKII-ΔB expression protects against oxidative stress, hypoxia, and angiotensin II–induced apoptosis.[117] Recent studies have determined that this cardioprotection is due in part to increasing inducible HSP70 through phosphorylation of HSF1.[117] These studies suggest that HSF1 may be a common mechanism by which cardiomyocytes induce a number of HSPs to protect against cardiac/ischemia reperfusion injury.

At least 13 chaperones and cochaperones regulated by at least 2 HSF transcription factors have been identified in the heart (the Table). Quite predictably, the expression of the proteins identified increases in cardiac disease and is generally cardioprotective. Although most studies have focused on the regulation of these chaperones, cochaperones, and transcription factors in heart failure, a growing number of studies have demonstrated more broadly their regulation in cardiac hypertrophy and ischemia reperfusion injury (the Table). This cardioprotection includes a host of mechanisms that regulate growth and inhibit apoptosis through a variety of systems, including the PI3K/Akt pathway, AMP kinase, protein kinase Cϵ, nitric oxide, and mTOR. Pharmacological enhancement of these endogenous cardioprotective mechanisms may prove to be simple yet effective strategies for reducing the morbidity and mortality associated with common cardiac diseases.

Cochaperones in the Heart: Chaperone Assistants and Protein Triage

Cochaperones have many functions in the heart, including assisting chaperones with protein folding and/or assisting with other functions, including targeting damaged proteins for degradation by the ubiquitin proteasome system in a process called protein triage.[118] Increased cochaperone expression in the heart has been found to be cardioprotective in ischemia and necessary to regulate proteins involved in long-QT syndrome. A number of cochaperones have been identified that control the activity of chaperones, including DnaJ, BAGs, Hop, CHIP, immunophilins, and prefoldin (the Figure). Of these 6 general types of cochaperones, only DnaJ, BAG-1, Hop, and CHIP have been described in the heart, and our understanding of their role is preliminary (the Table).

Through the use of a pig model of ischemia/reperfusion to identify genes participating in mechanisms of cell survival, the DnaJ-like cochaperone (pDJA1) was identified by microarray with subtractive hybridization.[82] pDJA1 is restricted to cardiomyocytes and is not present in skeletal muscle, liver, lung, kidney, aorta, stomach, or spleen.[82] pDJA1 increases slightly in response to ischemia but increases 4-fold after reperfusion and is protective against staurosporine-induced apoptosis in isolated rat cardiomyocytes.[82] Since the identification of pDJA1 in 2003, little more has been reported on it despite its potential role in limiting damage in the postischemic myocardium.

Studies on the role of BAG-1 in cardiac ischemia reperfusion injury have demonstrated the ability of BAG-1 to inhibit apoptosis and to induce autophagy to protect cardiomyocytes. BAG-1 interacts with HSC70 and HSP70 and promotes cell survival by coordinating the function of these chaperones with the degradation of proteins by the proteasome. Both BAG-1 isoforms (BAG-1S and BAG-1L) are rapidly induced after ischemia challenge in rat cardiomyocytes, with the

increase in BAG-1 being sustained after subsequent reperfusion.[83] The interaction of BAG-1 with HSC70 increases after ischemia/reperfusion injury,[83] and increasing BAG-1S and BAG-1L in cardiomyocytes reduces apoptosis after ischemia/reperfusion injury. When BAG-1S and BAG-1L are fused to a nuclear localization sequence to force their nuclear localization, they fail to protect cardiomyocytes, similar to BAG-1 deletion mutants that are unable to bind HSC70/HSP70.[83] BAG-1 deletion constructs missing the N-terminal ubiquitin-like domain, however, do not affect the ability of the protein to protect against ischemia/reperfusion injury.[83] These studies demonstrate a novel cardioprotective role for BAG-1, with a critical component related to its interaction with HSC70/HSP70 and cytoplasmic localization. In addition, subsequent studies have identified that autophagy plays an important role in the adaptation to ischemia/reperfusion injury in association with BAG-1.[84] BAG-1 associates with the autophagosomal membrane protein LC3-II and may induce autophagy using HSC70.[84,119] Intracardiac injection of BAG-1 siRNA attenuates the induction of LC3-II and abolishes the cardioprotection achieved by adaption.[119] The BAG-3 isoform participates in the induction of macroautophagy in association with HSP22,[84] demonstrating how BAG family members may shuttle damaged or oxidized proteins into the autophagy pathway to improve cell survival.[84]

The cochaperone CHIP is one protein that plays a key role in both the folding system (as a cochaperone regulating HSP70) and in the ubiquitin-proteasome system as a ubiquitin ligase. CHIP directs the degradation of aggregate prone proteins[120,121] such as polyglutamine proteins, which are prevalent in conformation diseases such as Alzheimer or Huntington disease.[122–124] Although CHIP binds HSP70, it can also target it for degradation in the absence of cargo, possibly as a feedback mechanism to adjust chaperone levels needed for the number of misfolded proteins (the Figure, B).[50] Recent studies have identified BAG-2, a specific inhibitor of CHIP-dependent ubiquitin ligase activity, as a common component of CHIP holocomplexes in vivo.[125] CHIP plays an important role in the heart in response to ischemia/reperfusion injury. When CHIP−/− mice are challenged with ischemia/reperfusion injury in vivo, the ratio of the infarct area to the area of risk is 50% greater than that found in sibling wild-type mice.[85] CHIP−/− hearts are more prone to cell death, indicating a critical role of CHIP in ischemia/reperfusion injury. These studies parallel the role of BAG proteins described above, indicating a critical role of CHIP in shuttling damaged and oxidized proteins into autophagic pathways after ischemia/reperfusion injury. The specific role of CHIP in autophagy has yet to be reported. Cardiomyocyte CHIP increases in response to high glucose and is responsible for the degradation of the prohypertrophic transcription factor GATA4.[81] The significance of these findings in cardiac disease has yet to be reported.

The cochaperone FKBP38 is an immunophilin-type sHSP that has recently been implicated in the maturation of HERG (human ether-à-go-go related gene, also known as KCNH2 in newer nomenclature).[126] The HERG gene encodes the voltage-dependent delayed rectifier potassium channel (I_{KR}), and mutations in HERG are among the most common underlying cause of hereditary long-QT syndrome. Recent studies have used proteomic screens to identify that HSC70, HSP90, HDJ2 (Human DnaJ 2), Hop (HSP-organizing protein), and BAG-2 are differentially expressed in models of long-QT syndrome caused by mutations in HERG.[126] However, the most relevant findings of these studies are that the cochaperone FKBP38 immunoprecipitates and colocalizes with HERG.[126] Additionally, siRNA knockdown of FKBP38 causes a reduction in HERG trafficking, and overexpression of wild-type FKBP38 partially rescues HERG trafficking in the presence of F805C disease-causing KCNH2 mutation.[126] These studies suggest an important role for the cochaperone FKBP38 in rescuing mutations in KCNH2 that lead to the long-QT syndrome.

A picture of the HSP system as a mediator of protein quality control is emerging. Specific cochaperones such as CHIP have the ability to ubiquitinate proteins that the chaperone/cochaperone complex is unable to refold (the Figure, B). The ubiquitination of key structural proteins such as sarcomere proteins and transcription factors is critical to the long-term health of the heart (see recent reviews[43,51,127,128]). These cochaperones represent a system of triage whereby protein quality is maintained and, in the long run, the health of the cardiomyocyte is maintained.

Future Directions: The Role of Drug Therapies in Cardiac Health and Pathophysiology

Drugs That Induce Heat Shock Proteins

A number of studies reviewed here have tested the hypothesis that increasing HSPs improves the outcome of cardiac diseases experimentally, particularly in the context of ischemia/reperfusion injury. Although these studies were primarily proof of concept that increasing HSPs were cardioprotective, it is interesting to note that the fold increase in these proteins was as little as 2. From a clinical standpoint, there are several drugs and herbal products that increase HSPs that may be beneficial in the treatment of cardiac diseases. However, their clinical utility has yet to be tested experimentally in the context of their regulation of HSPs.

Geranylgeranylacetone

Geranylgeranylacetone is a cyclic polyisoprenoid gastric ulcer drug that protects the gastric mucosa by inducing HSF1 and HSP70 mRNA.[129] It has recently been shown experimentally to be cardioprotective by inducing HSP72.[130,131] It has also been shown experimentally to suppress polyglutamine toxicity (see recent review[132]).

Arimoclomol

Arimoclomol, developed by CytRx, is a small molecule that acts by inducing HSF1, resulting in downstream increases in HSP70 and HSP90.[133] Experimentally, arimoclomol increases HSP70 and HSP90 ≈5-fold in an experimental model of amyotrophic lateral sclerosis.[134] Arimoclomol is currently in phase II/II clinical trials as a treatment for amyotrophic lateral sclerosis.

Celastrol

Celastrol is a triterpenoid compound with a retinoid skeleton extracted from *Tripterygium wilfordii* that is used in traditional Chinese medicine. It potently induces HSF1 and HSP70 expression, having both antioxidant and antiinflammatory activities.[135] Celastrol has been shown to ameliorate the neurodegeneration of SOD1 mutant mice; however, it has not been determined if this is through its antiinflammatory effects and/or its effect on HSF1 and HSP70 induction.[136]

Statins

In addition to their ability to decrease cholesterol synthesis via inhibition of HMG-CoA, statins have been shown to have many additional activities, including modulation of the immune system, reduction in apoptosis, and an effect on nitric oxide production.[137–139] Both simvastatin and lovastatin induce HSP27, but not HSP70 and HSP90, in an osteoblast-like cell line.[140] Simvastatin induces HSF1 in vascular endothelial cells to induce nuclear translocation and the transcription of HSP70 and HSP90.[141] Simvastatin induces HSP27 in axotomized retinal ganglion cells to enhance their survival after optic nerve transaction.[142] Statins increase HSF1 and HSF2 in retinal ganglion cells in vivo.[143] Statins act to increase HSFs, HSP70, HSP90, and sHSPs, possibly in a cell-dependent manner. Their effect on cardiac HSPs has not been identified to date. With the discovery of the ability of statins to induce HSPs, studies have identified a decreased prevalence of Alzheimer disease in patients taking statins[144,145] and a decrease in neurofibrillary tangles.[145] These studies suggest that several drugs, including the widely prescribed statins, have the potential to be cardioprotective because of their ability to prime critical HSPs in the heart. The use of most of these drugs has yet to be determined in human studies.

Conclusions

During the development of cardiac hypertrophy, heart failure, and ischemia/reperfusion injury, there is a general increase in a number of chaperones, cochaperones, and the transcription factors that regulate them. In this review, we discuss their uniformly protective mechanisms and the possibility that therapeutic regulation may enhance both short- and long-term health of the heart. With the recent discovery that increases in soluble preamyloid oligomers play a significant role in cardiac disease and are able to induce cardiomyopathy experimentally,[5] there is a need for a rational way to increase chaperone/cochaperone function to combat the accumulation of misfolded proteins. A number of drugs with the potential to increase HSPs are being developed for neurodegenerative diseases caused by misfolded proteins (ie, polyglutamine diseases such as Huntington disease). Given the parallel mechanisms found in cardiac diseases and the overwhelming evidence that increasing chaperones/cochaperones are cardioprotective against the most common cardiac diseases, there may be future clinical applicability in cardiology of these drugs.

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Disclosures

None.

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KEY WORDS: cardiovascular diseases ■ heart failure ■ heat shock protein ■ molecular chaperone

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