Minireview

Altered Hsp90 function in cancer: A unique therapeutic opportunity

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Abstract

Molecular chaperones or so-called heat shock proteins serve as central integrators of protein homeostasis within cells. In performing this function, they guide the folding, intracellular disposition, and proteolytic turnover of many key regulators of cell growth, differentiation, and survival. Recent data show essential roles for the chaperones in facilitating malignant transformation at the molecular level and support the concept that their altered utilization during oncogenesis is critical to the development of human cancers. The field is evolving rapidly, but it has become apparent that chaperones can serve as biochemical buffers at the phenotypic level for the genetic instability that is characteristic of many human cancers. Chaperone proteins thus allow tumor cells to tolerate the mutation of multiple critical signaling molecules that would otherwise be lethal. Much of the recent progress in understanding the complex role of heat shock proteins in tumorigenesis has been made possible by the discovery of several natural product antitumor antibiotics that selectively inhibit the function of the chaperone Hsp90. These agents have been used as probes to define the biological functions of Hsp90 at the molecular level and to validate it as a novel target for anticancer drug action. One of these agents, 17-allylamino,17-demethoxygeldanamycin (NSC 330507) has begun phase II clinical trials, and several second-generation compounds are now in late preclinical development. The best way to use Hsp90 inhibitors as anticancer agents remains to be defined. Trials accomplished to date, however, serve as proof of principle that Hsp90 function can be modulated pharmacologically without undue toxicity in humans. Given the redundancy and complexity of the signaling pathway abnormalities present in most cancers, the ability of Hsp90 inhibitors to alter the activity of multiple aberrant signaling molecules instead of just one or two (as most current-generation molecular therapeutics have been designed to do) may prove of unique therapeutic benefit. [Mol Cancer Ther 2004;3(8):1021 – 30]

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Introduction

Shortly after exposure to a significant increase over basal temperature, the cells in most tissues dramatically increase the production of a restricted class of proteins collectively termed heat shock or stress proteins. Extensive work over the past 20 years has revealed that these ''heat shock'' proteins are actually constitutively expressed molecular chaperones that prevent ''illicit or promiscuous interactions'' between proteins and help guide their folding, intracellular disposition, and proteolytic turnover within the cell. Chaperones are critical to maintaining the normal protein folding environment, and their increased expression enhances cell survival in tissues damaged by a variety of stressors including heat, heavy metals, hypoxia, or acidosis. The latter conditions are common within tumors, and the increased expression of chaperone proteins showed in several types of solid tumors (1–6) may reflect the ability of malignant cells to maintain homeostasis in a hostile environment. In addition to facilitating cell survival in the face of stressful environmental challenges, chaperone proteins also allow tumor cells to tolerate alterations from within, including mutation of critical signaling molecules that would otherwise be lethal (7). Chaperones thus can serve as biochemical buffers for the genetic instability that is characteristic of many human cancers. As our understanding of the role of chaperone proteins in facilitating and maintaining the transformed phenotype increases, so too does interest in pharmacologic modulation of chaperone function (8). Several small molecule inhibitors of the molecular chaperone Hsp90 have been identified over the past decade that have the unusual ability to decrease the activity of multiple receptors, kinases, and transcription factors known to be involved in human cancers. After a brief summary of chaperone biology to provide context, this review will discuss emerging insights into the role of chaperones in tumorigenesis and focus on the current status of Hsp90 inhibitors and their development as cancer chemotherapeutics with a unique mechanism of action.

Chaperone Biology

Although the amino acid sequence of a protein ultimately dictates its native conformation, most polypeptides would fail to fold effectively in the highly concentrated, complex environment of the cell without the assistance of an ancient and highly conserved group of molecular chaperones

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also known as heat shock proteins. The chaperone concept was first set forth by Ellis in 1987, and since then, a very active field of research spanning the disciplines of biophysics, structural biology, molecular and cellular biology, and whole organism physiology has developed. Several detailed reviews are available addressing the molecular and cellular biology of the heat shock response (9, 10), heat shock proteins in general (11, 12) and Hsp90 in particular (13, 14). Excellent reviews of Hsp90 as an anticancer drug target have also been published very recently (15, 16). For those interested in molecular anticancer therapy, the key principles of chaperone biology with their most immediate implications for further drug discovery and development efforts can be summarized as follows:

1. Despite the name heat shock or stress protein, most chaperones are produced under basal conditions at substantial levels, For example, even under nonstressed conditions, Hsp90 comprises as much as 1% to 2% of total cellular protein.

Chaperones therefore are ubiquitously expressed in both normal and malignant cells. The basis for a therapeutic index is likely to lie in the altered functions chaperone proteins are required to perform in cancer cells, not simply their presence or absolute levels.

2. Complementation studies have shown that the functions of most chaperones are highly conserved across great phylogenetic distances.

Work in genetically tractable model organisms such as yeast, plants, and fruit flies can provide valuable insights into chaperone function relevant to human cancers. Likewise, the small molecule natural products targeting chaperone function that microorganisms produce to enhance their survival in the wild are likely to retain activity against human chaperones.

3. Chaperones rarely, if ever, act alone. Instead, they function as components of large multiprotein complexes containing cochaperones and accessory proteins.

By using drugs to alter the recruitment of specific accessory molecules to complexes, it seems possible to modulate the actions of the chaperone machinery without globally disrupting its function.

4. By definition, the substrates on which chaperones act (so-called client proteins) are not covalently modified by the chaperones themselves. Chaperone interactions with their substrates and cochaperones are typically transient and are frequently driven by multiple rounds of ATP hydrolysis.

Targeting the nucleotide binding pockets of chaperones with small molecules can provide at least one means of disrupting otherwise ''undrugable'' chaperone protein-protein interactions that occur at multiple contact points over extended distances.

5. Chaperones are required for essential housekeeping functions such as *de novo* protein folding during nascent chain synthesis, translocation of proteins across membranes, quality control in the endoplasmic reticulum, and normal protein turnover.

Complete ablation of the function of an essential chaperone is likely to be lethal to both normal and malignant cells. Consequently, development of extremely high affinity drugs may not lead to improvements in therapeutic index. In addition, because chaperones are involved in multiple essential functions, their mutation leading to target-related drug resistance is highly disfavored.

6. Chaperones also participate in many higher-order functions such as the post-translational regulation of signaling molecules, the assembly/disassembly of transcriptional complexes, and the processing of immunogenic peptides by the immune system.

The potential of chaperone inhibitors to disrupt multiple oncogenic clients simultaneously is a unique and therapeutically attractive feature. At the same time, the pleiotropic effects of targeting chaperones may make it very difficult to identify specific markers that can serve as predictive indicators of anticancer activity in patients.

Chaperones and Cancer Biology

The increased expression of one or more heat shock proteins over the level seen in normal tissues seems to be a common finding in many human cancers, both solid tumors (1–6) and hematologic malignancies (17, 18). Studies have been done most extensively in breast cancer, in which overexpression of Hsp70 and Hsp90 correlates with poor prognosis (19, 20). Overexpression of Hsp70 and Hsp27 may also contribute to drug resistance and a poor response to combination chemotherapy regimens (2, 21, 22).

At a physiologic level, the increased heat shock protein levels seen in advanced cancers could simply reflect an appropriate stress response to the hostile microenvironment characteristic of hypoxic, acidotic, nutrient-deprived tumors. At a more basic level, however, chaperone protein activities may actually permit tumor cells to escape apoptotic death that would normally result from the imbalanced signaling associated with neoplastic transformation (reviewed in ref. 7). Impairment of apoptotic signaling is a common characteristic of cancer cells, which facilitates their survival and expansion by rendering them independent of normal regulatory factors and resistant to both host defense mechanisms and chemotherapeutic drugs (23–25). Hsp70 and its cochaperones, especially the Bcl-2–associated athanogene family proteins, are well-recognized antiapoptotic factors. The mechanisms by which they exert their effects, however, are just beginning to be understood in detail. Recent work *in vitro* (26) and in whole cells (27) has shown that Hsp70 blocks the assembly of a multiprotein complex termed the apoptosome.

This complex is essential for activating the cascade of cysteine-aspartyl proteases known as caspases that are responsible for executing the apoptotic program. Consistent with these findings, enforced overexpression of Hsp70 in stably transfected cells provides protection from stress-induced apoptosis at the levels of both cytochrome *c* release and initiator caspase activation. Furthermore, it has been

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shown that the actual chaperoning function of Hsp70 is required for this protection (28). Conversely, antisense-mediated inhibition of Hsp70 expression has been shown to cause massive death in multiple breast cancer cell lines, whereas nontumorigenic breast epithelial cells are not affected (29). This finding has led to the suggestion that the antiapoptotic function of Hsp70 might provide a useful target for anticancer therapy, but no small molecule inhibitors of this function have yet been reported.

Hsp90 and its cochaperones are also reported to modulate tumor cell apoptosis. Much of this activity seems to be mediated through effects on AKT (30), tumor necrosis factor receptors (31), and nuclear factor-κB function (32). However, Hsp90 may also play a more global role in facilitating neoplastic transformation than just inhibiting apoptosis. Among the heat shock proteins, Hsp90 is unique because it is not required for the biogenesis of most polypeptides (33).

Instead, many of its cellular substrates or client proteins are conformationally labile signal transducers that play a critical role in growth control, cell survival, and tissue development (34). A sample of the more prominent Hsp90 client proteins with relevance to cancer are SRC family kinases (SRC, LCK, and FYN), receptor tyrosine kinases (HER2, EGFR, IGF1R, and FLT3), serine/threonine kinases (RAF-1, AKT, and CDK4), cell cycle G_2 checkpoint kinases (WEE1, MYT1, and POLO-1), mutant fusion kinases (BCR-ABL and NPM-ALK), steroid hormone receptors (glucocorticoid, androgen, estrogen, and progesterone), transcription factors (p53, HSF-1, and HIF-1), and telomerase (hTERT). The number of proteins known to interact with Hsp90, however, is expanding rapidly. A more comprehensive, frequently updated list of Hsp90 client proteins can be viewed on a Web site maintained by the laboratory of D. Picard (http://www.picard.ch/DP/downloads/Hsp90interactors.pdf). Post-translational interaction with these targets allows Hsp90 to link the cell to its environment and couple the stress response to integrated changes in signal transduction pathways (35) and transcriptional responses (35, 36).

Figure 1 provides a cartoon representation of how the dynamic, low-affinity interactions of Hsp90 with client proteins such as hormone receptors, transcription factors, and kinases serve to maintain them in a latent state that is capable of activation (37). On oncogenic mutation, however, many of these same client proteins display unusually stable associations with Hsp90-containing chaperone complexes. These associations impair the normal proteolytic turnover of these cancer-related molecules and seem to be essential for their transforming activity (38–41). Much effort has been directed at understanding the impact on tumor cell growth and survival, which results from targeting the function of Hsp90 in these aberrant complexes (see below). At one level, the ability of small molecule Hsp90 inhibitors to disrupt multiple oncogenic clients simultaneously is a unique and therapeutically attractive feature of these compounds. On the other hand, it becomes very difficult to predict which patients are likely to benefit from such drugs if response is dictated by the constellation of molecular genetic defects present in their particular tumor (42, 43).

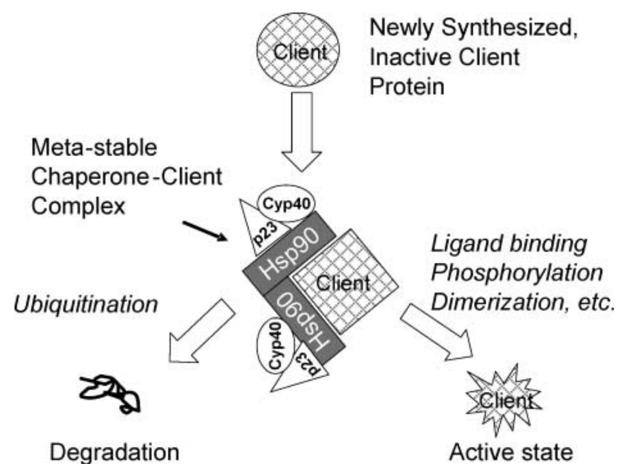

Figure 1. Simplified cartoon representation of the mechanisms by which heat shock proteins regulate the stability and function of their many client proteins. Newly synthesized, conformationally labile proteins associate with a Hsp90-containing multichaperone complex. The specific components of this complex seem to be client specific, but as indicated here, they can include other cochaperones such as p23 and the immunophilin Cyp40. Association with this complex maintains the client in a non-aggregated, metastable state, which allows it to be activated by specific stimuli such as ligand binding, phosphorylation, or association with other signaling molecules. In the absence of appropriate stimuli, the chaperone complex targets the client for degradation via the ubiquitin-proteasome pathway, thus regulating its steady-state level.

Chaperones and Cancer Evolution

By the time they are usually detected, most solid tumors display sufficient redundancy and heterogeneity to render them extremely robust to a wide range of perturbations, including those caused by conventional and molecularly targeted therapeutic agents (44). The total number of genomic alterations per typical colon carcinoma cell has been estimated at ~10,000, although only 5 to 10 specific genetic alterations seem to be necessary for the generation of the malignant phenotype (45, 46). This striking discrepancy means that clinical malignancies are not the end result of a pathway consisting of only a few discrete mutations. Instead, they are the consequence of cells with highly unstable genomes that accumulate numerous "nonpathway" mutations over years but evolve along certain conserved pathways in the face of intense selection pressure within the particular tissue of a host environment (47). From this perspective, the seemingly ordered sequence of events described by the classic Fearon-Vogelstein model of multistep carcinogenesis may actually result from the same pressures of natural selection that drive the ordered Darwinian macroevolution of species in nature.

In their role as molecular chaperones, it now seems that heat shock proteins, especially Hsp90, may play a pivotal but previously unrecognized role in evolutionary processes including those active in human cancers. Using *Drosophila* and *Arabidopsis* model systems, Lindquist et al. have recently provided compelling data that Hsp90 buffers inherent genetic variation within populations of organisms, thus canalizing (funneling into discrete, well-defined outcomes) their developmental processes and allowing the

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robust expression of a uniform phenotype under basal conditions (48, 49). Under stressful conditions, however, the client proteins of Hsp90 become more unstable, and there is an increased demand for Hsp90 to facilitate the refolding of denatured proteins. The cache of genetic variation exceeds the buffering capacity of Hsp90 to produce specific phenotypes. Previously hidden variation becomes available to be acted on by natural selection to enhance survival of the species (50). In an analogous fashion, Hsp90 may act as a biochemical buffer at the phenotypic level for the genetic heterogeneity characteristic of most cancers. When buffering capacity is exceeded as a result of normal aging, the higher load of mutant, misfolded oncoproteins, or the hostile tumor microenvironment (indeed, these all may occur in concert), canalization may break down. As a result, rapid evolution of the tumor to invasive, metastatic, and drug-resistant phenotypes may ensue (51). In addition to playing a role in cancer evolution as a biochemical buffer at the phenotypic level, Hsp90 may also contribute to tumor heterogeneity through direct effects on gene expression. It has now been shown that Hsp90 and its cochaperone p23 can regulate gene expression through effects on the disassembly of transcriptional regulatory complexes such as that involving the glucocorticoid receptor (37). Moreover, intriguing new data in *Drosophila* have shown that compromise of Hsp90 function can induce epigenetic alterations in gene expression and heritable alterations in chromatin state (52, 53).

In light of all the evidence accumulating in model systems, it would now seem that increased Hsp90 levels and the dramatically altered chaperone associations commonly seen in advanced solid tumors may represent a homeostatic response to their extensive genetic heterogeneity. This may explain their intriguing sensitivity to the pharmacologic inhibitors of Hsp90 that are currently in clinical development (54). Likewise, an evolutionary view of the cancer treatment problem suggests that definitive control is more likely to be achieved by altering the key determinants shaping the ability of a tumor to adapt and evolve such as Hsp90 rather than by targeting a single oncogenically activated but dispensable pathway within it. Whereas a critical evolutionary role for Hsp90 in cancer initiation and progression remains largely hypothetical at this time, work to provide rigorous scientific evidence for this concept is now being pursued by several groups.

Hsp90 Structure and Function

The very important initial discovery by Whitesell et al. (38) of geldanamycin as a selective inhibitor of Hsp90 has led to an explosive growth over the past decade in our molecular understanding of the complex biological functions of this chaperone. Detailed reviews of the extensive structural and biochemical studies that have been done since that time are available (55, 56). Only a brief overview is presented here. Hsp90 is essential in all eukaryotes, but knockout results in only a mildly thermosensitive phenotype in *Escherichia coli*. In vertebrates, two distinct genes encode constitutive and inducible isoforms of the protein (Hsp90β and Hsp90α, respectively), but functional differences between these isoforms are poorly understood. Homologues of Hsp90 are also found in the endoplasmic reticulum (Grp94) and mitochondrion (TRAP1). Recently, a Hsp90 variant (Hsp90N) has been identified that seems to be primarily membrane associated as a result of its unique hydrophobic NH_2-terminal domain. Limited information exists on its precise cellular functions (57).

Hsp90 resides primarily in the cytoplasm, where it exists predominantly as a homodimer. Each homodimer is made up of monomers consisting of three main functional domains that display important functional interactions. The NH_2-terminal domain contains an adenine nucleotide binding pocket of the GHKL superfamily that shares homology with bacterial gyrase B and MutL proteins (58). Structural alterations driven by the hydrolysis of ATP to ADP in this pocket are thought to play an essential role in the chaperoning activity of the protein. This pocket is also the binding site of the structurally unrelated natural products geldanamycin and radicicol as well as the growing number of semisynthetic derivatives and synthetic compounds summarized in Table 1. These drugs bind with higher affinity than nucleotides do, and they lock the domain in its ADP-bound conformation. These drugs thus alter many if not all of the normal functions of the chaperone (59). In particular, arresting Hsp90 in its ADP conformation seems to recruit E3 ubiquitin ligases such as CHIP to the many client proteins normally chaperoned by Hsp90-containing multiprotein complexes (60). This recruitment leads to enhanced proteasome-mediated degradation of the clients and depletion of their cellular levels. In eukaryotes, a flexible, highly charged linker sequence connects the NH_2-terminal domain to the ''middle region''

Table 1. Hsp90 inhibitors

Site of Action	Class	Members
NH_2-terminal ATPase	Benzoquinone	Geldanamycin
	Ansamycin	Herbimycin A
		Macbecins I and II
		17AAG
		17-(desmethoxy), 17-dimethylaminoethylamino geldanamycin
	Macrolide	Radicicol
		Monocillin I
		KF58333 (radicicol oxime derivative)
	Purine scaffold	PU3
		PU24FC1
	Pyrazole	CCT018159
COOH-terminal ATPase	Coumarin antibiotic	Novobiocin
	Cross-linker	Cisplatin
Other	Histone deacetylase inhibitor	Depsipeptide
		Suberoylanilide hydroxamic acid

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of Hsp90. The crystal structure of this middle region has very recently been determined, and it seems to play an important role in modulating ATP hydrolysis by interacting with the γ-phosphate of ATP molecules bound in the NH_2-terminal pocket of the chaperone (61, 62). In addition, it interacts with the recently discovered cochaperone Aha-1 to promote an association between the NH_2-terminal and the middle domains of Hsp90 that markedly accelerates its ATP hydrolysis rate (43, 61). Mutagenesis experiments also suggest that the middle region plays a key role in the binding of many client proteins to Hsp90. The far COOH-terminal end of this region has been implicated biochemically as the site of a possible second cryptic ATP binding site on Hsp90 that is revealed by nucleotide occupancy of its NH_2-terminal site (62). Structural data to corroborate these findings are not available. The contribution of this site to the overall regulation of chaperone function is not yet clear, but the antibiotic novobiocin has been reported to bind this site. Although it binds with poor affinity, novobiocin has been shown to destabilize Hsp90 client proteins at high concentrations (63, 64). The chemotherapeutic agent cisplatin has also been reported to adduct to Hsp90 at a site that overlaps the putative ATP-novobiocin binding site in this region and inhibit some of the activities of the chaperone (65, 66). Concentrations of cisplatin used in these studies were higher than those typically considered pharmacologically relevant. Whether cisplatin interaction with Hsp90 contributes to its potent anticancer activity is unknown at this time. A better understanding of the role of this site in regulating Hsp90 function and its potential as an anticancer drug target awaits further studies with a wider range of cisplatin concentrations as well as the identification of more potent site-specific inhibitors.

A second flexible linker connects the middle region of Hsp90 to a 12-kDa COOH-terminal domain that is responsible for its inherent dimerization. Removal of this domain drastically impairs the ATPase activity of Hsp90, emphasizing the role of highly cooperative intermolecular and intramolecular interactions in regulating the ATP utilization of the chaperone. In addition, this domain carries a conserved EEVD motif that is responsible for recruiting a variety of tetratricopeptide repeat domain–containing cochaperones such as the immunophilins Hop and PP5. These proteins are capable of modifying and increasing the specificity of Hsp90-containing complexes (67, 68). Whether targeting any of these interactions with small molecule drugs would be possible and/or therapeutically beneficial remains to be seen.

Hsp90 Inhibitors as Cancer Chemotherapeutics

Although Hsp90 function provides an attractive target conceptually for the treatment of cancer, the feasibility and efficacy of this approach has just begun to be explored in the clinic. A frequent criticism of targeting Hsp90 has been that drugs affecting such an essential chaperone will have prohibitive side effects due to impairment of normal cellular function. Based on phase I studies accomplished thus far, however, the Hsp90 inhibitor 17-allylamino,17-demethoxygeldanamycin (17AAG) can be administered to patients with tolerable toxicity, and alterations in Hsp90 client protein levels can be detected following drug therapy (69, 70). These promising observations emphasize that drug-mediated inhibition of the NH_2-terminal ATPase activity of Hsp90 is not functionally equivalent to constitutive genetic knockout, which is uniformly lethal in eukaryotes. Conditional genetic approaches to altering Hsp90 function that may be more relevant to drug effects have only been reported in yeast and flies. In these model organisms, impairment of chaperone function resulted in cell cycle arrest and male sterility, respectively, in a manner that was dependent on the extent of Hsp90 compromise (71, 72). Because it is very difficult to directly measure the extent of Hsp90 inhibition achieved in whole cells, it is quite possible that drug-mediated inhibition is sufficient to alter mutant client protein levels in tumor cells but is not lethal in normal cells until a more profound threshold for chaperone inhibition is exceeded. Such seems to be the case with inhibitors of the proteasome, another essential multiprotein complex that has recently been targeted for cancer therapy (73). Finally, very recent evidence suggests that Hsp90 in tumor cells has greater affinity for 17AAG than that found in normal cells. This difference may result from the bulk of Hsp90 being tied up in multiprotein complexes in tumor cells, whereas a substantial pool of free dimers with low ATPase activity and low drug affinity exists in normal cells (54, 74).

Both cytotoxic and cytostatic anticancer activities have been reported for Hsp90 inhibitors in cell culture and animal tumor models. Information regarding clinical responses in patients with cancer is limited, because the first in class Hsp90 inhibitor, 17AAG, has only been studied in the phase I setting thus far. To date, the only responses observed have been disease stabilization consistent with a cytostatic effect (16). Given the nature of Hsp90 function as described above, this is not surprising. The net effect of drug exposure on tumor cells in patients undoubtedly depends on their environment and the pattern of specific molecular genetic defects driving the particular tumor. At this point, the literature consists mostly of empirical observations describing the effects of destabilizing specific oncogenic Hsp90 client proteins in a variety of tumor cell types *in vitro*. The use of microarray expression profiling and proteomic approaches to investigate drug-induced changes that may predict sensitivity at a more global level is really just beginning (42, 43, 75).

Nevertheless, some molecular genetic and pharmacologic determinants of drug sensitivity with relevance to the clinical setting are beginning to emerge. In normal cycling cells and many cancer cell lines, Hsp90 inhibitors induce a predominant G_1 cell cycle arrest in a p53-independent manner (76). In certain cancer cell lines, however, a catastrophic pattern of mitotic arrest is induced that leads to loss of viability. In breast cancer cells, this pattern was observed only in RB-deficient cells (77), but we and others have found that such RB dependence may in fact be tumor

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type dependent[1] (78). It seems likely that the cell cycle effects of 17AAG and other inhibitors will depend on the constellation of checkpoint defects present rather than on the presence or absence of a single entity. Profound disruption of antiapoptotic signaling in tumor cells clearly occurs following exposure to Hsp90 inhibitors and enhances the proapoptotic effects of cytotoxic agents (79, 80). Whether such disruption is necessary and/or sufficient for the anticancer activity of Hsp90 inhibitors, however, remains less well defined. Indeed, it may depend on the specific cell type and perhaps its tissue environment. Because Hsp90 inhibitors destabilize multiple client proteins simultaneously, it has proven difficult to determine which ones are most important for drug activity. Most likely, some clients such as AKT will turn out to be relatively generic (80), whereas others such as BCR-ABL (81) and NPM-ALK (82) are obviously tumor specific. Steroid hormone receptors in both breast cancer (83) and prostate cancer (84) are also examples of tumor-specific clients that are disrupted by Hsp90 inhibitors and play an important role in the malignant behavior of these tumors. Destabilization of receptor tyrosine kinases as a class is an important mechanism of drug action in many tumor types that results in both antiproliferative and proapoptotic effects. As an example, depletion of amplified, overexpressed erbB2 in breast cancers correlates with exquisite sensitivity to 17AAG (85, 86).

In addition to its various client proteins, Hsp90 and its associated cochaperones may themselves be important determinants of drug sensitivity. A correlation between 17AAG cytotoxicity and basal levels of Hsp90 in colon cancer cells has been described. Moreover, failure to upregulate heat shock protein expression following drug exposure correlates with increased cytotoxicity in colon cancer cells (75) and in knockout fibroblasts lacking HSF-1, the major transcriptional regulator of the vertebrate heat shock response (87).

At a pharmacologic level, two potentially important determinants of 17AAG activity have been identified. The quinone reductase NQO1 (DT-diaphorase) has been shown to metabolize 17AAG to a more potent Hsp90 inhibitor in cell culture, but it is not clear whether polymorphic expression of this enzyme will affect 17AAG activity in patients because the major active metabolite of 17AAG *in vivo* is not potentiated by NQO1 (88). Hepatic metabolism of 17AAG via the cytochrome *P*450 isoform CYP3A4 leads to the rapid generation of 17-amino,17-demethoxygeldanamycin, which retains Hsp90 inhibitory activity (89). Polymorphisms for this enzyme in the general population are believed to be a major cause of variability in the metabolism of 17AAG seen in clinical trials thus far. Although implications for the activity and toxicity of 17AAG in patients are not yet clear, the use of known inducers of CYP3A4 has been prohibited in trials of 17AAG to date.

Future Directions and Conclusions

It is an exciting time in the development of Hsp90 inhibitors as cancer chemotherapeutics. Proof of principle that Hsp90 function can be modulated in patients has been established through recently completed phase I trials of 17AAG (16, 69). Several National Cancer Institute (NCI)–sponsored phase II trials of 17AAG are now beginning that will focus on specific malignancies in which Hsp90 clients are known to play an important role. These trials are summarized in Table 2.[2] In addition to the NCI, the company Kosan Biosciences is sponsoring a single-agent trial in multiple myeloma and a combination therapy trial of 17AAG and Herceptin. Two pediatric phase I trials that incorporate both leukemia and solid tumor strata have also begun. These trials incorporate novel, minimally invasive approaches to assessing the modulation of Hsp90 function in tumor-derived materials, which should provide critical insights into the value of specific client proteins in predicting anticancer activity in this patient population.

Another emerging area of interest involves the feasibility and efficacy of combining 17AAG treatment with radiation therapy, conventional cytotoxins, or new molecularly targeted agents. Preclinical data in both adult and pediatric cancer cell lines indicate that 17AAG can sensitize cells to the induction of programmed cell death by ionizing radiation (90, 91) and conventional chemotherapeutics (79). A summary of the current phase Ib trials under way to examine specific drug combinations is provided in Table 2. Of note, significant schedule dependence has been observed in some cell lines, especially when a Hsp90 inhibitor is combined with taxol (79). Given the prominent effects of 17AAG on cell cycle progression discussed above, schedule dependence for combination with cycle-specific agents is not surprising. Conflicting findings regarding synergy versus antagonism have been reported for the combination of 17AAG with cisplatin (92, 93). This may be due to differences in the cell lines and techniques used to perform the studies. Nevertheless, the combination of these two drugs remains quite intriguing in light of the evidence that cisplatin itself is a Hsp90 binding drug (see above). Occupancy of the NH_2-terminal ATP binding site of Hsp90 by geldanamycin clearly enhances the ability of cisplatin to bind the chaperone (62), but the extent to which this contributes to its anticancer activity is not known. Interesting interaction has also been reported for the combination of 17AAG with proteasome inhibitors such as bortezomib (PS-341). At a molecular level, the effect may result from increased protein misfolding induced by 17AAG coupled to impaired clearance of proteins by the ubiquitin proteasome pathway (94, 95). Lastly, synergistic anticancer activity has also been reported for Hsp90 inhibitors combined with histone deacetylase inhibitors (96). The precise mechanisms underlying this effect are still not clear, but it is intriguing that increased acetylation of Hsp90 has been showed following exposure of cells to

[1] R. Bagatell, J. Beliakoff, C. David, M. Marron, L. Whitesell. Hsp90 inhibitors deplete key antiapoptotic proteins in pediatric solid tumors and demonstrate synergistic anticancer activity with cisplatin. Int J Cancer. In press.

[2] P. Ivy, personal communication.

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histone deacetylase inhibitors, and hyperacetylation of the protein seems to inhibit its ATP binding and chaperone activities. Cellular consequences including client protein depletion are reminiscent of the effects of classic Hsp90 inhibitors such as 17AAG (97). The extent to which effects of histone deacetylase inhibitors on Hsp90 contribute to their anticancer activity or their ability to alter chromatin structure are just beginning to be explored. Given the unique properties of Hsp90 as a therapeutic target and the tolerable toxicity associated with 17AAG in phase I trials, considerable efforts are now being directed at developing new Hsp90 inhibitors with better pharmacologic and toxicity profiles. A variety of approaches are being pursued by academic laboratories as well as the pharmaceutical industry. These include structure-based screens targeting the Hsp90 nucleotide binding pocket (98), a colorimetric screen for inhibitors of Hsp90 ATPase activity (99), a stress response–guided natural product screen,[3] a forward chemical genetic approach (100), directed synthesis of various ansamycin (101, 102) and radicicol derivatives (103), and synthesis of modified purine scaffolds (104). This work is primarily at the stages of lead optimization and preclinical development, but next-generation compounds are expected to become available for clinical trial shortly. For example, the more potent, water-soluble geldanamycin analogue 17-(desmethoxy),17-dimethylaminoethylamino geldanamycin (NSC 707545) is now beginning NCI-sponsored phase I testing at four sites [NCI, University of Pittsburgh, Memorial Sloan-Kettering Cancer Center, and Royal Marsden (United Kingdom)]. An overview of the various classes of Hsp90-active agents currently under investigation is provided in Table 1.

In conclusion, advances in structural biology, medicinal chemistry, and high throughput technologies have made it possible to generate small molecule modulators of oncologically relevant molecular targets with increasing speed and efficiency. With a few notable exceptions such as early-stage chronic myeloid leukemia, however, it has proven quite difficult to exploit the power of these technologies for the development of clinically effective anticancer therapeutics. The key to the problem seems to lie in identifying appropriate targets for intervention. By the very manner in which they arise, human cancers display sufficient redundancy and heterogeneity to render them extremely robust to many perturbations including those caused by conventional and molecularly targeted drugs. Molecular chaperones, especially Hsp90, clearly play a major role in enabling this problematic robustness. As a result, they constitute a truly unique class of anticancer target. In addition, the apparent increased affinity of Hsp90 for inhibitors such as 17AAG in tumor cells as compared with normal cells makes this chaperone a particularly attractive target for anticancer drug development. Clinical trials of 17AAG that have been completed to date serve as proof of principle that the functions of Hsp90 can be modulated pharmacologically without undue toxicity in humans. The best ways to use Hsp90 inhibitors as anticancer agents, however, remain to be defined. Through the ongoing multidisciplinary efforts of basic scientists and clinicians, our understanding of this target and our ability to exploit it are continuing to evolve rapidly.

[3] L. Whitesell, unpublished data.

Table 2. NCI-sponsored clinical trials of 17AAG

Phase I Pediatric Single Agent Disease	Trial Site
Hematopoetic and solid tumors	POIETIC Consortium
Hematopoetic and solid tumors	COG Consortium
Phase II Adult Single Agent **Disease**	**Trial Site**
Melanoma	Royal Marsden (United Kingdom), Memorial Sloan-Kettering Cancer Center
Renal cell	Memorial Sloan-Kettering Cancer Center, NCI
Ovarian	Mayo Clinic
Thyroid	Mayo Clinic with Washington University
Breast	Wayne State University
Mastocytosis	NCI with Mayo Clinic
Prostate (hormone refractory)	Wayne State University
Phase Ib Adult Combination Regimens **Combination Agent**	**Trial Site**
Gemcitabine/*cis*-diamminedichloroplatinum	Mayo Clinic
Docetaxel	Memorial Sloan-Kettering Cancer Center
Paclitaxel	Pittsburgh University
Imatanib	Wayne State University
Rituximab	Ohio State University
AraC/Daunomycin	Mayo Clinic
Irinotecan	Memorial Sloan-Kettering Cancer Center

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