Effectiveness of Hsp90 Inhibitors as Anti-Cancer Drugs

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Abstract: Hsp90 is a chaperone with over 100 identified client proteins. What makes Hsp90 especially promising as a target for anti-cancer drugs is that many of its client proteins are in signaling and chromatin-remodeling pathways, and these pathways are often disrupted in many types of cancers. Recently, it was determined that Hsp90 bound to a client protein in a co-chaperone complex has a higher ATPase activity and binds to the geldanamycin inhibitor with over 100-fold higher affinity than the low-ATPase form. Consequently, despite Hsp90 being an abundant protein in most cell types, Hsp90 inhibitors accumulate at high levels primarily in tumor cells because tumor cells are “oncogene addicted” and require especially high levels of the high-ATPase form of Hsp90. Numerous classes of Hsp90 inhibitors have recently been developed, such as the anasamysin geldanamycin and derivatives 17-AAG and 17-DMAG; the macrolide radicicol and derivatives; purine-scaffold derivatives; pyrazoles; and shepherdins that bind to the N-terminal high-affinity ATP-binding domain of Hsp90. Other inhibitors have recently been shown to bind to the C-terminal dimerization domain of Hsp90, such as cisplatin and novobiocin, or modify Hsp90 postranslationally, such as histone deacetylase or proteasome inhibitors. In this mini-review, we present hypothetical mechanisms for Hsp90 inhibitors in treating cancers, preliminary studies in early clinical trials, and potential tumor-killing and tumor-promoting activities of Hsp90 inhibitors.

Key Words: Hsp90, cancer.

1. INTRODUCTION

Genetically unstable cancer cells live under certain stresses, such as mutated and postranslationally-dysregulated signaling proteins, chromosome and microsatellite instability, hypoxia, and low nutrient concentrations. Cancer cells are able to survive in such stressed microenvironments by quickly selecting for adaptive mutations or activating alternative signaling pathways. The effectiveness of anti-cancer drugs that specifically target individual cancer-promoting proteins or signaling pathways may be gradually decreased, or even totally lost, due to the genetic and epigenetic variation of cancer cells, even within a tumor. This is a major concern in cancer therapy. One strategy to address this problem is to identify drug targets, such as Hsp90, that affect multiple signaling pathways or the basic machinery required for cancer cells to survive under stress.

The molecular chaperone heat shock protein 90 (Hsp90) has recently emerged as a promising target for anti-cancer drugs. Inhibition of Hsp90 functions affects multiple oncogenic substrates simultaneously and has shown obvious anti-cancer effects in vitro and in vivo. One of the Hsp90 inhibitors, 17-allylamino, 17-demethoxygeldanamycin (17-AAG), has recently finished several phase I clinical trials and entered phase II trials [1, 2]. In the phase I clinical trials, 17-AAG achieved satisfactory anti-cancer activities with well tolerated doses [3]. Many other Hsp90 inhibitors are under preclinical development.

2. Hsp90 AND CANCER

Hsp90 is one of the most abundant cellular chaperone proteins that play important roles in maintaining cellular homeostasis. It is a critical molecule for cancer development because it acts in regulating proteins involved in apoptosis and survival, buffering mutations and maintaining transformations [4, 5]. Hsp90 functions through numerous multicomponent complexes containing co-chaperones and various “client” proteins. The number of identified Hsp90 client proteins is over 100 and is still quickly increasing. An extensive list of Hsp90 client proteins can be found on the Web site of Dr. Didier Picard (http://www.picard.ch/downloads/Hsp90 facts.pdf). The client proteins include protein kinases, transcription factors, and other signaling proteins. Many of the client proteins, such as steroid receptors, epidermal growth factor receptor (EGF-R) family members, the MET oncogene, Raf-1 kinases, AKT kinase, BCR-ABL fusion proteins in leukemia, mutant p53, cyclin dependent kinase 4 (CDK4), hypoxia-inducible factor 1α (HIF1α), and matrix metalloproteinase 2 (MMP2) [6], are known to be involved in cancer signaling pathways. Two Hsp90 cofactors recently identified in yeast, Tah1 and Pih1, interact with DNA helicases, which are key factors in chromatin remodeling [7]. Thus Hsp90 affects the activity of multiple signaling pathways which are essential for cancer cells to survive and proliferate [8]. This unique and varied role of Hsp90 makes it an especially promising target for anti-cancer drugs.

3. Hsp90 INHIBITORS AS ANTI-CANCER DRUGS

Hsp90 is responsible for the correct folding, activation, assembly, or disassembly of the client proteins. It has three domains: the N-terminal ATP-binding domain, the middle domain, and the C-terminal dimerization domain. The C-terminal domain also has a second, low affinity ATP-binding
Hsp90 exists as a homodimer via its C-terminal region. The Hsp90 chaperone complex cycles between two major conformational states: the “open” state and the “closed” state [10]. In the “open” state, Hsp90 is nucleotide-free and able to capture client proteins. Co-chaperones, such as Hsp70, Hsp40, Hop and Hip, bind to Hsp90 and are important in helping to bind client proteins. Upon ATP loading, the Hsp90 complex switches to the “closed” state [10]. The “closed” or “tense” state is thought to have both C- and N-terminal domains associated, whereas, the “open” or “relaxed” state has only the C-terminal domains associate. It is thought that the ATP-bound structure of Hsp90 functions as a “clamp” to hold client proteins that are released upon ATP hydrolysis and opening of the clamp [11, 12].

Another group of co-chaperones, Cdc37, p23 and immunophilins, take the place of the original co-chaperones and help Hsp90 in the process of maturation of the client proteins and of maintaining client proteins in their active state. The Hsp90 complex associated with Hsp70 and Hop is thought to be the main proteasome-targeting form [13, 14] and the Cdc37 and p23 associated complex is another stable form that regulates the timing of mitosis [15, 16]. Hsp90 inhibitors disrupt the transition of the complexes towards the stable forms, and lead to enhanced proteosomal degradation of the “client” proteins [17]. The enhanced proteosomal degradation results in the simultaneous disruption of multiple oncogenic signaling pathways that are important for tumor survival and progression.

Based on the unique and therapeutically attractive effect of Hsp90 in regulating multiple tumor survival and progression pathways, extensive studies in developing Hsp90 inhibitors are being performed, and the number of inhibitors is rapidly increasing. Among them, 17-AAG is the first Hsp90 inhibitor to enter phase II clinical trials, and several others are in preclinical development. We will discuss the mechanisms and effectiveness of 17-AAG and several other major Hsp90 inhibitors in the following sections (Table 1).

3.1. Inhibitors that Bind to the Hsp90 N-Terminal Domain

a. Ansamycins

GA, a benzoquinone ansamycin antibiotic, is a natural product of the fungus *Streptomyces hygroscopicus*. GA binds to the N-terminal ATP binding domain of Hsp90 with high affinity and causes the destabilization and degradation of many Hsp90 client proteins [18]. Unfortunately, GA has significant hepatotoxicity in mammals [19], primarily due to its C-17 methoxy group. Fortunately, GA analogues containing a substitution on 17-alkylamino groups keep the excellent biological activity but have reduced hepatotoxicity. These analogues include: 17-AAG, 17-DMAG, and many more (Fig. 1). Structural analyses show that the N-terminal domain of Hsp90 functions as a “clamp” to hold client proteins that are released upon ATP hydrolysis and opening of the clamp [20].

17-AAG binds to Hsp90 with lower affinity than GA, but displays similar anti-cancer effects as GA and is less toxic [21, 22]. More important, elegant studies have shown that 17-AAG binds to Hsp90 in cancer cells with about 100-fold higher affinity than it does in normal cells [17]. The difference might come from the different states of Hsp90 in cancer cells and normal cells because the tumor Hsp90 is mostly in multi-protein complexes with high ATPase activity, probably due to the increased load of mutant client proteins [17]. However, Hsp90 from normal cells are mainly in an uncomplexed, latent state that has low ATPase activity [17]. This
specific inhibition of “oncogene addicted” tumor cells also minimizes the major concern that inhibiting such an important basic molecule might affect normal cell survival [17].

17-AAG is currently in phase I and phase II clinical trials in several centers worldwide [1, 2, 23]. Preliminary data obtained from these trials demonstrate that 17-AAG shows anti-cancer activity, such as stabilization of the disease, higher apoptosis, and less proliferation of the tumors, at concentrations below the maximum tolerated dose [24-31]. 17-AAG has been used in these trials to treat various solid tumors and leukemia. The reported down-regulated “client” proteins in animal xenograft models include Raf-1, CDK4, Erb-B2, Src, h-Tert, BCR-ABL, Akt, estrogen receptor and androgen receptor [32]. 17-AAG is metabolized to 17-amino-geldanamycin (17-AG) by cytochrome P450 3A5 (CYP3A5), androgen receptor [32]. 17-AAG is metabolized to 17-amino-geldanamycin (17-AG) by cytochrome P450 3A5 (CYP3A5), but 17-AG retains Hsp90 inhibitory activity [33, 34]. 17-AAG can also be metabolized by the quinone reductase NQO1 (DT-diaphorase) to a more potent Hsp90 inhibitor in cell culture [35]. A recent phase I study reported that human polymorphisms of CYP3A5 and NQO1 are not associated with 17-AAG toxicity [36].

There is increasing interest in combining 17-AAG with other cancer therapies, such as radiation therapy and conventional chemotherapy. Data shows that 17-AAG can sensitize cells to the induction of apoptosis by other therapies [37, 38]. Hawkins et al. (2005) have shown that when combined with 17-AAG, the inhibitory concentration 50% (IC50) of most of the common chemotherapeutic agents decreased [39]. For example, The IC50 of geldanamycin, the major Hsp90 inhibitor of the purine-scaffold series published to date, has been reported to be 10-50 times higher compared to that of GA. PU24FCl also shows higher affinity for tumor Hsp90 than for normal cell Hsp90 (700 to 3000-fold). However, oxidation of the sulfides to sulfoxides or sulfones, which can occur in vivo, leads to decreased activity of these compounds [50].

d. Pyrazoles (CCT018159)

3,4-diarylpyrazole CCT018159 is a pyrazole derivative identified by Workman and colleagues [51]. Crystal structure shows that CCT018159 binds yeast Hsp90 in the N-terminal domain with a similar binding mode as the ansamycins [52, 53]. The pyrazole scaffold is highly synthetically accessible and, therefore, a third group has designed and synthesized oxime derivatives to have considerable Hsp90 inhibitory effects and cytotoxicity [49].

Despite the promising anti-cancer properties, the main drawback of 17-AAG is its low solubility in water which makes it difficult to formulate for high absorption and bioavailability. A second-generation analogue of GA, 17-DMAG was developed. 17-DMAG shows similar in vivo and in vitro activity as 17-AAG in preclinical models. 17-DMAG is water soluble and orally bioavailable. It is widely distributed to tissues, but retains longer in tumors than in normal tissues. 17-DMAG is quantitatively metabolized much less than 17-AAG [33, 34, 42-45]. This inhibitor has now entered Phase I clinical trials.

b. Macrolide: Radicicol and its Analogues

Radicicol, a 14-member macrolide, is a natural product of the fungus Monosporium bonorden and is also able to bind the N-terminal ATP pocket of Hsp90 [22, 46, 47]. Among all of the known Hsp90 inhibitors, radicicol is the most potent. Radicicol is structurally unrelated to GA and the co-crystal structure of radicicol with Hsp90 shows that radicicol interacts with Hsp90 differently when compared with GA. Radicicol has similar cellular effects as the ansamycins in vitro, but is inactive in vivo, presumably due to metabolic instability. Efforts to modify this agent led to the discovery of radicicol analogues. The oxime-derivatives have potent activity both in vitro and in vivo with less liver toxicity than radicicol [4, 5]. Another group identified pochonin family members, such as pochonin D, a secondary metabolite of the fungus Pochonia chlamydospora, to be nearly as potent of an Hsp90 inhibitor as radicicol [48]. Radester, a hybrid that contains the quinone ring of GA and the resorcinol ring of radicicol (Fig. 1), was recently reported to have considerable Hsp90 inhibitory effects and cytotoxicity [49].

c. Purine-Scaffold Derivatives

PU3, PU24FCI, 8-arylsulfonyl adenine derivatives.

Choisis and colleagues designed and synthesized these small ATP analogues as Hsp90 inhibitors using purine as a scaffold [4, 5]. The first member, called PU-3 (Fig. 1), mimics the effects of ansamycins with modest potency. Another PU-compound, PU24FCI (Fig. 1), binds Hsp90 with a similar affinity as that of GA. PU24FCI also shows higher affinity (10-50 times higher) for tumor Hsp90 than for normal one. PU24FCI has broad anti-cancer activity at similar doses in cell lines, although it does not act preferentially in tumors over-expressing Her2 [4, 5]. Recently, the Chiosis group reported a series of new purine-scaffold compounds, 8-arylsulfonyl adenine derivatives, as new Hsp90 inhibitors [50]. The study identifies derivative 11v as the most potent Hsp90 inhibitor of the purine-scaffold series published to date (EC50 = 30 nM), and with highest selectivity for tumor vs normal cell Hsp90 (700 to 3000- fold). However, oxidation of the sulfides to sulfoxides or sulfones, which can occur in vivo, leads to decreased activity of these compounds [50].
ATP pocket [52]. *In vitro* evaluation of this agent indicates that it inhibits the growth of colon, ovarian and melanoma tumor cells. CCT018159 depletes Hsp90 client proteins in cancer cells and induces expression of Hsp70. New 3,4-diarylpyrazole class analogues have recently been reported [51, 52].

e. *Shepherdin*

Shepherdin is a cell-permeable peptide of Survivin K79-K90 [53]. It is specifically designed for blocking the interaction between Hsp90 and Survivin. Shepherdin makes extensive contacts with the Hsp90 N-terminal ATP pocket. It destabilizes Hsp90 “client” proteins, such as Survivin, Akt and CDK-6, and induces massive death of tumor cells by apoptotic and nonapoptotic mechanisms. Shepherdin kills tumor cells with more efficiency when compared with 17-AAG. Like 17-AAG, Shepherdin also has high tumor selectivity. Animal experiments showed that Shepherdin is well tolerated. According to the properties above, Shepherdin may serve as a new potent and selective anti-cancer agent [53].

3.3. Posttranslational Modification Inhibitors

a. *Cisplatin*

Cisplatin is a chemotherapeutic agent that interacts with the C-terminal domain of Hsp90 and interferes with nucleotide binding to this region [54]. Cisplatin treatment of neuroblastoma cells lead to the degradation of the androgen and glucocorticoid steroid receptors but does not affect other Hsp90 “client” proteins, such as Raf-1, Lck and c-Src [18]. Since inhibition of Hsp90 with GA-type inhibitors is thought to lead to nonspecific proteolysis of all of its client proteins [55, 56], the steroid-receptor-specific proteolysis induced by cisplatin suggests that cisplatin specifically inhibits steroid receptor-Hsp90 complexes and not complexes between Hsp90 and other client proteins. Cisplatin is thought to be cytotoxic mainly because it forms double-strand breaks in replicating DNA [57-59]. The results with steroid receptors show that cisplatin also functions independently of its DNA-mediated effects and, thus, suggest novel modes of action for this cytostatic drug [18].

b. *Novobiocin*

Novobiocin is a coumarin antibiotic isolated from *Streptomyces* species and has been clinically used as an anticancer drug for more than a decade. It was recently found to be an Hsp90 inhibitor [13, 14, 60, 61]. Novobiocin binds to the C-terminal ATP-binding pocket of Hsp90 and alters the conformation of Hsp90 [62]. The binding affinity of novobiocin to Hsp90 is poor and studies to synthesize higher-affinity novobiocin analogues are underway [63]. Yu et al. recently reported that a new synthetic novobiocin derivative, A4, has dramatically improved potency over the parent compound [64].

3.3. Posttranslational Modification Inhibitors

There are an increasing number of findings that indicate that posttranslational modification of Hsp90 is important for its function. Phosphorylation of Hsp90 leads to the release of the chaperone from the target protein, while geldanamycin (GA) blocks the whole process [8, 65]. Two recent studies showed that Hsp90 is a substrate of histone deacetylase 6 (HDAC 6) and the dynamic acetylation status of Hsp90 is an important regulatory mechanism for the chaperone activity [66]. Ubiquitination and S-nitrosylation of Hsp90 have also been demonstrated to inhibit its activity [67, 68]. Epigallocatechin gallate (EGCG), a compound in green tea that inhibits tumor growth, has been shown to inhibit the aryl hydrocarbon receptor (AhR) transcriptional activity through an indirect mechanism that involves binding to Hsp90 [69].

Studies using different cell lines indicated that HDAC inhibitors, such as FR901228 (FK228), LAQ824, LBH589, and trichostatin A (TSA), induce acetylation of Hsp90, resulting in inhibition of Hsp90 binding to ATP, disassociation of Hsp90 from the co-chaperone and loss of the chaperon activity [7, 66, 70, 71]. A recent study showed that application of LAQ824 depleted functional androgen receptor, which is important in the development of prostate cancer, via inactivation of Hsp90 [72]. However, their broad specificity is a major concern about using HDAC inhibitors to inhibit Hsp90. It is known that application of HDAC inhibitors can simultaneously acetylate other important cellular proteins, such as histones and tubulin.

4. SUMMARY AND DISCUSSION

The molecular chaperone Hsp90 is a promising target of anti-cancer drugs. Many of the Hsp90’s “client” proteins are involved in cancer signaling pathways or chromatin remodeling. Inhibition of Hsp90 simultaneously affects multiple oncogenic signaling pathways. Several Hsp90 inhibitors, such as 17-AAG, purine-scaffold derivatives and shepherdin, show very high selectivity for Hsp90 in tumor cells rather than in normal cells. 17-AAG has entered phase I/II clinical trials with very good effectiveness. Many other inhibitors are under preclinical development.

Recent studies indicate that combination of low dose Hsp90 inhibitors with conventional chemotherapeutic agents or radiation provides an effective strategy in cancer therapy. We would anticipate more exciting results from these combination studies. One concern in clinical application of Hsp90 inhibitors as anti-cancer drugs is that Hsp90 inhibitors may act as inducers of all heat shock proteins, including Hsp40 and Hsp70, via activation of heat shock factor 1 (HSF-1) [3, 73, 74]. Therapies that combine Hsp90 and Hsp70 inhibition, for instance, might be necessary to improve efficacy of either treatment alone [75]. Alternatively, drugs such as cisplatin inhibit steroid-receptor-Hsp90 complexes but do not induce the heat shock proteins [18].

Another potential concern is that Hsp90 inhibitors might be effective in killing end-stage tumors, but they might promote progression of early stage tumors. Two similar hypotheses have been made in considering this possible effect. We have proposed that geldanamycin “epigenetically destabilizes” cells and that this might promote the progression of benign tumors to aggressive tumors [76-78]. Similarly, Feinberg et al. (2006) have proposed an alternative to the classical “clonal genetic model” that they call the “epigenetic progenitor model” in which epigenetic has a critical role in early stages of neoplastic transformation [79]. The model of Feinberg et al. (2006) proposes that the first step in cancer forma-
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1. Introduction

The term “epigenetic disruption” of stem cells, and that this can occur by either cytosolic stress or inhibition of Hsp90 [79]. This epigenetic reprogramming of stem cells then gives rise to a pool of progenitor cells which are hyper-sensitive to mutations in oncogenes or tumor suppressor genes [79]. In other words, Feinberg et al. (2006) propose that mutations in oncogenes or tumor suppressor genes have little effect unless they occur in an “epigenetically sensitized” stem cell environment. If the “epigenetic progenitor model” for the origins of cancer is correct, this would be a paradigm shift in the understanding of cancer initiation and suggests that early cytosolic damage to early tumors might speed up the formation of malignant tumors in the future [79]. These potential disadvantages of Hsp90 inhibitors or other anti-cancer therapies that cytosolically stress cells should be considered in the future studies.

It is important to note that the hypothetical role of Hsp90 in “epigenetically destabilizing” cells of Feinberg et al. (2006) is currently unsupported by any experimental data [79]. The arguments that they used to support this hypothesis came from our studies reporting that Hsp90 inhibitors produce heritable phenotypic changes in Drosophila, and our studies focused on such epigenetic changes in offspring of flies fed the Hsp90 inhibitor [76, 78]. However, while no papers have shown epigenetic effects in adult flies fed Hsp90 inhibitors, several papers have shown that long-term exposure of adult flies to Hsp90 inhibitors are able to suppress the phenotypes of several models of human diseases, such as Huntington disease and Parkinson disease [80, 81]. Finally, our warning that Hsp90 inhibitors might promote cancer in the offspring of cancer patients also needs to be put in better context. It is well-accepted in oncology that cancer treatments of any type, including Hsp90 inhibitors, may be harmful to the fetus of a pregnant woman suffering from cancer.

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ABBREVIATIONS

GA = geldanamycin
17-AAG = 17-allylamino, 17-demethoxygeldanamycin
17-DMAG = 17-(Dimethylaminoethylamino)-17-demethoxygeldanamycin
17-AG = 17-aminogeldanamycin
IC50 = 50% inhibitory concentration

Table 1. Summary of Hsp90 Inhibitors

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<tr>
<th>Inhibitors</th>
<th>Hsp90 binding site</th>
<th>Tumor selectivity (fold)</th>
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| Ansamycins
  geldanamycin
  17-AAG
  17-DMAG                     | N-terminal         | ~100                      |
| Macrolide
  Radicicol
  Pochonin
  Radester                   | N-terminal         | 10-50                     |
| Purin-scaffold derivatives
  PU-3
  PU24FCI
  8-arylsulfanyl adenine derivatives (11v) | N-terminal         | 700-3000                  |
| Pyrazoles
  CCT018159                   | N-terminal         | high                      |
| Shepherdin                   | N-terminal         | high                      |
| Cisplatin                     | C-terminal         |                           |
| Novobiocin                    | C-terminal         |                           |
| HADC inhibitors
  FR901228 (FK228)
  LAQ824
  LBH589
  trichostatin (TSA)      |                   |                           |
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