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# Heat shock proteins 90 as a novel target for cancer therapy

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**ABSTRACT** *Heat shock proteins 90 (HSP90) is essential for the maintenance of appropriate folding and conformation of many cell signaling proteins, which are involved in cell proliferation and survival and already several HSP90 inhibitors are under clinical evaluation[1]. A major attraction of HSP90 inhibitors is their potential to inhibit a range of oncogenic client proteins and cancer pathways, thereby blocking all of the 'hallmark traits' of cancer and exhibiting broad-spectrum antitumor activity2. Many studies have suggested that targeting of the HSP90 molecular chaperone has great potential for cancer therapy[1].*

**Heat shock proteins 90**

Heat shock protein 90 (HSP90) is one of the most abundant cellular proteins. HSP90 accounts for 1–2% of total protein in unstressed cells and increases to 2–10% under stress[3]. HSP90 is a member of the family of heat shock proteins (HSPs) which function as molecular chaperones and play a critical role in protein folding, stabilization, transportation, and degradation[4]. These processes are essential for the normal functions of cellular proteins. As their name indicates, HSPs are expressed in response to heat shock and other conditions of stress such as environmental changes, genetic mutations, oxidative stress, heavy metals, hypoxia, acidosis, and metabolic toxins[3,5]. Heat shock proteins vary in their cellular localization and functions and mammalian HSPs have been classified according to their molecular weights: HSP90, HSP70, HSP60, HSP40 and the small HSPs such as HSP27[5].

Unlike other HSPs, HSP90 was found to interact specifically with many proteins involved in transcriptional regulation and signal transduction, such as steroid hormone receptors, transcriptional factors, and protein kinases. Furthermore, HSP90 is abundantly expressed in primary and cultured Hodgkin's lymphoma (HL) cells[6]. This review, therefore, will focus on HSP90 as a target for cancer therapy.

**Heat shock protein 90 structure**

HSP90 is a homodimer containing two to three covalently bound phosphate molecules on each monomer. Each homodimer is made up of monomers that consist of three distinct domains that have important functional interactions: a highly conserved 25 kDa ATP binding N-domain, a 35 kDa M-domain involved in client protein binding and the 12 kDa C-domain, the main region for dimer interaction with co-chaperones such as the stress induced phosphoprotein 1 (Sti1/Hop). Binding and hydrolysis of ATP at the N-terminal site results in a conformational change in HSP90 which enables conformational changes in the client protein. The presumed site of binding for client proteins, the "client site", is in the V-shaped pocket formed between the C domains, while the main site for the interaction with essential cofactors of HSP90, so called cochaperones and immunophilins, resides between the N and M domains[7] (Figure 1).

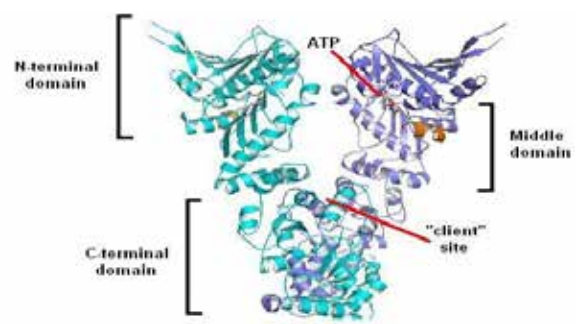


**Figure 1. HSP90 structure[8]**

In humans, two closely related HSP90 isoforms have been described so far, HSP90α and HSP90β. Both isoforms are induced by stress and contain intronic sequences, a rather unique feature of HSP90 compared to the other HSP proteins that lack introns. Although no major differences in the functions of the two isoforms have been determined yet, differences in their respective modes of regulation have been observed: HSP90α is more inducible than HSP90β. HSP90α seems to be a fast-response, cytoprotective isoform, while HSP90β seems to be associated with long-term cellular adaptation and facilitated cellular evolution[4]. Furthermore, HSP90β expression was shown to be associated with the development of drug resistance. HSP90β is generally expressed at higher levels than HSP90α, and is therefore considered the major form of HSP90 involved in normal cellular functions, such as maintenance of differentiation and cytoprotection. Additional HSP90 isoforms include HSP90N, which is associated with cellular transformation[9], Grp94 in the endoplasmic reticulum and HSP75/TRAP1 in the mitochondrial matrix[10] were added to the HSP90 family. HSP90 isoforms, functions and major expression status are summarized in Table 1. HSP90 isoforms have five highly conserved regions, called "signature

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sequences", of which three are in the N-terminal domain (amino acids 38-59, 106-114, 130-145 of human HSP90α) and two in the middle domain (amino acids 360-370 and 387-401)[2].

**Table 1. HSP90 isoforms, functions and major expression status[11]**

| Isoforms | Major expression status | Specific Function |
|---|---|---|
| HSP90α | Induced | Growth promotion<br>Cell cycle regulation<br>Stress induced<br>Cytoprotection |
| HSP90β | Constitutive | Early embryonic development<br>Germ cell maturation<br>Cytoskeletal stabilization<br>Cellular transformation<br>Long term cell adaption |
| HSP90N | Constitutive | Cellular transformation |
| HSP75/ TRAP1 | Constitutive | Cell cycle regulation |

**Heat shock protein 90 function**

Malignant cells have been shown to express high levels of HSP90 and to be dependent upon HSP90 for the correct folding and function of mutated and overexpressed client proteins[1]. HSP90 protects client proteins from degradation and environmental stress, including heat, hypoxia, free radicals, radiation, and chemotherapy. An elevated HSP90 level allows the accumulation of proteins of mutated genes in the cell, which may be evolutionarily advantageous to genetic diversity. In addition, increased HSP90 activity would also permitted the survival of genetically unstable cancer cells[12]. In normal cells, HSP90 exists in an inactive state, whereas in cancer cells HSP90 is present entirely in multiprotein complexes with high ATPase activity.

Since many of HSP90's client proteins are involved in cell signaling, proliferation and survival, HSP90 has received considerable attention as a potential target for cancer therapy. HSP90's client proteins, including several protein kinases, steroid hormone receptors and transcription factors, are involved in signal transduction, cell cycle control and transcriptional regulation and are thus important in controlling cell growth, proliferation, differentiation and survival[13]. Interestingly, most of these proteins are of major interest in cancer therapy. These include NF-kB, Akt, Her2, androgen receptor and HIF-1 alpha. The number of reported HSP90 client proteins now exceeds 100.....

HSP90 client proteins are involved in all six features of malignant cells, that have been suggested as "hallmarks of cancer": self-sufficiency in growth signaling, insensitivity to antigrowth signals, evasion of apoptosis, sustained angiogenesis and tissue invasion and metastasis[2] (Figure 2). Specific mutations in the client proteins or the abnormal expression of these client proteins promote the growth of malignant cells, resulting in tumor and disease progression. Although there are numerous known client proteins for HSP90, the HSP90 binding process has only been studied with a few of these proteins. The binding between HSP90 and the progesterone receptor (PR) has provided a useful model for HSP90 clients[14].

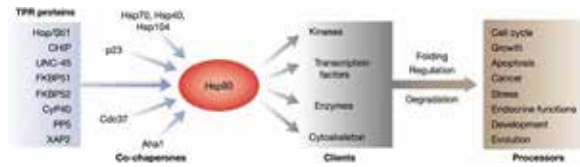


**Figure 2. Examples of roles of HSP90 and its co-chaperones in different cellular processes through their interactions with different client proteins [15]**

**HSP90 inhibitors**

Since HSP90 client proteins played critical roles in tumor growth and maintenance, inhibition of HSP90 has emerged as a promising approach for the treatment of cancer. Understanding the crystal structure of HSP90 allowed researchers to develop several small molecules, including 17-AAG and others, that bind to the ATP pocket in the N-terminal domain of HSP90 and inhibit the essential ATPase activity of HSP90 leading to inactivation, destabilization, and proteasomal degradation of many HSP90 client proteins by the ubiquitin-dependent proteasome pathway[16]. Recent studies suggest that HSP90 found in tumor cells exists in a highly active state that is more susceptible to HSP90 inhibitors compared to HSP90 in nonmalignant cells[17].

Inhibitors of HSP90 such as 17-AAG exhibited biologic activity against many human tumor cell lines through downregulation of client proteins, such as ErbB2, mutant p53, C-Raf, and Bcr-Abl, and has shown antitumor activity in preclinical experiments. 17-AAG is currently being tested in various solid and hematological malignancies, either as a single agent or in combination with conventional chemotherapeutics. 17-AAG has completed Phase I clinical trials, and several Phase II trials are in progress for different types of solid and hematological tumors.

Although 17-AAG has entered clinical trials with some promising early data, it demonstrated limitations including poor solubility, stability, and hepatotoxicity. In addition, dose-limiting toxicities of 17-AAG included gastrointestinal disturbances, anemia, thrombocytopenia, dehydration, and hyperglycemia [12]. In addition, expression of P-Glycoprotein (P-GP) or loss or mutation of the NQO1 gene that is required for the bio-reduction of 17-AAG to the more potent hydroquinone 17-AAGH2, have been suggested as mechanisms of de novo or acquired resistance to 17-AAG. Therefore, second and third generation HSP90 inhibitors that are not substrates for P-glycoprotein and do not require NQO1 metabolism could be preferable [12]. Other HSP90 inhibitors under clinical evaluation include 17-DMAG, IPI-504, and KOS-953, radicicol, novobiocin, LY294002, SNX2112 and BIIB02. Each of the HSP90 inhibitors was active *in vitro*, sometimes leading to more potent disruption and degradation of HSP90 client proteins than 17-AAG.

Consequently these small molecule inhibitors have demonstrated to be of great value in identifying new HSP90 client proteins and in understanding the biology of HSP90. Furthermore, novel HSP90 inhibitors could have antitumor activity in a variety of tumors. However, it remains to be elucidated, whether these compounds will offer greater clinical benefit than 17-AAG while maintaining an acceptable toxicity profile.

**BIIB021**

BIIB021 is a synthetic novel purine-base that binds to the ATP binding site in the N-terminal domain of HSP90. Inhi-

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bition of the ATPase activity by BIIB021 results in degradation of various HSP90 client proteins including HER-2, AKT, and Raf-1[18]. BIIB021 is a promising new oral inhibitor of HSP90 with antitumor activity in preclinical models. Recent studies showed that BIIB021 is more potent than 17AAG and has cytotoxic activity against many cancer cell lines[18]. Furthermore, BIIB021 is not susceptible to tumor cell resistance mediated by MDR, MRP and NQO1 that limit the potency of 17-AAG[18]. BIIB021 was administered to humans for the first time at "MD Anderson Cancer Centre" (USA) for relapsed patients with ZAP-70 positive chronic lymphocytic leukemia (CLL), in October 2005[19]. BIIB021 is currently in phase I/ II clinical trials in CLL (NCT00345189) and in gastrointestinal Stromal tumors (GIST) (NCT00618319). Preliminary data obtained from these trials revealed that BIIB021 is a promising novel oral HSP90 inhibitor with improved solubility, bioavailability and cytotoxicity in cancer cells, encouraging us to investigate the inhibitory effect of BIIB021 on Hodgkin"s Lymphoma cells compared to 17-AAG[20].

A number of studies have suggested that HSP90 plays a crucial role in the progression of diseases, as many proteins which control cell survival, proliferation, and apoptosis are client proteins of HSP90, Hsp90 function is closely related to human health[21]. Recently, there are about than ten different HSP90 inhibitors in various stages of clinical development[22].

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