Hsp90 in Cardiac Diseases

Subjects: Pathology | Cardiac & Cardiovascular Systems

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Heat shock protein 90 (Hsp90) is a molecular chaperone that interacts with up to 10% of the proteome. The extensive involvement in protein folding and regulation of protein stability within cells makes Hsp90 an attractive therapeutic target to correct multiple dysfunctions in the heart.

Hsp90 hypertrophy cardiomyopathy fibrosis heart failure signal transduction

1. Introduction

1.1. Hsp90 as a Chaperone

The primary roles of chaperones in the cell are to help stabilize proteins during folding, assisting them to reach their active conformation, and regulate their degradation. Many proteins require chaperone activity to assume their active conformation, with 20–30% of mammalian proteins lacking native three-dimensional structure [1]. Chaperones are also critical in the heat shock response by preventing protein unfolding and misfolding due to environmental stressors, intracellular stressors, and mutations [2]. This stabilization allows cells to continue functioning in these suboptimal conditions. Heat shock proteins (Hsps) function to prevent protein aggregation by dissembling and refolding aggregates, labeling peptides for proteasomal degradation, and sequestering proteins via the spatial protein quality control mechanism [3][4]. This balance between protein stabilization and degradation is called proteostasis (protein homeostasis) and is vital to cell survival [1].

There are five major categories of heat shock proteins: small heat shock proteins (sHsps), Hsp60, Hsp70, Hsp90, and Hsp100. Each class has multiple isoforms with their own function [2]. This review is focused on Hsp90. The name heat shock protein 90 refers to the role it plays in the heat shock response as well as its molecular weight (90 kDa) which distinguishes it from other heat shock proteins. Hsp90 is highly conserved across many species ranging from *E. coli* to humans [5]. The Hsp90 chaperone family contains four isoforms in mammalian cells. These isoforms are Hsp90α, Hsp90β, glucose response protein 94 (Grp94), and tumor necrosis factor type 1 receptor-associated protein (TRAP1) [6]. Hsp90α/β operates in the cytosol while Grp94 localizes to the endoplasmic reticulum and TRAP1 to the inner mitochondrial space [7]. Hsp90α expression is inducible and regulated by heat shock factor 1 (HSF1), while Hsp90β is constitutively expressed [8]. Together, these two isoforms make up 1–2% of cytosolic proteins in normal homeostasis, and up to 4–6% when a cell is stressed [9]. It has been suggested that Hsp90α/β potentially interact with 10% of all cytosolic proteins [10] which demonstrates how important it is to understand the roles they play. Hsp90α/β have the same general function and interact with the same cochaperones

across different cell types. The major difference between the two is how much Hsp90α is upregulated following heat shock relative to Hsp90β [8][11].

1.2. Hsp90 Structure

Hsp90 is expressed as a monomer, however, the homodimerization of these monomers is required for chaperone activity [12]. There has also been evidence of Hsp90α/β heterodimers in HEK293 cells, however, evidence for these heterodimers is not abundant [13]. The Hsp90 monomer contains four major domains that are critical in its function: n-terminal (NTD), charged linker (CL), middle (MD), and c-terminal (CTD) domains. The NTD is responsible for the ATPase activity which drives the conformation cycle of the enzyme. There is also a small part of the NTD that is referred to as the "lid" which closes ATP into the active site [14]. The MD interacts with substrate (or client) proteins, acts as a binding site for co-chaperones, and is involved in ATP hydrolysis. Upon ATP binding, the MDs undergo a dramatic shift in position and eventually cross over each other [14]. Connecting the MD and NTD is a charged linker (CL) which contributes flexibility during conformational shifts. The CL also seems to play a role in the regulation of Hsp90 conformation and chaperone cycle [15][16][17]. The CTD is largely involved in the dimerization of Hsp90 monomers to form the functional Hsp90 enzyme. Much like the "lid" in the NTD, the CTD contains a motif called MEEVD, which is derived from the single letter amino acid code. The MEEVD motif is important in many co-chaperone interactions. These cochaperones contain tetratricopeptide repeat (TPR) domains which facilitate the binding to MEEVD [18]. The various co-chaperone interactions play a huge role in driving Hsp90 function and ATPase activity.

1.3. Hsp90 Chaperone Cycle and Function

Hsp90 function is best represented as a cycle involving various co-chaperones that facilitate conformational changes (see **Figure 1**). The cycle begins with an Hsp90 dimer in an open conformation. Here, the middle domains are split far apart and the ATPase catalytic site in the NTDs are empty. The open conformation is stabilized by CDC37 (cell division cycle 37), HOP (Hsp70-Hsp90 organizing protein), and PPIase (peptidyl-prolyl cis-trans isomerase). CDC37 binds to the NTD of HSP90 and inhibits ATPase activity of the homodimer. The main function of CDC37 is the activation of kinase clients [19][20][21][22]. HOP inhibits ATPase activity and aids in the recruitment of various client proteins via Hsp70 recruitment [23][24]. There is also evidence that HOP interacts with components of the proteasome which may contribute to HSP90 client degradation [25]. Lastly, PPIases help fold client proteins via their activity [18][26]. PPIases commonly associated with Hsp90 are FKBP51, FKBP52, and CYP40 (FK506-binding proteins 51, 52, and peptidyl-prolyl cis-trans isomerase 40) [14].

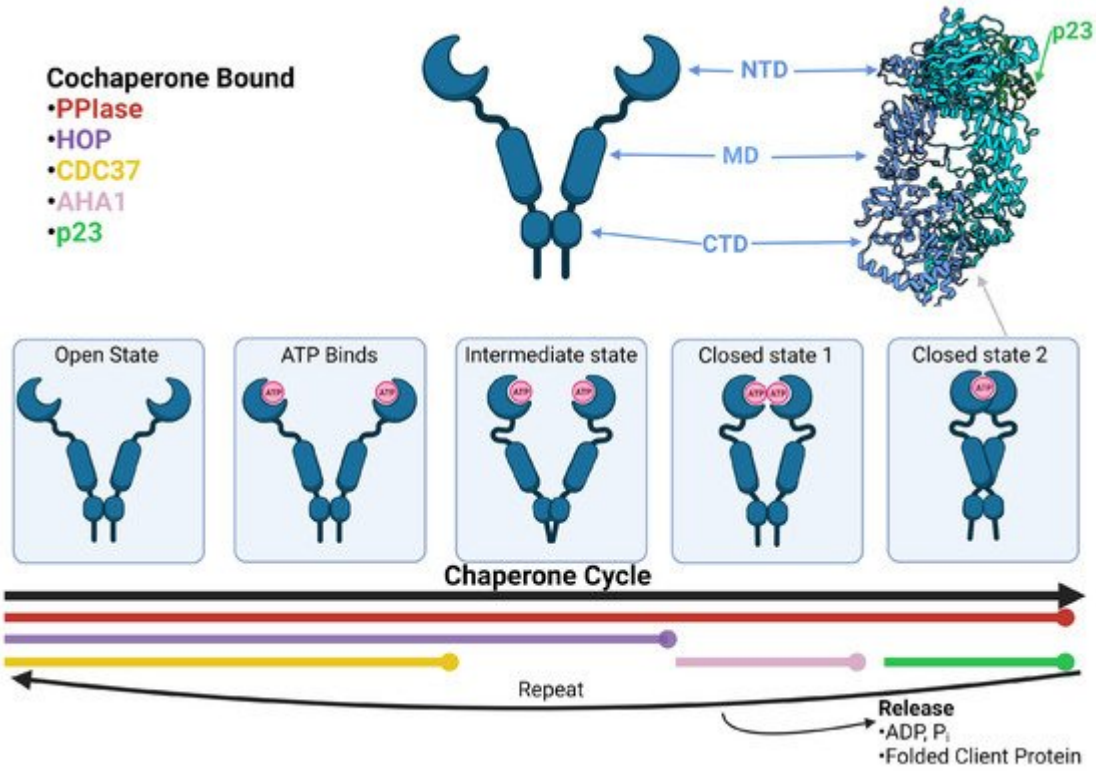

Figure 1. Hsp90 structure and chaperone cycle. The Hsp90 homodimer goes through multiple conformational changes while folding client proteins. The phases when certain cochaperones, PPIase, HOP, CDC47, AHA1, and p23, bind are labeled by color. In the top right is the Cryo-EM structure of Hsp90 complexed with p23 in closed state 2 (PDB ID: 7L7J from [27]). The domains of Hsp90 are labeled NTD, MD, and CTD.

2. Hsp90 in Cardiomyopathy

Cardiomyopathy refers to electrical or muscular dysfunction in heart tissue, which can be induced by a diverse set of pathological conditions or genetic factors [28]. Cardiomyopathies come in five general classifications: ischemic, dilated (DCM), hypertrophic (HCM), arrhythmogenic (ACM), and restrictive (RCM) [29]. Ischemic cardiomyopathy occurs when the heart muscle is damaged from a lack of oxygen typically from coronary artery disease and atherosclerosis [30]. DCM, HCM, ACM, and RCM are typically caused by genetic factors affecting the myocardium, which can be further exacerbated by pathophysiological conditions like hypertension [29]. Fibrotic and hypertrophic signaling in these conditions becomes imbalanced leading to their development and advancement [31][32]. Uncontrolled cardiomyopathy will ultimately lead to congestive heart failure (HF). The clinical syndrome of HF places a considerable burden on the United States healthcare system. Estimates pin the total cost of HF to increase to 70 billion dollars annually by 2030 [33]. In this regard, Hsp90 plays an important role in many of these cardiomyopathy-related pathways. Our current understanding of the Hsp90 interactome highlights the potential for targeting Hsp90 in the prevention of fibrosis, hypertrophy, and cell death response, which are crucial contributors of cardiomyopathy development (see **Figure 2**) [22].

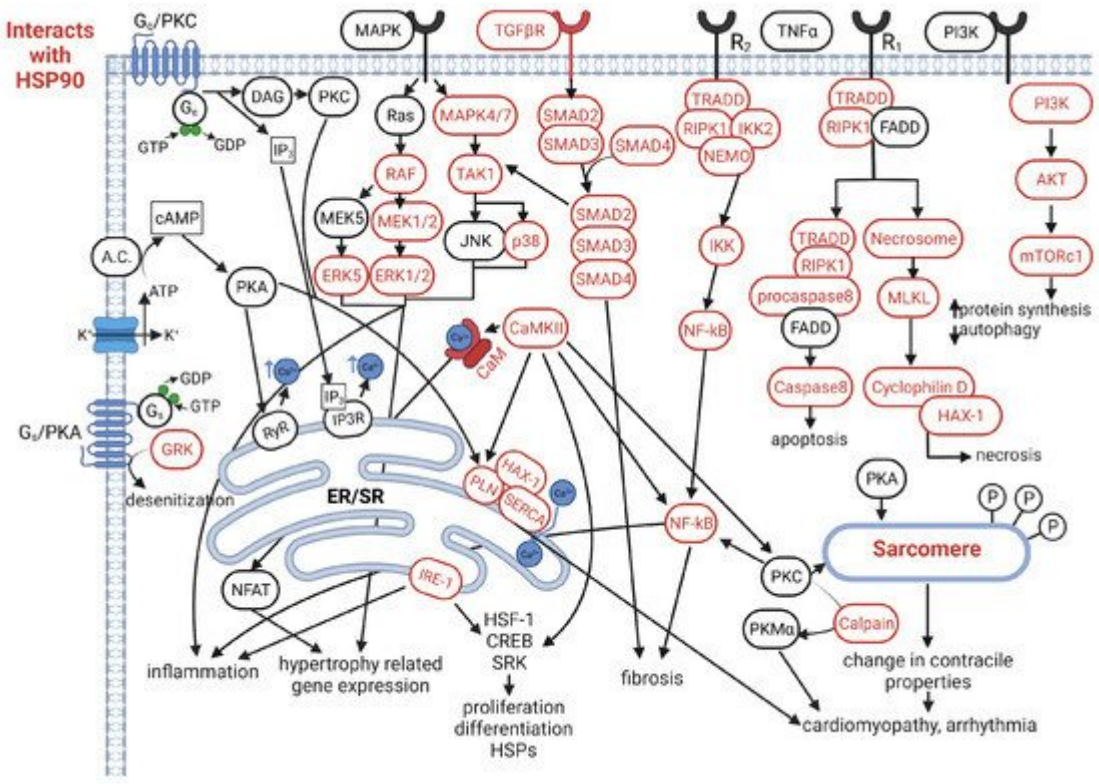

Figure 2. Signaling pathways related to cardiomyopathy. Proteins highlighted in red interact with Hsp90.

2.1. Pathways Regulated by Hsp90 in the Heart

2.1.1. TGF-β Signaling

Transforming growth factor β (TGF-β) is a potent cytokine which plays an important role in cellular responses, such as angiogenesis, fibrosis, and immune response [34]. Induction of higher extracellular TGF-β levels can occur via mechanical overload typically in the form of hypertension, myocardial infarction, as well as ischemia/reperfusion (IR) injury [35]. TGF-β has been shown to induce cardiac hypertrophy as well as cardiac fibrosis [36]. These two responses occur from the difference between canonical and non-canonical TGF-β signaling. In the canonical cascade, the receptor is activated via autophosphorylation upon ligand binding. Another protein called activin receptor-like kinase 1 (ALK1) dimerizes with the receptor and is also phosphorylated. ALK1 is a kinase which aids in the phosphorylation of Smad1 and Smad5 proteins. The Smad1/5 complex joins with Smad4. This trimer is then transported to the nucleus where it acts as a transcription factor, activating genes involved in the fibrotic response and extracellular matrix (ECM) production [37]. Hsp90 has been shown to stabilize Smads and potentially aid in their translocation to the nucleus [38]. It has also been implicated in the stabilization of the TGF-β receptor which prevents the degradation of the receptor via SMURF-mediated ubiquitination [39][40].

2.1.2. MAPK Signaling

MAPK signaling is responsible for expression of proteins involved in cell proliferation, differentiation, development, apoptosis, and inflammation [41]. In the heart, MAPK signaling is induced by growth factors [42]. The varying responses depend on which arm of the signaling cascade is activated. As mentioned before, TGF-β is able to

activate the p38 pathway of MAPK which goes on to express proteins involved in all categories previously listed. Both p38 and the kinase which activates it, mitogen-activated protein kinase kinase kinase 7 (MAP3K7 or TAK1), have been found to be Hsp90 clients [43][44]. Another part of MAPK signaling relevant in heart tissue is extracellular-signal-regulated kinase 1 and 2 (ERK1/2), Here, signaling is activated in the well-known MAPK cascade Ras-Raf-Mek-Erk typically through activation of a tyrosine kinase receptor (RTK) [45]. This pathway is known to upregulate proliferative genes as well as those involved in differentiation and development [41]. Within this pathway, Hsp90 has been shown to chaperone for MEK1, A-Raf, B-Raf, Raf-1, ERK, p90RSK, STAT3, and STAT5 [46][47].

2.1.3. PI3K/AKT(PKB)/mTOR Signaling

PI3K signaling is typically initiated by RTK or cytokine receptor activation [48]. Upon receptor activation, PI3K (p85 & p110) binds to the receptor via IRS and is phosphorylated. This complex phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP2) which then phosphorylates phosphatidylinositol (3,4,5)-trisphosphate (PIP3). PIP3 activates PDK proteins which go on to phosphorylate protein kinase B (PKB) activating it. PKB acts as a kinase for many different proteins which control autophagy (mTOR), glucose metabolism (mTOR), protein synthesis (mTOR), proliferation, and cell survival [49]. It is clear that a major part of PI3K signaling consists of mTOR and its downstream targets. The mTOR protein is found in a complex with many others which aid in its function including RAPTOR in mTORc1. This complex inhibits ULK1 via phosphorylation thereby inhibiting autophagy [50]. It activates protein synthesis via p70S6K activation which activates S6, a ribosomal protein. It also inhibits 4E-BP1 which allows the elongation factors to form around the 5’ cap of mRNA [49]. Of these proteins, p85, p110, PKB, mTOR, RAPTOR, S6K, and eIF4E (translation elongation factor) are all Hsp90 clients [51][52][53]. It is also seen that inhibiting Hsp90 severely downregulates PKB and mTOR signaling [51][54]. There is also evidence that higher expression levels of Hsp90 preserve mitochondrial function through phosphorylation of Bcl2 in cardiomyocytes exposed to heat shock conditions via PKB and PKM2 signaling [55].

2.1.4. G_s/PKA Signaling and Calcium (Ca^{2+}) Regulation

Hsp90 has been shown to mediate interactions between PLN, SERCA, and HAX-1. By recruiting Hsp90 to the SR Ca2+ uptake complex, the function of IRE-1, another Hsp90 client protein, was impaired [56][57][58]. Furthermore, the function of PLN and ryanodine receptor can be regulated by Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) phosphorylation [59][60]. This kinase is also stabilized by Hsp90 [22]. CaMKII is relevant in intra-nuclear phosphorylation of transcription factors including HSF-1, CREB, and SRF. It also may activate NF-kB signaling leading to inflammatory response [61]. Given the role of CaMKII and SR Ca^{2+} cycling in the development of heart diseases, it is intriguing to examine if Hsp90 can be targeted to correct these dysfunctions. When Ca^{2+} levels increase in the cytosol, two important Ca^{2+}-dependent proteins can be activated, calcineurin and calmodulin. Upon Ca^{2+} binding, these enzymes dimerize to form a functional phosphatase [62]. Hsp90 is found to stabilize both calcineurin and calmodulin and inhibition of Hsp90 leads to decreased nuclear factor of activated T-cells (NFAT) signaling [63][64]. Once the calcineurin/calmodulin phosphatase (CaM) is active, it dephosphorylates NFAT which is translocated to the nucleus as a transcription factor. Here, NFAT can activate genes controlled by MEF2 and GATA which are implicated in cardiac hypertrophy [65]. NFAT has been shown to be relevant in pathological cardiac hypertrophy and may also cross-talk with MAPK to accentuate pathological effects [66][67].

2.1.5. G_q/PKC Signaling

A different GPCR pathway is activated via angiotensin and endothelin receptors. The heterotrimeric g protein associated with these receptors is G_q. Once activated through phosphorylation of the receptor, the α subunit goes on to activate protein lipase c (PLC), which ultimately activates protein kinase c (PKC) [68]. PKC has four isoforms in humans (α, β, δ, and ϵ) with α being the most abundant in heart [69]. Each of these isoforms has been found to have slightly different activity, for simplicity, this review will refer to all of them as PKC [70]. PKC has a wide range of targets which it phosphorylates. Some of the targets that are phosphorylated are sarcomere proteins which will alter the stiffness of the myocardium and can contribute to the onset of cardiomyopathy if dysregulated [70]. PKC affects phospholamban (PLN) indirectly through phosphorylation of I-1, this inhibits PP1 which directly regulates PLN [71]. This leads to a decrease in phosphorylation causing a decrease in Ca^{2+} uptake by SERCA2 and cardiac dysfunction [72]. There is also crosstalk between PKC and MAPK through ERK1/2 which implicates PKC in the expression of hypertrophy-related gene expression [73]. There is also evidence of PKC activating NF-kB in cardiomyocytes, causing expression of pro-inflammatory proteins implicated in fibrosis [74]. Hsp90 is known to regulate NF-kB through stabilization of IkB kinase [75]. Lastly, PKC can also be cleaved by calpain (a Ca^{2+} dependent protease) which is stabilized by Hsp90 in the cytosol. This cleavage makes a fragment called PKMα which is implicated in dilated cardiomyopathy [76].

2.1.6. TNFα Signaling

Tumor necrosis factor α (TNF-α) signaling is known to activate apoptosis, necrosis, proliferation, and inflammation responses in cells. In the heart, this type of signaling is relevant in myocardial remodeling and is typically induced in myocytes by IR injury and HF [77]. Initially, the cytokine TNFα binds to its receptors, TNFR1 or TNFR2. Both receptors are expressed in the heart and are upregulated following IR injury [78]. TNFR1 signaling is more associated with apoptosis and necrosis response, while TNFR2 response results in proliferative and inflammatory genes, suggesting TNFR1 is cardiotoxic and TNFR2 is cardioprotective in response to injury [79]. In both pathways, many of the signaling proteins are stabilized by Hsp90.

TNFR2 signaling is most commonly associated with inflammation. TNFα binds TNFR2 and a complex forms around the intracellular portion of the receptor, similar to TNFR1. However, there are differences in the proteins recruited [80]. The TNFR2 forms a complex with multiple proteins, including TAK1, IKK2 (IKKa/IKKb), and NEMO, which are crucial in the initiation of NF-kB. Hsp90 is required for the recruitment of IKK2 to the receptor [81] and is also important in IKKa/IKKb stabilization in cardiomyocytes [75]. Studies have shown that treatment with geldanamycin (Hsp90 inhibitor) disrupts TNFα induced NF-kB signaling [82][83].

2.2. Pathophysiological Significance in Cardiomyopathy

Hsp90 plays a role in all of the aforementioned pathways by stabilizing or folding various proteins in each cascade. These pathways have all been implicated in pathological fibrosis, hypertrophy, cell death responses in the heart and the effects of inhibiting Hsp90 have been studied in each as well. Hsp90 in the context of the TGF-β pathway has been studied due to the pro-fibrotic gene expression it causes in the heart. It has been found that inhibiting

Hsp90 using either geldanamycin or an inhibitory peptide prevents pro-fibrotic TGF-β signaling in cardiomyocytes and cardiac fibroblasts [40][84].

The effects of Hsp90 inhibition on the MAPK pathway has also been studied recently. Rats treated with 17-AAG ((17-(allylamino)-17-dimethoxy-geldanamycin) two weeks after undergoing coronary artery ligation survived better and maintained better cardiac function compared to rats not receiving 17-AAG treatments [85]. This shows a direct link between Hsp90 function, MAPK signaling, and development of cardiomyopathy following I/R injury. The PI3K/AKT/mTOR pathway and Hsp90 were studied following heat shock damage in vitro. Here, it was seen that Hsp90 inhibition in mice using geldanamycin prevented the cardioprotective action of AKT under heat shock conditions leading to more apoptosis [55]. While this may not be a direct link to cardiomyopathy, it shows that Hsp90 and AKT may protect against apoptosis in the heart.

In the G_s/PKA pathway, Hsp90 is found to be involved in the regulation of SERCA2 via HAX-1, which has implications in arrhythmia in cardiomyocytes [57][58]. In the G_q/PKC pathway, there is one study that shows hypertrophic angiotensin II was prevented with administration of geldanamycin. This occurs from Hsp90's role in stabilizing the IKK complex which is required for NF-kB. Inhibiting this signaling pathway using geldanamycin prevents hypertrophic signaling in cardiomyocytes [75].

Lastly, the TNF-α pathway was demonstrated to be affected by geldanamycin treatment in ischemic postconditioning. In rats, the postconditioning treatment was able to reduce infarct size partially through reducing TNF-α signaling. Rats that underwent postconditioning and given geldanamycin saw the infarct size return to the same levels as rats that received no postconditioning treatment showing that the inhibition of Hsp90 increases TNFα via JNK signaling [86]. Necrosis in mouse heart has also been shown to be regulated by Hsp90 via HAX-1, cyclophilin D, and mPTP [87]. Hsp90 also plays a cardioprotective regulatory role in apoptosis through HAX-1 and IRE-1 in mouse heart. Here, inhibition of Hsp90 prevented additional cardioprotective effects of HAX-1 [56]. Indeed, systemic inhibition of Hsp90 is associated with development of cardiomyopathy [88][89][90][91]. Thus, instead of affecting every Hsp90 complex in the cardiac cells, developing a strategy to target a subset of Hsp90 client protein may be a better approach.

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