HSP90 AND THE CHAPERONING OF CANCER

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Abstract | Standing watch over the proteome, molecular chaperones are an ancient and evolutionarily conserved class of proteins that guide the normal folding, intracellular disposition and proteolytic turnover of many of the key regulators of cell growth, differentiation and survival. This essential guardian function is subverted during oncogenesis to allow malignant transformation and to facilitate rapid somatic evolution. Pharmacologically 'bribing' the essential guard duty of the chaperone HSP90 (heat-shock protein of 90 kDa) seems to offer a unique anticancer strategy of considerable promise.

CHAPERONE
A class of proteins that prevent improper associations and assist in the correct folding and maturation of other cellular proteins collectively termed clients and substrates. In most cases, chaperones do not participate in the final mature structures that their clients form.

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"Quis custodiet ipsos custodes?"

"Who will guard the guardians themselves?"

Attributed to the Roman poet and ethicist Juvenal, First century AD

Following exposure to environmental insults, the cells in most tissues dramatically increase the production of a small group of proteins that are collectively known as 'heat-shock' or stress proteins. Many groups over the past 30 years have shown that these heat-shock proteins (HSPs) and their close, constitutively-expressed relatives are actually molecular CHAPERONES that guard against illicit or promiscuous interactions between other proteins. Their basal levels facilitate normal protein folding and guard the proteome from the dangers of misfolding and aggregation[1]. Their increased expression in tissues that are subjected to various proteotoxic stressors (including heat, heavy metals, hypoxia and acidosis) is an adaptive response that enhances cell survival. Both functions are needed in tumours. So, the increased expression of chaperone proteins that is observed in many tumour types undoubtedly reflects the efforts of malignant cells to maintain homeostasis in a hostile environment. However, in addition to facilitating the survival of tumour cells within their stressful microenvironments, other evidence indicates that chaperone proteins also allow tumour cells to tolerate alterations from within. Mutations in crucial signalling molecules that would otherwise be lethal are not only tolerated but actually help to drive oncogenesis[2]. In this sense, at the phenotypic level, chaperones seem to serve as biochemical buffers for the numerous genetic lesions that are characteristic of most human cancers.

As our understanding of the roles that chaperone proteins have in initiating and maintaining transformed phenotypes increases, so too does our interest in the pharmacological modulation of chaperone function for the treatment of cancer and other diseases[3,4]. Over the past decade, several small-molecule drugs that target the molecular chaperone HSP90 have been identified as potential anticancer agents. These drugs have the unusual ability of disrupting the activity of numerous receptors, kinases and transcription factors that are known to be involved in oncogenesis. Here, we provide a brief summary of normal chaperone biology, and we describe emerging insights into the ways that the essential guardian roles of HSP90 and other chaperones are subverted in cancer. We then discuss how HSP90 functions at the molecular level and how its function can be targeted by drugs. We close with issues that relate to the clinical development of HSP90 inhibitors, their current status and their prospects as useful cancer chemotherapeutics.

Summary

- Most heat-shock proteins (HSPs) are constitutively expressed molecular chaperones that guide the normal folding, intracellular disposition and proteolytic turnover of many of the key regulators of cell growth and survival. Their levels of intracellular expression increase in response to protein-denaturing stressors, such as temperature change, as an evolutionarily conserved response to restore the normal protein-folding environment and to enhance cell survival.
- The essential chaperoning functions of HSPs are subverted during oncogenesis to make malignant transformation possible and to facilitate rapid somatic evolution.
- Functioning as biochemical buffers for the numerous genetic lesions that are present within tumours, chaperones, especially HSP90, allow mutant proteins to retain or even gain function while permitting cancer cells to tolerate the imbalanced signalling that such oncoproteins create.
- Highly specific inhibitors of HSP90 have been identified that redirect its chaperoning activity and decrease cellular levels of the many cancer-related client proteins that depend on it for their function.
- The use of HSP90 inhibitors has proven invaluable at a basic level in probing the complex cellular functions of this chaperone.
- The modulation of client protein levels has been demonstrated in animal models and cancer patients following systemically well-tolerated exposure to the HSP90 inhibitor 17AAG, which is the first in its class.
- Work is ongoing to identify and develop new HSP90 inhibitors with improved pharmacological properties.
- The best way to exploit the novel mechanism of action of HSP90 inhibitors for anticancer therapy remains to be defined, but probably involves combination with conventional cytotoxic drugs or other molecularly targeted agents.

Normal chaperone biology

Since the chance discovery of the heat-shock response by Ritossa in the 1960s, the study of chaperone expression, structure and function has become an active area of research, spanning the disciplines of biophysics, structural biology, molecular biology, cellular biology and whole-organism physiology. Several detailed reviews are available that address the molecular and cellular biology of the heat-shock response[5,6], HSPs in general[7,8], and HSP90 in particular[1,9]. Despite the name 'heat-shock' protein, most chaperones, or their close relatives, are ubiquitously expressed under normal conditions. For example, even under non-stressed conditions, HSP90 comprises as much as 1–2% of total cellular protein content, and this amount increases only about twofold under stress. Chaperones are required for essential housekeeping functions, such as *de novo* protein folding during nascent polypeptide-chain synthesis, translocation of proteins across membranes, quality control in the endoplasmic reticulum, and normal protein turnover[1]. Chaperones also participate in many higher-order functions, such as the post-translational regulation of signalling molecules, the assembly/disassembly of transcriptional complexes[10,11], and the processing of immunogenic peptides by the immune system[12,13]. The most important functions of molecular chaperones in mediating post-translational protein homeostasis are summarized schematically in FIG. 1.

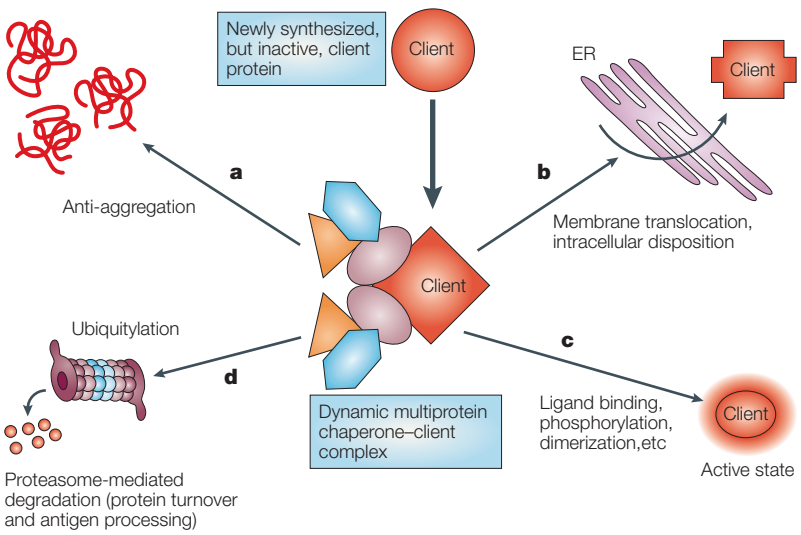

Figure 1 | **Participation of molecular chaperones in regulating many aspects of post-translational protein homeostasis.** Newly synthesized, conformationally labile client proteins associate with multi-protein complexes that contain various chaperones, co-chaperones and accessory molecules (different coloured shapes). The particular components of a complex vary according to the client and also help specify the function of a particular complex. Dynamic association of a client with chaperone complexes can prevent its aggregation (**a**) and assist in its intracellular trafficking, especially its translocation across membranous structures such as the endoplasmic reticulum (ER) (**b**). For many clients involved in signal transduction pathways, association with the chaperone machinery maintains the protein in a meta-stable state that allows it to be activated by specific stimuli such as ligand binding, phosphorylation or assembly into multisubunit signalling complexes (**c**). In the absence of appropriate stimuli, chaperone complexes can target the client for degradation through the ubiquitin–proteasome pathway, thereby regulating its steady-state cellular level (**d**).

Although they are relatively abundant, chaperones rarely, if ever, function alone. Instead, they typically function as components of larger machines that contain other chaperones, co-chaperones, modulators of ATPase activity and various accessory proteins. The most important components of the HSP90-based chaperone machinery are summarized in TABLE 1. In contrast to the kinases and other enzymes that are being targeted for the development of new anticancer drugs at present, chaperones do not covalently modify the substrates on which they act (their so-called 'client' proteins) to prevent/resolve aggregation or alter conformation. Instead, chaperones typically interact with their clients in a cyclical, iterative fashion that was first

Table 1 | **Important components of the HSP90 chaperone machinery**

Protein family	Classification	Function
HSP90	Chaperone	Supports meta-stable protein conformations, especially in signal transducers
HSP70	Chaperone	Helps fold nascent polypeptide chains; participates in assembly of multiprotein complexes
HSP40	Co-chaperone	Stimulates HSP70 ATPase activity
HIP, HOP	Adapters	Mediate interaction of HSP90 and HSP70
CDC37/p50	Co-chaperone	Modulates interactions with kinases
AHA1	Co-chaperone	Stimulates HSP90 ATPase activity
p23	Co-chaperone	Stabilizes HSP90 association with clients
Immunophilin	Prolylisomerase	Modulates interactions with hormone receptors

AHA1, activator of HSP90 ATPase homologue 1; CDC37, cell division cycle 37 homologue; HIP, HSP70-interacting protein; HOP, HSP70/HSP90-organizing protein; HSP, heat-shock protein.

described in detail for the steroid-hormone receptors by Smith and colleagues[14]. Such chaperone cycling is driven by multiple rounds of ATP hydrolysis (FIG. 2). As a result, targeting the nucleotide-binding pockets of chaperones with small molecules can provide a means of altering otherwise 'undruggable' chaperone–protein interactions that occur at multiple contact points over extended distances.

Among the chaperones, HSP90 is unique because it is not required for the biogenesis of most polypeptides[15]. Instead, many but not all of its cellular substrates or client proteins are conformationally labile signal transducers that have a crucial role in growth control, cell survival and developmental processes[16]. A sample of the many HSP90 client proteins that are known to be involved in cancer, and organized according to their contributions to the malignant phenotype, is provided in TABLE 2.

The number of proteins thought to interact with HSP90 is expanding rapidly. A recent study using global proteomic and genomic methods in yeast to map HSP90 interactions has identified an extended network consisting of 198 putative physical interactions and 451 putative genetic and chemical–genetic interactions. These were detected by screening a library of 4,700 viable-yeast gene mutants for hypersensitivity to GELDANAMYCIN, a small-molecule inhibitor of HSP90 function[17]. A well-annotated and frequently updated list of bona-fide HSP90 client proteins is maintained as a web page by the Picard laboratory (see Online links box). Post-translational interactions with its clients allows HSP90 to link the cell to its environment, and couple the stress response to integrated changes in signal-transduction pathways[18] and transcriptional responses[11,18]. Moreover, intriguing data from *Drosophila melanogaster* have shown that compromising the function of HSP90 can also induce epigenetic alterations in gene expression, as well as heritable alterations in chromatin state[19,20]. Consistent with these findings, HSP90 co-factors that interact with the DNA helicases RVB1 and RVB2 have now been identified in yeast. These helicases are key components of general chromatin-remodelling complexes, providing further evidence linking HSP90 to the epigenetic regulation of gene function[17].

As a consequence of its far-reaching effects on signal transduction and gene expression, it now seems that HSP90 has an important but previously unrecognized role in evolutionary processes. Compelling data have been reported from *D. melanogaster* and *Arabidopsis thaliana* model systems that HSP90 can conceal inherent genetic variation within populations of organisms[21,22]. As a consequence of its protein-chaperoning function, HSP90 allows polymorphic variants of crucial signalling pathways to accumulate while the pathway as a whole retains sufficient function to maintain wild-type phenotypes. This 'buffering' at the protein level by HSP90 funnels complex developmental processes into discrete, well-defined outcomes despite underlying genotypic variation, and it seems to be essential for the robust expression of uniform phenotypes under basal conditions[23]. Under stressful conditions, however, some of the unstable client proteins of HSP90 might become even more unstable. This situation creates an increased demand for HSP90 to facilitate the refolding of its usual client proteins as well as new, stress-destabilized clients. The cache of genetic variation in certain individuals can thereby exceed the buffering capacity of HSP90 and produce diverse, genotype-specific phenotypes[24]. In this way, previously hidden genetic variations become available for natural selection to enhance the survival of distinct genotypes within a population[25].

In an analogous fashion, we have proposed that, at the protein level, HSP90 might function as biochemical buffers of the extensive genetic heterogeneity that is characteristic of most cancers[24,26]. During cancer progression, however, CANALIZATION of the malignant phenotype might break down when the buffering capacity of HSP90 is exceeded in tumour cells as a result of normal ageing, the increasing load of mutant and/or misfolded oncoproteins, or the hostile tumour microenvironment — or indeed, all of these factors in concert. As a result, phenotypic diversity within the tumour cell population would increase and accelerate the evolution of invasive, metastatic and drug-resistant biologies[27]. Such an evolutionary view of the malignant progression problem suggests that definitive control of a cancer will probably be achieved most effectively by altering the key determinants that shape its ability to adapt and evolve. Consequently, HSP90 might provide a broader, more effective target for anti-cancer therapies than single, oncogenically activated but dispensable signalling pathways that are the focus of most current drug-discovery efforts. However, on a cautionary note, compromising HSP90 function in advanced malignancies might also reveal their underlying genotypic diversity and accelerate the process of malignant progression in highly unpredictable ways. Little or no data are available to address these issues at present, but work is underway to better define the role(s) of HSP90 in cancer evolution.

GELDANAMYCIN
Natural-product antibiotic of the benzoquinone ansamycin class that inhibits the chaperone activity of HSP90.

CANALIZATION
The funnelling of variation into discrete, well-defined outcomes, especially during developmental processes.

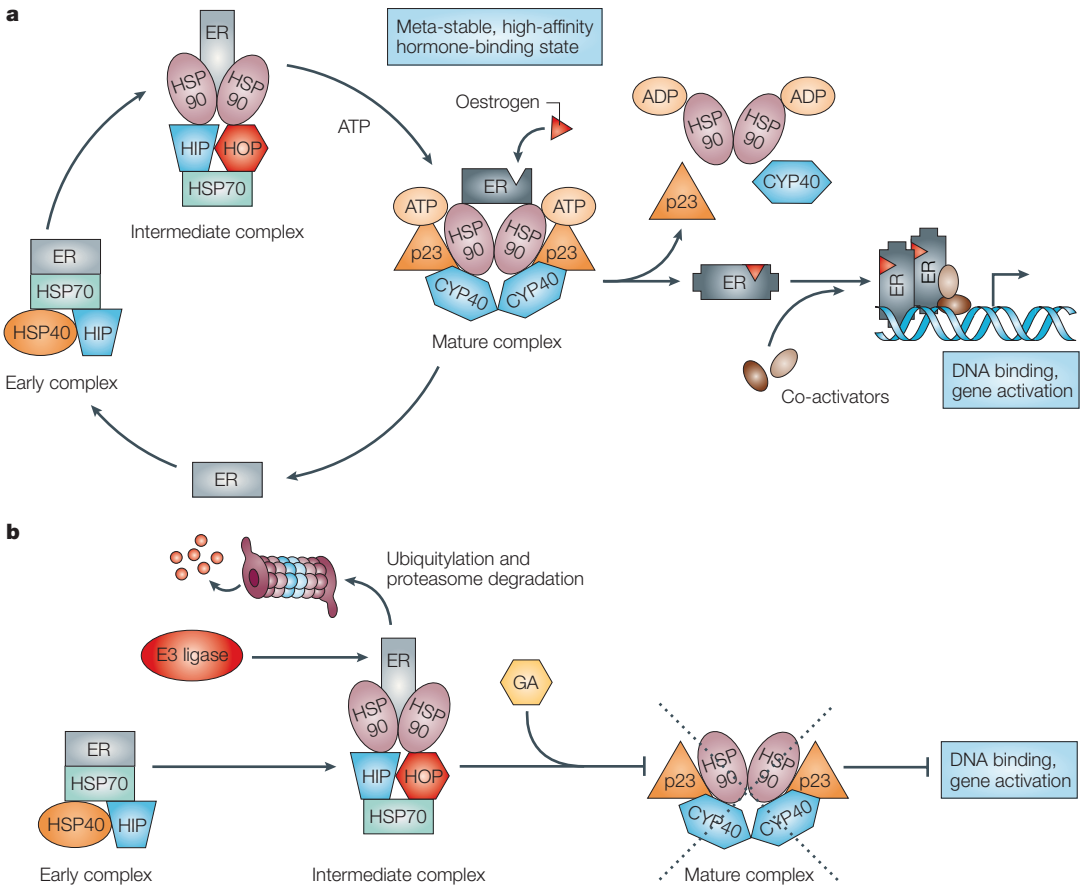

Figure 2 | **The role of chaperone cycling in regulating the function and turnover of client proteins such as the oestrogen receptor. a** | The newly synthesized oestrogen receptor (ER) associates with HSP70 (heat-shock protein of 70 kDa), HSP40 and the adapter HIP (HSP70-interacting protein) to form an early complex. The hydrophobic hormone-binding domain is partially exposed in this complex and HSP90 binds to this region in association with the adapter protein HOP (HSP70/HSP90-organizing protein) and displaces HSP40 to form an intermediate complex. In an ATP-dependent manner, HSP90 fully exposes the hormone-binding domain, and the co-chaperone p23 stabilizes the ATP-bound HSP90. Cyclophilin 40 (CYP40) fills the open tetratricopeptide repeat (TPR) acceptor site on HSP90 to complete a mature complex. In the absence of oestrogenic ligands, the ER is released from the mature complex to undergo additional cycles of chaperone interactions. Oestrogen binding, however, leads to a conformational change in the ER, which releases chaperone components and leads to tight binding of the receptor protein to oestrogen response elements and the recruitment of the co-activators needed to drive transcription. **b** | Geldanamycin (GA) binding to HSP90 locks the chaperone in an alternative conformation that prevents normal cycling and the formation of mature chaperone complexes. The ER accumulates in an intermediate complex that recruits E3 ubiquitin ligase and drives proteasome-mediated degradation of the protein, thereby dramatically lowering cellular levels of the receptor and disrupting its function.

Chaperone alterations in cancer

The increased expression of one or more HSPs above the level observed in normal tissues is a common feature of human cancers, both solid tumours[28–33] and haematological malignancies[34,35]. In breast cancer, in which the phenomenon has been examined most closely, overexpression of HSP70 and HSP90 correlates with poor prognosis[36,37]. Overexpression of HSP70 and HSP27 might also contribute to drug resistance and a poor response to combination-chemotherapy regimens[29,38,39]. Overall, the available data indicate that increased chaperone expression contributes to oncogenesis at several levels. At a physiological level, the increased abundance of HSPs in advanced cancers reflects an appropriate cytoprotective stress response to the hostile hypoxic, acidotic and nutrient-deprived microenvironment that is characteristic of tumours. At the molecular level, increased chaperone activities might allow tumour cells to cope with the imbalanced signalling that is associated with neoplastic transformation, and thereby escape the apoptotic death that would normally ensue (reviewed in REFS 2,40). Impairment of apoptotic signalling is a common characteristic of cancer cells. It facilitates their survival and expansion by rendering them independent of normal regulatory factors and resistant to both host defence mechanisms and chemotherapeutic drugs[41–43]. HSP70-family members are well-recognized anti-apoptotic factors. In addition, several HSP70 co-chaperones, especially members of the BAG (BCL2-binding athanogene) family, have been shown to inhibit apoptosis in their own right as well as through their modulation of HSP70 function[2].

Table 2 | **HSP90 clients and the malignant phenotype**

Phenotype	Clients	References
Uncontrolled proliferation	Receptor tyrosine kinases, serine/threonine kinases, steroid hormone receptors	110,142,143,108
Immortalization	Telomerase	144
Impaired apoptosis	AKT	49
Angiogenesis	HIF1α	145
Invasion/metastasis	MMP2	63

HIF1α, hypoxia-inducible factor 1α; HSP90, heat-shock protein of 90 kDa; MMP2, matrix metalloproteinase 2.

Inhibition of stress-induced apoptosis by HSP70 seems to require its intact chaperone activity[44], but the precise mechanisms by which it functions remain controversial[45]. Numerous studies *in vitro* and in whole cells have implicated HSP70 in various processes. These include the regulation of apoptotic signalling through the JNK–SAPK (JUN N-terminal kinase–stress-activated protein kinase) pathway, and caspase activation through effects on the assembly of the multiprotein apoptosome complex and its involvement in events downstream of caspase activation, such as binding and inhibition of AIF (apoptosis-inducing factor)[40]. Consistent with these diverse findings, enforced overexpression of HSP70 in stably transfected cells provides protection from stress-induced apoptosis[46]. Conversely, inhibiting the expression of certain HSP70 isoforms has been shown to selectively cause cell death in breast cancer cell lines, whereas non-tumorigenic breast epithelial cells are not affected[47,48]. It seems that the role of HSP70 in helping organisms balance their response to stressors and damage is subverted in tumour cells, allowing them to survive when they should otherwise die. This insight has led to the suggestion that the anti-apoptotic function of HSP70 might provide a useful target for anticancer therapy, but so far no small-molecule inhibitors of this function have been reported.

HSP90 and its co-chaperones also modulate tumour cell apoptosis. Much of this activity seems to be mediated through effects on AKT[49], tumour-necrosis factor (TNF)-receptors[50] and nuclear factor-κB (NF-κB) function[51]. However, HSP90 has a more complex role in facilitating neoplastic transformation than simply inhibiting apoptosis. The dynamic, low-affinity interactions of HSP90 with its client proteins — such as hormone receptors, transcription factors and kinases — maintain them in a latent but readily activated state[14] (FIG. 2). Oncogenic mutation of such clients, however, leads to higher requirements for HSP90 function, presumably because of an exaggerated conformational instability of the mutant.

The earliest and perhaps most dramatic example of this phenomenon is provided by the SRC tyrosine kinase. Most oncogenic SRC mutations involve truncation of the C-terminus of the protein, resulting in the deletion of its crucial regulatory domain. This domain normally undergoes an intramolecular interaction with an SH2 domain in the protein that stabilizes its structure and represses its kinase activity. Truncation leads to a constitutively active but conformationally unstable kinase[146]. Normal c-SRC requires only limited assistance from the HSP90 machinery for its maturation and function within cells[52]. By contrast, v-SRC mutants display unusually stable physical association with HSP90, which was noted soon after their discovery as the first oncogenes[53,54]. Using genetic and pharmacological approaches, this aberrant chaperone interaction was eventually shown to be essential for both acquisition and maintenance of the increased kinase activity that underlies the transforming activity of v-SRC[55,56].

Another example of altered chaperone use by mutant versus wild-type proteins is provided by the tumour-suppressor protein p53 (which is encoded by *TP53*), mutations of which are the most common molecular genetic defect found in human cancers. Most *TP53* mutations result in the expression of a protein with an altered conformation and impaired cell-cycle-checkpoint activity. As depicted in FIG. 3, wild-type p53 is a short-lived protein that undergoes transient interactions with elements of the HSP90 machinery that maintain it in an activation-competent state and regulate its degradation through the ubiquitin–proteasome system[57,58] (FIG. 3a). Presumably because of their aberrant conformations, most p53 mutants display extended interactions with the chaperone machinery that prevent their normal ubiquitylation and subsequent degradation. As a result, increased cellular levels of dysfunctional protein (a pathological hallmark of mutation) accumulate within the tumour cell[59–61] (FIG. 3b). Mutant p53 proteins that are bound to HSPs do not function as tumour suppressors. They might also interfere with the function of normal p53 by forming heterodimers (dominant-negative effect) or inappropriately transactivating other target genes (positive tumour-promoting effect).

As the only chaperone for which clinically relevant small-molecule inhibitors have been reported, the remainder of this review will focus on HSP90 and efforts to target it for anticancer therapy.

HSP90 structure and function

HSP90 is essential for the survival of all eukaryotes tested, whereas HSP90-knockout results in only a mild thermosensitive phenotype in *Escherichia coli*. In vertebrates, two distinct genes encode inducible and constitutively-expressed isoforms of the protein (HSP90α and HSP90β, respectively), but the functional differences between these isoforms are poorly understood[62]. Homologues of HSP90 are also found in the endoplasmic reticulum (glucose related protein 94, GRP94) and the mitochondria (TNF receptor-associated protein 1, TRAP1). Secretion of HSP90α into the extracellular matrix surrounding tumour cells has been reported and seems to assist in the activation of matrix metalloproteinase-2 as well as contributing to tumour cell invasiveness[63]. Recently, an HSP90 variant (HSP90N) has been identified that seems to be primarily membrane-associated as a result of its

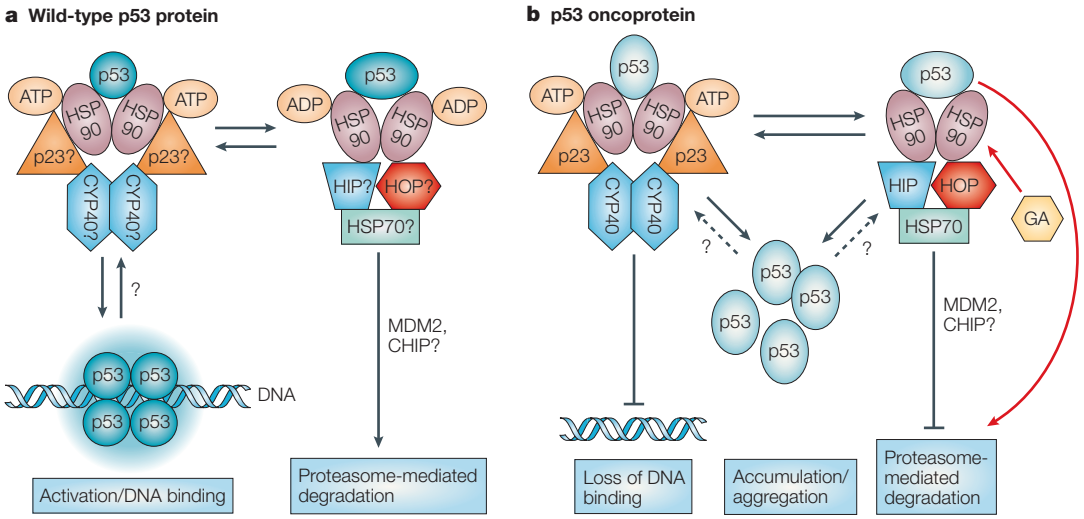

Figure 3 | **Depiction of chaperone interactions that modulate the function of p53 and how they are altered on mutation of this tumour suppressor. a** | Wild-type p53 is held in dynamic equilibrium by transient association with HSP90 (heat-shock protein of 90 kDa)-containing complexes (presumably containing p23 and CYP40 (cyclophilin 40)) that maintain it in a conformation that can be activated for DNA binding. In the absence of DNA damage, HSP90 (presumably in conjunction with its usual co-chaperones HIP (HSP70-interacting protein), HOP (HSP70/HSP90-organizing protein) and HSP70) presents p53 for degradation by recruiting ubiquitin ligases, such as MDM2 and CHIP (carboxy-terminus of HSP70-interacting protein), to the protein and stimulating its proteasome-mediated degradation. This maintains the low, steady-state level of wild-type p53 in normal cells. **b** | Most mutant p53 proteins are not able to achieve a conformation that is capable of DNA binding despite extended chaperone interactions. Presentation of p53 for ubiquitylation by MDM2, and possibly CHIP, is also impaired, which leads to the accumulation of aggregation-prone, dysfunctional protein. The binding of geldanamycin (GA) to HSP90 inhibits normal chaperone cycling and drives the degradation of p53 mutants, which leads to a decrease in their cellular levels. It remains unclear whether this decrease is sufficient to disrupt the dominant-negative and positive tumour-promoting activities of such mutants.

unique hydrophobic N-terminal domain. There is limited information on the precise cellular functions of this variant[64].

Detailed reviews of the many structural and biochemical studies that have been done on HSP90 are available[65,66]. Only a brief overview is presented here to provide the necessary background for understanding its inhibitors. HSP90 resides primarily in the cytoplasm, where it exists predominantly as a homodimer. Each homodimer is made up of monomers that consist of three main domains and that have important functional interactions (FIG. 4). Because of the intrinsic conformational flexibility of the intact protein, atomic resolution crystal structures have only been solved for its individual structural domains. The N-terminal[67,68] and middle domains[69] of eukaryotic HSP90 have been reported and structures for the N-terminal, middle and C-terminal domains of the bacterial HSP90 homologue htpG have recently been elucidated[70,71]. The N-terminal domain contains an unusual adenine-nucleotide-binding pocket known as the Bergerat fold[72]. This structural motif belongs to the GHKL (bacterial gyrase, HSP90, histidine kinase, MutL) superfamily but has no similarity to the ATP-binding domains found in other kinases or the chaperone HSP70. Extensive structural alterations driven by the hydrolysis of ATP to ADP in the Bergerat fold clearly have an essential role in the chaperoning activity of the HSP90 dimer, but conflicting models have been proposed as to the specifics of what these might be[69,73].

Recently, the structure of a fragment of the co-chaperone p50 (also known as CDC37, cell-division-cycle 37 homologue) complexed to the N-terminus of HSP90 has been reported[74]. This co-chaperone has an important role in recruiting kinases to the HSP90 machinery, and it has been proposed that its association with HSP90 might provide a target for the design of new HSP90 inhibitors with selectivity for kinases[75]. In eukaryotes, a flexible, highly charged linker sequence connects the N-terminal domain to the 'middle region' of HSP90. The structure of this middle region indicates that it has an important role in modulating ATP hydrolysis by interacting with the γ-phosphate of ATP molecules that are bound in the N-terminal pocket of the chaperone[69,76]. In addition, HSP90 interacts with the recently discovered co-chaperone AHA1 (activator of HSP90 ATPase homologue 1) to promote an association between the N-terminal and middle domains of HSP90 that markedly accelerates its ATP-hydrolysis rate[77,78]. Mutagenesis experiments also indicate that the middle region has a key role in the binding of many client proteins to HSP90. A second flexible linker connects the middle region of HSP90 to a 12 kDa C-terminal domain that is responsible for its inherent dimerization. Removal

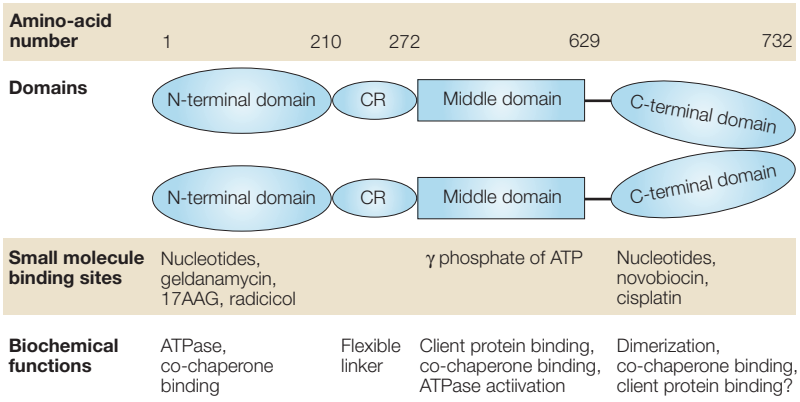

Figure 4 | **Structure of the HSP90 dimer.** The numbering 1–732 indicates the approximate positions in the amino acid sequence of the human protein that define its functional domains. 'CR' refers to a charged region which serves as a flexible linker between the N-terminal and middle domains. The locations where various small molecules bind HSP90 (heat-shock protein of 90 kDa) and modulate its function are indicated. The biochemical functions of each domain are also shown. 17AAG, 17-allylaminogeldanamycin; GA, geldanamycin.

of this domain drastically impairs the ATPase activity of HSP90, emphasizing the role of highly cooperative intermolecular and intramolecular interactions in regulating the use of ATP by the chaperone. This region has also been implicated biochemically as the site of a possible second, cryptic ATP-binding site on HSP90 that is shown by nucleotide occupancy of its N-terminal site[76,79]. These findings remain controversial as structural data to corroborate these findings are not available[73]. Finally, this domain carries a conserved EEVD motif that is responsible for recruiting various tetratricopeptide-containing repeats (TPR)-domain-containing co-chaperones such as the immunophilins, HSP70/HSP90-organizing protein (HOP) and protein phosphatase 5 (PP5). These proteins are important for modifying and increasing the specificity of HSP90-containing complexes[80,81].

Inhibiting HSP90 function

The N-terminal ATP-binding pocket of HSP90 is also the binding site of the structurally unrelated natural products geldanamycin and RADICICOL as well as a growing number of semi-synthetic derivatives and synthetic compounds (summarized in TABLE 3). These compounds bind with higher affinity than the natural nucleotides and prevent the chaperone from cycling between its ADP- and ATP-bound conformations. It has also been proposed that ISOMERIZATION of geldanamycin can be catalyzed by the HSP90-binding pocket itself and serves to stabilize drug binding[82]. Drug binding at the N-terminus of HSP90 has now been shown to alter many of its normal functions[83]. In particular, drug-bound HSP90 seems to recruit E3 ubiquitin ligases such as CHIP (carboxy-terminus of HSP70-interacting protein) to the many client proteins that are normally chaperoned by HSP90-containing multi-protein complexes[84]. This recruitment leads to increased proteasome-mediated degradation of the clients and depletion of their cellular levels. Drug-mediated inhibition of the N-terminal ATPase activity of HSP90, however, is not functionally equivalent to constitutive genetic knockout, which is uniformly lethal in eukaryotes. Conditional genetic approaches to altering HSP90 function that might be more relevant to drug effects in multicellular organisms have only been reported in *Saccharomyces cerevisiae*, *Caenorhabditis elegans* and *D. melanogaster*. In these model organisms, impairment of chaperone function resulted in cell-cycle arrest, DAUER DEVELOPMENTAL ARREST and male sterility, respectively, in a manner that was dependent on the extent of HSP90 compromise[85–87].

As it is difficult to directly measure the extent of HSP90 inhibition in whole cells, it is possible that drug-mediated inhibition is sufficient to alter mutant-client-protein levels in tumour cells, but is not lethal in normal cells until a threshold for chaperone inhibition is exceeded. This seems to be the case with inhibitors of the proteasome, another essential multiprotein complex that has recently been targeted for cancer therapy[88]. Finally, recent evidence indicates that HSP90 has an approximately 100-fold greater affinity for its inhibitors in cancer cells than in normal cells, leading to accumulation of the drug within tumours[89,90]. This difference might result from the bulk of HSP90 being engaged in multiprotein complexes in tumour cells, possibly because of their increased load of mutant client proteins. By contrast, in normal cells, there is a substantial pool of free HSP90 dimers that have low ATPase activity and low drug affinity[91,92].

The C-terminal end of the middle domain of HSP90 has been implicated biochemically as the site of a possible second, cryptic ATP-binding site on HSP90, which is shown by nucleotide occupancy of its N-terminal site, as mentioned above. The contribution of this site to the overall regulation of chaperone function is not clear, but the antibiotic novobiocin has been reported to bind this site and alter the conformation of the chaperone[93]. Although it binds with poor affinity, novobiocin destabilizes HSP90 client proteins at high concentrations[94,95]. The chemotherapeutic agent cisplatin has also been reported to bind to HSP90 at a site that overlaps the putative ATP/novobiocin binding site in this region and to inhibit some of its activities[96,97]. The concentrations of cisplatin used in these studies were higher than those typically considered as pharmacologically relevant. Whether interaction of cisplatin with HSP90 contributes to its potent anticancer activity is unknown at this time. A better understanding of the role of this putative HSP90 middle-domain site in regulating the function of the chaperone, as well as its potential as an anticancer drug target, requires further work. The identification of more potent site-specific inhibitors is also needed.

Issues in preclinical development

Both cytotoxic and cytostatic anticancer activities have been reported for HSP90 inhibitors in cell-culture models and animal tumour models. At this point, however, the literature consists mostly of empirical observations describing the short-term effects on growth/survival of destabilizing specific oncogenic HSP90 client proteins in various tumour cell types

RADICICOL
Natural-product antibiotic of the macrolide class that inhibits the chaperone activity of HSP90.

ISOMERIZATION
A change in the structural organization of a compound without altering its underlying chemical composition.

DAUER DEVELOPMENTAL ARREST
An alternate third stage of larval development in *C. elegans* that enhances survival of the organism under harsh conditions.

Table 3 | **HSP90-binding drugs**

Binding site	Chemical class	Selected examples
N-terminal ATP-binding pocket	Benzoquinone ansamycin	GA, 17AAG, 17DMAG
N-terminal ATP-binding pocket	Macrolide	Radicicol and related oxime derivatives, β-zearalenol
N-terminal ATP-binding pocket	Purine scaffold	PU24FC1
N-terminal ATP-binding pocket	Pyrazole	CCT018159
N-terminal ATP-binding pocket	Hybrid	Radamycin, GA dimer, GA-testosterone, GA-oestrogen
C-terminus	Noviosylcoumarin crosslinker	Novobiocin, coumermycin, cisplatin
Unknown	Histone deacetylase inhibitor	Depsipeptide, SAHA

17AAG, 17-(demethoxy). 17-allylamino geldanamycin; 17DMAG, 17-(demethoxy),17-dimethylaminoethylamino geldanamycin; GA, geldanamycin; HSP90, heat-shock protein of 90 kDa; SAHA, suberoylanilide hydroxamic acid.

in vitro and in mouse models. Little or no attention has been paid to the potential effects on tumour evolution and progression. In normal cycling cells and many cancer cell lines, HSP90 inhibitors induce a predominant G1 cell-cycle arrest in a p53-independent manner[98]. In certain cancer cell lines, however, a catastrophic pattern of mitotic arrest is induced that leads to loss of viability[99]. In breast cancer cells, this pattern was observed only in retinoblastoma protein (RB)-deficient cells[100], but we and others have found that such RB-dependence might, in fact, be tumour-type dependent[101,102]. It seems likely that the cell-cycle effects of HSP90 inhibitors will depend on the particular array of checkpoint defects that are present rather than on the presence or absence of a single molecule. Disruption of anti-apoptotic signalling in tumour cells occurs following exposure to HSP90 inhibitors and can enhance the pro-apoptotic effects of cytotoxic agents[103,104]. Whether such disruption is necessary and/or sufficient for the anticancer activity of HSP90 inhibitors, however, remains less well-defined.

The ability of HSP90 inhibitors to affect multiple oncogenic pathways simultaneously is a unique and therapeutically attractive feature of these compounds. However, the possibility exists that inhibiting HSP90 buffering activity at certain stages of malignant progression, although deleterious to most cells in a tumour, might also reveal mutations that enhance the survival and malignant progression of some cells within the population. Another problem in developing HSP90 inhibitors has been predicting which patients are likely to benefit from anti-HSP90 therapy since the response(s) might be dictated by the constellation of molecular genetic defects that are present in a particular tumour[78,105]. It is likely that some HSP90 clients, such as AKT, will turn out to be relatively generic[104], whereas others, such as BCR–ABL[106], which characterizes chronic myeloid leukaemia, and nucleophosmin–ALK (anaplastic lymphoma kinase)[107], which is found in lymphomas, are obviously tumour specific. Steroid-hormone receptors in breast cancers[108] and prostate cancers[109] are also examples of tumour-specific clients that are disrupted by HSP90 inhibitors, and they have an important role in the malignant behaviour of these tumours. Destabilization of receptor tyrosine kinases as a class is an important mechanism of HSP90-inhibitor action in many tumour types, which results in both anti-proliferative and pro-apoptotic effects. As an example, drug-induced depletion of the overexpressed growth factor receptor ERBB2 in certain breast cancer lines correlates with sensitivity to 17-allylamino-17-demethoxygeldanamycin (17AAG), a geldanamycin derivative that retains activity *in vivo* and the first HSP90 inhibitor to undergo clinical testing[110,111].

In addition to its various client proteins, HSP90 itself and the cellular stress response seem to be important determinants of drug sensitivity. It has been shown that HSP90 inhibitors alter the multi-chaperone complexes associated with heat-shock factor 1 (HSF1), the dominant transcription factor controlling induction of the stress response, thereby stimulating HSF1-activated heat-shock gene expression. Induction of this heat-shock response provides a measure of protection to non-transformed cells from the toxicity of HSP90 inhibitors[112], whereas tumours that fail to upregulate HSP levels seem to be particularly sensitive to HSP90 inhibition[113,114]. Induction of the stress response, as measured by increased levels of HSP72 in peripheral blood lymphocytes, is currently being used as a sensitive pharmacodynamic endpoint to confirm drug-induced target-modulation in patients. Given the cytoprotective effect of the stress response, however, it might prove important to administer HSP90 inhibitors in a pulsed fashion with sufficient time between doses to allow drug-stimulated stress responses to resolve and to avoid the induction of a tolerant, relatively drug-resistant state in the tumour. Finally, deliberate manipulation of the stress response using HSP90 inhibitors is beginning to be explored in models of non-oncological diseases in which chaperones play an important part. These include hypoxic-ischaemic injury[115] and protein aggregation disorders such as Huntington disease and Parkinson disease[116,117].

Clinical evaluation

Although modulation of HSP90 function provides a mechanistically attractive target for the treatment of cancer, the feasibility and efficacy of this approach has just begun to be explored in the clinic. In whole-animal pharmacological studies, two potentially important modulators of 17AAG activity have been identified. Hepatic metabolism of 17AAG through cytochrome P450 (CYP450) enzymes leads to the rapid generation of 17-amino, 17-demethoxygeldanamycin (17AG), which retains HSP90 inhibitory activity[118]. To minimize variability, patient use of known inducers of the CYP450 system has been prohibited in trials of 17AAG. A recently reported phase I trial of 17AAG found that polymorphisms of *CYP3A5* were associated

with altered drug clearance but not overall toxicity. The quinone reductase NQO1 (DT-diaphorase) has been shown to metabolize 17AAG to a more potent HSP90 inhibitor in cell culture[119]. However, polymorphisms in *NQO1* did not affect 17AAG pharmacokinetics or systemic toxicity in the small number of patients reported so far[120].

A frequent criticism of targeting HSP90 has been that drugs affecting such an essential chaperone will have prohibitive side-effects because of the impairment of normal cellular function(s). Similar concerns were raised during the development of proteasome inhibitors that, nevertheless, have demonstrated useful anticancer activity, especially against multiple myeloma[121]. Based on phase I studies, it is clear that 17AAG can be given to patients with acceptable levels of toxicity, and that pharmacodynamic alterations in HSP90 client protein levels can be induced following drug administration[120,122,123]. Interestingly, myelosuppression was not a dose-limiting toxicity in these trials, which supports the feasibility of combining HSP90 inhibitors with conventional cytotoxic agents in the future. As 17AAG is the first inhibitor in its class, information regarding clinical responses in patients with cancer is limited because studies of this drug have only completed phase I testing so far. To date, the only activity reported has been prolonged disease stabilization, most notably in malignant melanoma, consistent with a cytostatic drug effect[123].

Future directions

The clinical development of HSP90 inhibitors as cancer chemotherapeutics is progressing rapidly. Now that proof-of-principle has been established by several phase I trials, National Cancer Institute (NCI)-sponsored phase II trials of 17AAG are beginning. These will focus on specific malignancies in which certain HSP90 clients are known to have an important role, such as ERBB2-overexpressing breast cancer. The NCI is also sponsoring two paediatric phase I trials that incorporate both leukaemias and solid tumours. These trials incorporate novel pharmacodynamic endpoints to assess the modulation of HSP90 function in tumours, and should provide insights into the value of specific client proteins in predicting anticancer activity. In addition, Kosan BioSciences are sponsoring a single agent trial of 17AAG in multiple myeloma, as well as a combination therapy trial of 17AAG with trastuzumab (Herceptin).

There is now interest in the feasibility and efficacy of combining 17AAG treatment with radiation therapy, conventional cytotoxic agents or new molecularly targeted agents. Preclinical data from both adult and paediatric cancer cell lines indicate that 17AAG can sensitize cells to the induction of programmed cell death by ionizing radiation[124,125] and by conventional chemotherapeutics[103]. Several phase Ib trials are planned, or are underway, to examine combination therapy with 17AAG and conventional agents such as gemcitabine, cytarabine, cisplatin and taxanes. However, significant dependence on the sequence of drug administration has been observed in some cell lines, especially when a HSP90 inhibitor is combined with taxanes[103]. Given the prominent effects of 17AAG on cell-cycle progression, as discussed above, treatment-schedule dependence for combination with cell-cycle-specific agents is not surprising. Conflicting findings regarding synergy versus antagonism have been reported for the combination of 17AAG and cisplatin[126,127]. This might be due to differences in the cell lines and techniques that are used to carry out the studies. Nevertheless, the combination of these two drugs remains intriguing in light of the evidence that cisplatin itself is an HSP90-binding drug (see above). Occupancy of the N-terminal ATP-binding site of HSP90 by geldanamycin clearly enhances the ability of cisplatin to bind the chaperone, but the extent to which this contributes to its anticancer activity is not known[76,102].

Enhancement of 17AAG activity has also been reported for proteasome inhibitors such as bortezomib. This effect might result from the increase in protein misfolding that is induced by 17AAG, coupled to the impaired clearance of proteins by the ubiquitin–proteasome pathway[128,129]. Synergistic anticancer activity has also been reported for HSP90 inhibitors combined with histone deacetylase (HDAC) inhibitors, which are compounds that alter the acetylation of many proteins in addition to histones[130]. The precise mechanisms underlying this effect are still not clear, but it is intriguing that increased acetylation of HSP90 has been demonstrated following exposure of cells to HDAC inhibitors, and hyperacetylation of the protein seems to inhibit its ATP-binding and chaperone activities. Cellular consequences, including client-protein depletion, are similar to the effects of classic HSP90 inhibitors such as 17AAG[131]. The extent to which the effect of HDAC inhibitors on HSP90 contributes towards 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 levels of toxicity that are associated with target modulation in phase I trials, considerable efforts are now being directed at developing new HSP90 inhibitors with better pharmacological and toxicity profiles. Various approaches are being pursued by academic laboratories as well as the pharmaceutical industry. These include the structure-based design of purines that target the HSP90 nucleotide-binding pocket[132,133], a high-throughput colorimetric screen for inhibitors of HSP90 ATPase activity[134], a stress-response-guided natural-product screen[135], and a FORWARD CHEMICAL-GENETIC APPROACH[136] as well as chemical and biosynthetic approaches to generate geldanamycin[137–139] and radicicol derivatives[140]. Much of this work has been summarized in a detailed review by Dymock *et al.*[3] Whereas new agents are primarily at the stages of lead optimization and preclinical development, next-generation compounds are expected to become available for clinical trial shortly. For example, the more potent, water soluble geldanamycin analogue 17-(demethoxy),17-dimethylaminoethylamino geldanamycin (17DMAG) is now beginning NCI-sponsored phase I testing[141].

FORWARD CHEMICAL-GENETIC APPROACH
The use of small-molecule chemical libraries to generate phenotypes of interest in a manner analogous to mutagenesis in classical genetic screens. Any active compounds found can be used to identify the proteins involved in regulating biological processes, to provide molecular insights into how specific proteins perform their biological functions and to identify lead compounds for the development of useful therapeutics.

Conclusions

Since their initial discovery about a decade ago, tremendous progress has been made in developing HSP90 inhibitors as anticancer agents. These compounds have also proven useful in defining the complex role of HSP90 in diverse biological processes. In the realm of cancer therapy, however, two important challenges remain. The first is to improve the poor pharmacological properties of current agents while retaining target specificity. Although this problem is far from trivial, the diversity of approaches being pursued and the progress achieved so far make success in this area seem highly likely. The second, more profound challenge is to define the appropriate way in which to deploy HSP90 inhibitors in the treatment of cancer. Except in rare specific circumstances, it is unlikely that HSP90 inhibitors will prove useful as primary cytoreductive agents in the setting of bulk disease. Rather, their known biological activities and early clinical results indicate that they will be more useful as modulators of the response of a cancer to other therapeutic interventions. They might also be useful as modulators of the intrinsic ability of the tumour to evolve, which at present is an important barrier to curative therapy. Given its crucial role in 'guarding' the proteome, it is safe to say that HSP90 as a therapeutic target in cancer is now here to stay.

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Competing interests statement
The authors declare no competing financial interests.

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