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A review of process on the Heat Shock Protein 90 (Hsp90) structural specificity and functional diversity

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Abstract

Heat Shock Protein 90 (Hsp90) is a molecular chaperone which plays an active role in maintaining protein homeostasis. Hsp90 is known to be highly expressed in tumour cells where it regulates stability and function of several key oncogenic client proteins including Akt kinase, EGFR, CDK and PDGFR. These client proteins are mutated or overexpressed in tumours and are involved in tumour progression and metastasis due to their roles in signaling pathways, cell cycle and apoptosis. Hsp90 has two isoforms, namely Hsp90α and Hsp90β and share 85% sequence homology. Hsp90β is the constitutive isoform, however, Hsp90α is highly induced in many cancers and is responsible for tumorigenesis.

Keywords: Heat Shock Protein 90 (Hsp90); Hsp90α and Hsp90β; Hemeprotein; Oncoproteins and metastasis

1. Introduction

The molecular chaperone Hsp90 is highly abundant molecular chaperone and constitutes 1-2% of total cellular proteins under non-stressed conditions [1]. While the amino acid sequence of a protein dictates its native conformation, most proteins fail to fold efficiently in the highly concentrated and complex environment of the cell without the help of heat shock molecular chaperone proteins [2]. Since its discovery in the 1960s, study of chaperone structure and function has been the active area of research due to its essential house-keeping functions including protein folding and translocation across membranes, and most importantly the post-translational regulation of cell signaling molecules [2].

Hsp90 is unique since it is not required for the de novo synthesis of most polypeptides but it is required for folding of particular subset of proteins that have difficulty in reaching the active conformation. Alternatively, most but not all of its client proteins are conformationally labile signal transducers and have vital role in growth and survival processes [2]. Over the past decade, Hsp90 has emerged as an important biomolecule responsible for cancer cell survival under obnoxious conditions [3]. Although, Hsp90 is present in all cells, Hsp90 protein level is induced by heat shock, oxidative stress and nutritional deficiencies in many cancers including glioma but not in normal human brain tissue. This over-expression has been attributed to the hostile conditions and stressful environment prevalent in tumors [3].

2. Glioma: Role of Hsp90

An increased expression of heat shock protein seen in cancer cells, in addition to the levels seen in normal cells, is common among solid tumor's including gliomas [4]. Molecular chaperones act as biochemical buffers for the molecular and genetic instability commonly observed in solid tumors. In addition, chaperones also permit tolerance to genetic alterations including mutations of fundamental signaling molecules which would otherwise be fatal [4]. This exemplifies the vital role of Hsp90 in maintaining cellular homeostasis in the hostile environment imminent in gliomas. Moreover, gliomas have induced levels of Hsp90α (inducible isoform of Hsp90) protein as compared to its normal

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counterpart which has minimal or no presence of the chaperone protein [4]. Furthermore, oncogenic client proteins of Hsp90 such as Akt kinase, C-RAF, MET oncogene, CDK4, hypoxia inducible factor 1α (HIF-1α), human telomerase reverse transcriptase (hTERT), PDGFR, EGFR and mutant p53 hugely contribute to the pathogenesis of glioblastomas [5]. The pharmacologic inhibition of Hsp90 in tumor cells has also been reported to cause client protein degradation via the ubiquitin-proteasome pathway [6].

The ability to achieve multiple effects via a single drug target may potentially prove to be remarkably promising in the treatment of cancer. CDK4 is a serine/ threonine kinase that plays a pivotal role in cell cycle progression from G1 to S phase whose function is reliant on active Hsp90 and inhibition of Hsp90 causes destabilization and degradation of CDK4 which further leads to cell cycle halt at G1 phase [5]. Recently, an overwhelming array of Hsp90 client proteins have been identified. Hsp90 mainly involved in protein chaperoning of client proteins that actively participate in glioma progression include CDK4, EGFR, FAK, Akt, hTERT, p53, PDGFR, MAPK, MMP2, PI3K, EF-2 kinase, and HIF-1α and appear to play vital roles in cell cycle regulation and signal transduction [6].

Akt is a serine/threonine protein kinase (also known as protein kinase B, PKB) activated by growth factor stimuli such as insulin and PDGF [7]. Akt is present in the cell as an enzymatically active complex in association with Hsp90. Hsp90 prevents Akt inactivation by evading its dephosphorylation by phosphatases. Association with functional Hsp90 is essential for Akt stability and binding of inhibitors to Hsp90 results in its destabilization and is subsequently targeted for proteasomal degradation [7]. Additionally, the ability of Hsp90 to bind to hTERT is crucial for assembly of telomerase complexes responsible for cancer cell immortalization [7]. The anti-apoptotic signaling is regulated by the association of hTERT and Akt with Hsp90, and subsequent phosphorylation of both the client proteins [7].

3. Structure and functions of Hsp90 protein

Hsp90 belongs to a family of molecular chaperones known to be highly conserved from prokaryotes to eukaryotes [8]. Hsp90 mainly resides in the cytoplasm and exists predominantly as a homodimer [7]. It has three domains: N-terminal domain with ability to bind ATP, middle domain and C-terminal domain which facilitates the homo-dimerization of Hsp90 monomers [7]. The N-terminal ATP binding domain requires the binding and hydrolysis of the ATP to execute and maintain the proper chaperoning of its client proteins. The conformational change in Hsp90 as a result of the phosphorylation at the N-terminal domain of Hsp90 further assists the conformational activation of its client proteins [8].

In eukaryotes, a versatile, highly charged linker sequence connects the N-terminal domain to the middle domain [9]. The length and composition of this linker region is variable among the different organisms. Structural analysis of the N-terminal domain of Hsp90 shows the existence of an ATP-binding site. Moreover, biochemical studies of this domain also suggest that the intermolecular interaction between the two N-terminal domains of the Hsp90 homodimer occur in an ATP-dependent manner which gives Hsp90 characteristic ATP-driven chaperoning activity [5]. Inhibition of ATPase dependent Hsp90 activity using inhibitors such as radicicol and geldanamycin that binds to the N-terminal domain of Hsp90 was one of the important findings of the 20th century in anticancer research [9]. For the past decade, Hsp90 has been considered as primary therapeutic target for the treatment of cancer. The middle domain is proteolytically resistant and contains a catalytic loop that accepts the terminal phosphate of ATP bound to the N-terminal ATP binding pocket of Hsp90 (also called the γ-phosphate) [10].

Structural studies of Hsp90 indicate that the middle domain is involved in the interface of many client proteins including p53 and Akt[6]. Also, a recently discovered cochaperone AHA1 (activator of Hsp90 ATPase homologue 1) interacts with Hsp90 and accelerates ATP hydrolysis by promoting association between the N-terminal and the middle domains [4]. The middle domain and the C-terminal domain of the Hsp90 are associated via another versatile linker region that drastically facilitates the ATPase activity of Hsp90 [10]. C-terminal truncations of Hsp90 eliminate its ability to hydrolyze ATP, therefore signifying the importance of the dimeric nature of C-terminal domain for Hsp90 activity [11]. More importantly, the C-terminal domain contains the MEEVD motif (highly conserved pentapeptide) which is responsible for recruiting several co-chaperones containing the tetratricopeptide repeats, such as immunophilins and Hsp90 organizing protein (Hop), which corroborates the C-terminal domain as an essential subunit for Hsp90/cochaperone complex formation [11].

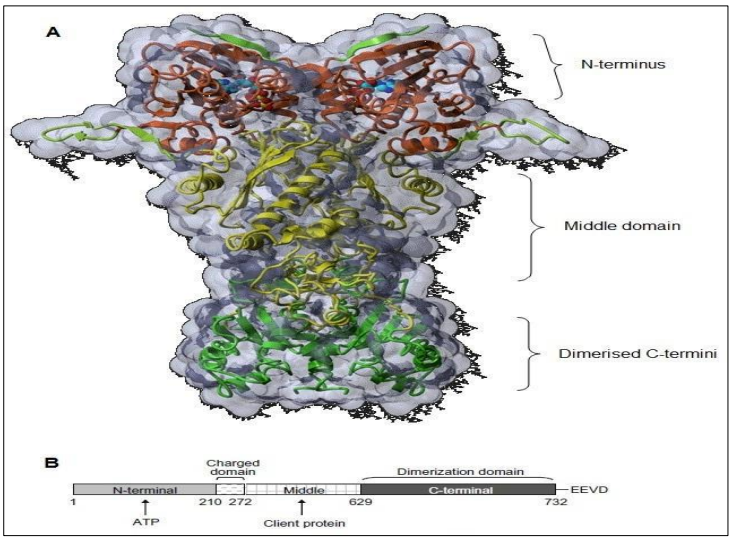

Figure modified from (Fukuyo et al., 2010).

Figure 1 Structure of Hsp90 showing the different domains in the homodimer

The structural mechanism adapted by Hsp90 for its chaperone activity resembles a 'molecular clamp'. In the absence of ATP, the N-terminal domain of the Hsp90 homodimer binds its client proteins by maintaining an open-state [11]. Once bound to ATP, Hsp90 homodimer undergoes conformational changes that permit transitory interaction between the N-terminal domains of the homodimer which ultimately leads to the formation of the closed-form of Hsp90 homodimer and clamping of the substrate protein. Ultimately, the client proteins are activated through this ATPase-driven cycle involving Hsp90/co-chaperone complex [14].

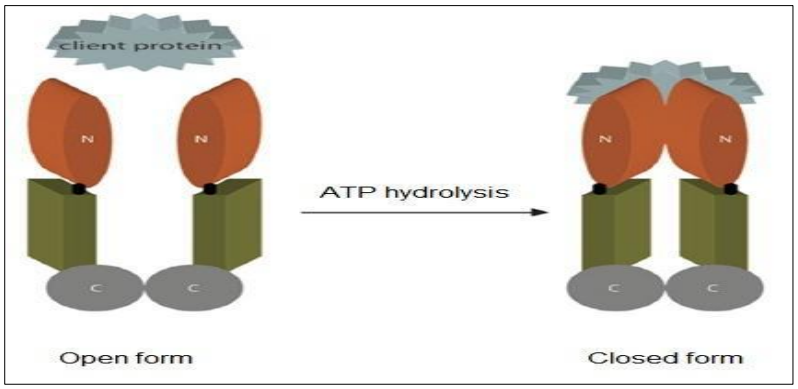

Figure modified from (Brown *et al.*, 2007)

Figure 2 ATPase driven Hsp90 dependent activation of client protein

The Hsp90 homodimer maintains an open state which facilitates capture of client proteins. Once associated with ATP, Hsp90 homodimer undergoes conformational changes including transitory interaction between the two N-terminal domains with concomitant activation of the captured client protein. Two cytoplasmic isoforms of Hsp90 exists in humans, namely Hsp90α (inducible expression) and Hsp90β (constitutively expressed), who share sequence homology of 85% [15]. Hsp90 is mainly a homodimer, but monomers (α or β) and heterodimers can also exist [14].

The α isoform readily forms dimers, whereas β isoform dimerizes with less efficiency. The C terminal of Hsp90 is mainly responsible for dimerization. Another distinctive isoform, Hsp90N is associated with cellular transformation. Hsp90N shares sequence homology with Hsp90α, but it lacks the N-terminal domain which is a mandatory requirement for the ATP-driven Hsp90 chaperoning [16]. Gene sequencing experiments identified hsp90α cDNA sequence in chromosome 14 and hsp90β cDNA sequence in chromosome 6 at the genomic locations at 14q32-33 and 6p21 for hsp90α and hsp90β, respectively, have been documented as functional [16].

4. Transcriptional regulation of hsp90α.

The transcription of human hsp90α gene is regulated by the 5' upstream promoter sequences bearing the heat shock elements (HSEs) known to regulate gene expression of hsp90α [17]. The heat shock factor (HSF1) binds to the HSEs and marks the initiation of hsp90α gene transcription. Under normal conditions, HSF1 is bound to cytosolic Hsp90 and Hsp70 and hence avoids transcription of heat shock genes. However, under stress, or upon inhibition of Hsp90, the Hsp90 chaperone refolds partially denatured client proteins which liberates HSF1 consequently resulting in its translocation to the nucleus where it initiates transcription of hsp90 gene [17]. Hence, through a feedback mechanism, Hsp90 regulates its own gene expression whereby under normal conditions, HSF1 binds to Hsp90 and prompts gene transcription to a halt [17].

The exact mechanism responsible for the dissociation of Hsp90 from HSF1 under stress or in response to its inhibition of Hsp90 inhibitors is still unclear. However, under stressful conditions it may alter protein stability by increasing the concentration of partially folded proteins which compete for Hsp90, consequently increasing the concentration of the active HSF1 [19]. Figure 3illustrates the negative feedback mechanism between Hsp90/Hsp70 and HSF1 which regulates transcription of the hsp90 genes.

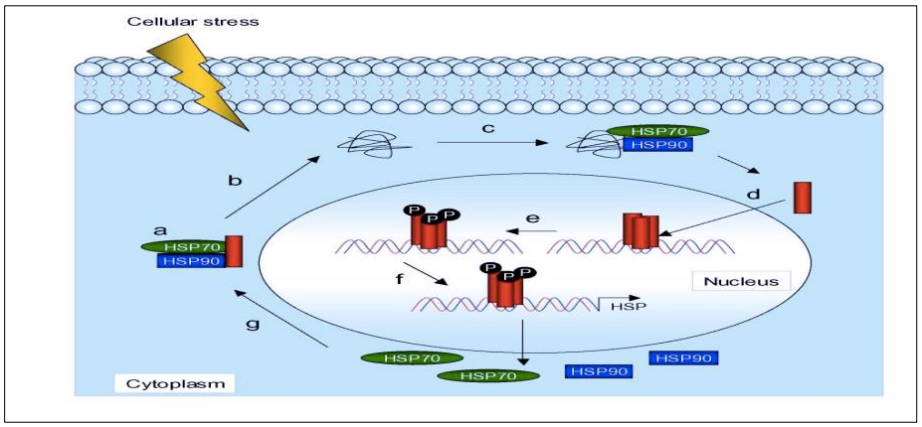

Figure 3 Transcriptional regulation of Hsp90

A) Under normal condition, HSF1 exists as a complex with Hsp90 and Hsp70. b) Under stress, Hsp90/Hsp70 dissociates from HSF1 to stabilize denatured proteins (c). d) Monomeric HSF1 now translocate to the nucleus where it can trimerize. e) HSF1 undergoes a series of post-translational phosphorylation events before it activates hsp90 gene transcription (f). g) HSF1 is inactivated when cellular concentrations of Hsp90 and Hsp70 increases. Figure adapted from [19].

5. Rationale for targeting the Hsp90α protein

Hsp90 is involved in cell survival and signaling pathways that regulate cell proliferation. High levels of Hsp90 protein have been reported in several cancers including gliomas and has been correlated with tumor progression in glioma [20]. In addition, Hsp90 client proteins including PDGFR, EGFR and Akt play central role in survival and growth signaling pathways in glioma which validates targeting Hsp90 as an anti-cancer approach [21]. The inducible isoform Hsp90α is highly expressed in brain tumors and is known to be involved in cell cycle progression, apoptosis, malignant invasiveness and metastasis [19]. Furthermore, not only does Hsp90 facilitate tumor cell survival in noxious tumor environments, but in its presence tumor cells tolerate genetic alterations to vital signaling molecules that would otherwise be fatal [22]. Therefore, down regulation of Hsp90α mRNA and protein levels in glioblastoma may induce tumor cell death [23].

A recent study on glioma cell lines showed that hsp90α was elevated (27-fold increase) in 3/3 glioma cell lines and 8/8 glioma tissue specimens as compared to extremely low or absent in normal brain cell lines and tissue specimens analyzed[24].The inducible isoform, Hsp90α has also been reported to be released into the extracellular matrix surrounding tumor cells where it assists in activation of MMP2, in addition to contributing to tumor metastasis [25].Due to the important role played by Hsp90α in tumor survival and progression as discussed above, and its induced protein levels in glioblastoma validates Hsp90α as an important therapeutic molecular target in glioblastoma, whose etiology is so multifaceted [26].

Various methods exist to silence/downregulate Hsp90 levels *In-vitro*. Established approaches involve the use of Hsp90 inhibitors such as geldanamycin (GA), radicicol (RA) and 17-allylamino-17-demethoxygeldanamycin (17-AAG) that target the Hsp90 protein [27]. These inhibitors bind to the N-terminal domain of Hsp90 and interfere with its heat shock chaperoning by eradicating its ability to hydrolyze ATP [28, 29].Another approach using small interfering RNA (siRNA) has emerged as an effective silencing tool for post-transcriptional down regulation of target gene since the discovery of RNA interference (RNAi) pathway in invertebrates [30].RNAi mediated down regulation of hsp90α gene in glioblastoma in vitro using siRNA has been shown to induce chemosensitivity in glioblastoma [31].

6. Conclusion

Cancer is a disease of exceptional heterogeneity in which populations of tumor cells are remark- ably adaptable and withstand an array of severe intrinsic and extrinsic stresses. These stresses reshape numerous cellular processes including metabolism, signaling, the folding and degradation of proteins (both wild type and mutant), and interactions with the microenvironment and immune system. By supporting adaptation, the upregulation of protein folding capacity enables heterogeneous cancers to evolve into progressively more malignant states. Consequently, the protein folding machinery, and HSP90 in particular, has been identified as a promising therapeutic target for the treatment of cancer. Despite great success in generating potent and selective in-hibitors of HSP90, however, the therapeutic potential of modulating this unique target has yet to be realized.

The 90 kDa HSPs constitute a class of highly conserved proteins implicated in a plethora of physiological, as well as pathological, processes. Due to their abundance and distinct cellular distribution, the function of HSP90 members attracted researchers to extensively explore their complexity. Although we present here the most significant knowledge about HSP90 family members, it only represents a fraction of the HSP90 literature. In this review we shed light on the major diverse functions of HSP90 family members in terms of structure, regulation, and function in health and disease conditions. The use of HSP90 inhibitors is of critical clinical importance especially in cancer and neurodegenerative diseases. However, the challenge remains to identify and establish the most effective and least toxic drug. Moreover, the chances of drug resistance decrease with the application of rationale drug combinations, for the inhibition of Hsp90 offers a wide range of opportunities to enhance the anticancer effects of drugs used in combination therapies.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declare that they have no conflicts of interest.

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