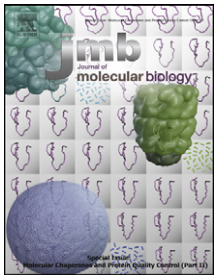
Review



The Chemical Biology of Molecular Chaperones—Implications for Modulation of Proteostasis

Kristoffer R. Brandvold and Richard I. Morimoto

Department of Molecular Biosciences, *Rice Institute for Biomedical Research, Northwestern University, Evanston, IL 60208, USA*

Correspondence to Richard I. Morimoto: *Department of Molecular Bioscience, Rice Institute for Biomedical Research, Northwestern University, 2205 Tech Drive, Hogan 2-100, Evanston, IL 60208, USA. r-morimoto@northwestern.edu*
http://dx.doi.org/10.1016/j.jmb.2015.05.010
Edited by J. Buchner

Abstract

Protein homeostasis (proteostasis) is inextricably tied to cellular health and organismal lifespan. Aging, exposure to physiological and environmental stress, and expression of mutant and metastable proteins can cause an imbalance in the protein-folding landscape, which results in the formation of non-native protein aggregates that challenge the capacity of the proteostasis network (PN), increasing the risk for diseases associated with misfolding, aggregation, and aberrant regulation of cell stress responses. Molecular chaperones have central roles in each of the arms of the PN (protein synthesis, folding, disaggregation, and degradation), leading to the proposal that modulation of chaperone function could have therapeutic benefits for the large and growing family of diseases of protein conformation including neurodegeneration, metabolic diseases, and cancer. In this review, we will discuss the current strategies used to tune the PN through targeting molecular chaperones and assess the potential of the chemical biology of proteostasis.

Introduction

All free-living prokaryotes and eukaryotes rely on the proteostasis network (PN), a conserved cellular machinery to support all aspects of protein biogenesis, to adapt to a complex and changing environment, and to determine organismal lifespan. Quality control processes are central for biological fidelity and, together with cell stress responses that detect and react to acute and chronic imbalances, are essential for evolutionary robustness. For proteins, this is exemplified by proteostasis, the process that integrates signals and regulates flux from synthesis through folding, transport, and clearance, which altogether ensures cellular, tissue, and organismal health [1].

At the core of the PN are the molecular chaperones that detect fluctuations in protein conformation and can determine the functional properties of client substrates. Many molecular chaperones were discovered as heat shock proteins (HSPs) whose expression is induced by transient exposure to acute elevated temperatures and other forms of environmental and physiological stress [2]. HSPs were initially subdivided into six groups based upon their molecular weight: sHSP, HSP40, HSP60 (chaperonins), HSP70, HSP90, and HSP100, although an updated classification based upon function has been recently proposed [3]. In addition to the role of molecular chaperones in macromolecular assembly and disassembly of proteins, interactions of chaperones with clients can confer alternative conformational intermediates that influence function.

An intrinsic feature of protein folding is metastability and the sampling of alternative conformational states. This freedom to sample structural conformations, therefore, allows for the probability that non-functional and potentially toxic folds are accessed during aging and disease. Adjusting the cellular balance of chaperones to drive folding equilibrium toward functional conformers or to promote degradation of non-functional states compensates for perturbations in the protein-folding landscape. As modern medicine identifies an increasing number of diseases that result from the aberrant expression of metastable proteins, strategies that modulate the PN could have broad beneficial consequences. Furthermore, the composition of, and need for, PN components varies among

tissues and changes over time, which infers opportunities to tune and re-tune the PN to achieve tissue-specific effects.

The concept that molecular chaperones could be targeted for therapeutic benefit was initially met with concern because of their evolutionary conservation, ubiquitous expression, high cellular abundance, and central roles in all aspects of protein biogenesis. However, the therapeutic value of shifting the protein-folding landscape through pharmacological effects on molecular chaperones is highlighted by the recent development of small molecule proteostasis regulators for neurodegeneration diseases and cancer. Much emphasis has been placed upon finding small molecules that regulate the expression of multiple molecular chaperones simultaneously or by directly influencing the activity of isolated chaperone proteins.

From a rational ligand-design perspective (Fig. 1), current approaches to directly target molecular chaperones with small molecules largely fall into three classes: (1) ATP-competitive inhibition, (2) modulation of substrate binding, and (3) modulation of co-chaperone interactions. Small molecule binding of the ATP pocket disrupts the hydrolysis event that allosterically regulates the conformation of the substrate-binding domain in chaperones such as HSP70, HSP60, and HSP90 (Fig. 2). Conversely, substrate binding and co-chaperone interactions must be modulated through small molecule targeting of protein–protein interactions. Fewer small molecule modulators have been identified from these latter approaches due to inherent difficulties of targeting sites that are typically flat and solvent exposed, but targeting proteins via such sites is becoming increasingly feasible [4,5]. Although modulating chaperone activity through targeting co-chaperone binding offers the opportunity to selectively enhance or inhibit chaperone function, an important consideration is that this may have unintended consequences on the folding landscape of other chaperone clients.

Finally, the development of novel chemical tools to modulate the protein-folding landscape will help unravel the cellular roles of various PN components. This represents an essential step toward efficacious pharmacological modulation of the PN. In this review, we draw from the recent literature to highlight small molecules with “drug-like” properties that are reported to affect specific chaperone targets in human and other relevant model systems, with a focus on the HSP90, HSP70, and HSP60 machines. We present examples of available chemical strategies, followed by discussion of how these probes shape the current outlook on the pharmacology of molecular chaperones, and highlight, where appropriate, under-developed areas. Other strategies for pharmacological modulation of proteostasis, such as targeting stress-inducible transcription factors

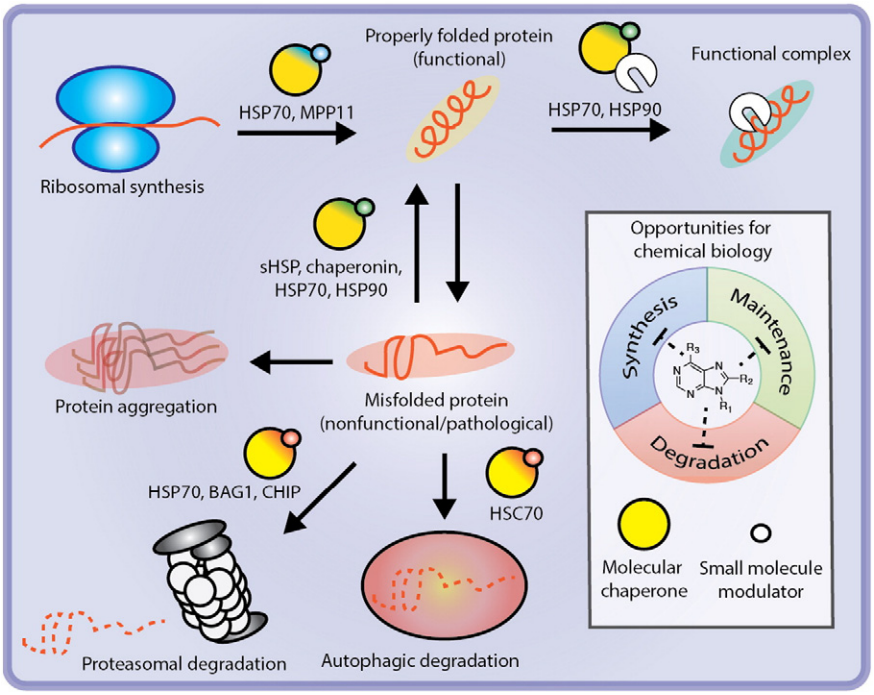


Fig. 1. Molecular chaperones offer a multidimensional approach to pharmacological modulation of the PN. Representative molecular chaperones that respectively support each of these components are illustrated. Molecular chaperones are generically represented as yellow circles. The smaller blue, green, or red circles represent a pharmacological reagent that impacts synthesis, maintenance, or degradation, respectively.

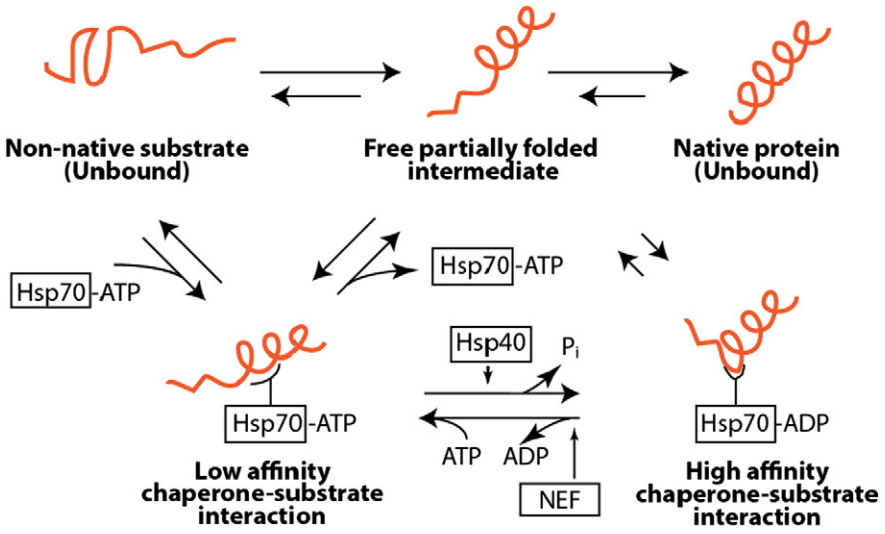


Fig. 2. The HSP70 folding cycle. HSP70 relies on a complex collection of co-chaperones and nucleotide exchange factors to determine both activity level and substrate specificity. The relative ratio of co-chaperones and nucleotide exchange factors serves as a means to fine-tune the PN, and therefore, targeting these protein–protein interactions remains an attractive approach to pharmacological modulation.

(i.e., HSF1) [6], will not be discussed and are beyond the scope of this review.

HSP90

Function

HSP90 molecular chaperones are among the most abundant cellular proteins and correspond to approximately two percent of the cellular proteome under normal conditions of cell growth. The human HSP90 family is composed of four members that exhibit distinct subcellular localization, with two isoforms HSP90AA1 (inducible) and HSP90AB1 (constitutive) in the cytosol and nucleus, GRP94 (94-kDa glucose-regulated protein) localized to the endoplasmic reticulum, and TRAP1 (tumor necrosis factor receptor-associated protein 1) in mitochondria. HSP90 interacts with a wide spectrum of protein clients that includes many protein kinases [7–9], nuclear receptors [10–12], and transcription factors [13–16] and thus has a pivotal role in cell signaling. For example, HSP90 regulates transcriptional responses through the chaperone-dependent assembly of steroid aporeceptors such as the glucocorticoid receptor [17] and chaperone-regulated control of HSF1 and the heat shock response [16].

Structure

HSP90 is composed of a C-terminal dimerization domain that is coupled to an ATPase cycle directed by the N-terminal domain [18,19] and a middle regulatory domain that links the termini [19–21] (Fig. 3). The capacity for substrate binding is dependent upon HSP90 cycling between “open” and “closed” conformations, which requires a large conformational transition that is ATP dependent [22–24]. ATPase cycling is regulated by co-chaperones that influence the hydrolysis reaction rate [25–27] and facilitate substrate association as observed with the HSP90–progesterone receptor interaction that requires HOP [28], as well as in the case of protein kinases, which are delivered to HSP90 via the co-chaperone CDC37 [29].

Co-chaperones

The largest family of HSP90 co-chaperones is the tetratricopeptide repeat (TPR)-containing proteins [26] although not all of these proteins have been analyzed for co-chaperone activity. TPR co-chaperones do not directly bind non-native proteins but have an integral role in directing the activity of HSP90; for example, HOP serves as an adaptor and facilitates the shuttling of non-native substrates from HSP70 to HSP90 [30]. The N-terminal TPR domain (TPR1) of HOP is responsible for interaction with the C-terminus of HSP70, whereas the C-terminal TPR domain (TPR2) facilitates interaction with HSP90 [31]. An EEVD motif in the C-termini of HSP90 and HSP70 anchors the TPR–chaperone interaction in the case of both TPR1 and TPR2, with specificity conferred by residues on the N-terminal side of the EEVD sequence [32]. TPR1 and TPR2 form helical bundles that cradle the EEVD-containing peptide substrates in defined grooves [32].

Roles in pathology and disease

Given its central role, it is unsurprising that HSP90 is implicated in many human pathologies. Deregulation

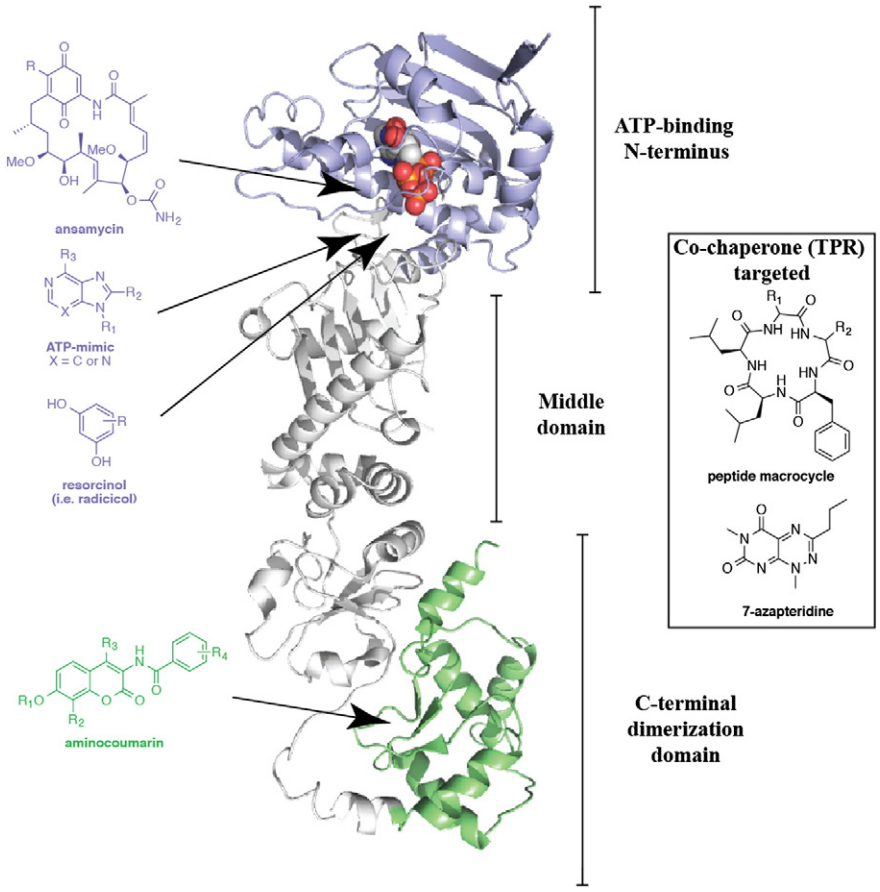


Fig. 3. HSP90 and its pharmacological modulators. The structure of the yeast HSP90 homolog in ribbon presentation. The image represents half of the HSP90 dimer, which is shown in complex with an ATP molecule. The three respective domains are labeled and color-coded. The ATP molecule is represented in sphere form. The image was generated with PyMOL using PDB structure 2CG9. Representative chemical scaffolds for HSP90 modulators are included, and arrows indicate binding sites.

of HSP90 supports oncogenic transformation [33], which is largely attributed to its role in the stabilization of signaling proteins involved in cell growth and proliferation [34], evasion of apoptosis [35], and metastasis [36]. HSP90 expression is increased in tumor cells, and high expression is correlated with poor prognosis [37]. The degradation of the mutant cystic fibrosis transmembrane conductance regulator (CFTR) protein is also dependent upon HSP90, as demonstrated in a yeast-based model for cystic fibrosis [38,39]. In neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and the polyglutamine diseases (e.g., Huntington disease, spinobulbar muscular atrophy, dentatorubral-pallidoluysian atrophy, and spinocerebellar ataxias), HSP90 has been observed to stabilize the toxic oligomers associated with these conditions [40], presumably through inhibition of HSP70-promoted ubiquitination [41].

Pharmacological targeting of HSP90

The small molecule natural product geldanamycin, which was initially identified for antimicrobial activity, was shown to have potent cytotoxic activity against two cancer cell lines [42] and later shown to be broadly active in a screen against a panel of 60 cancer cell lines (NCI-60) [43]. Although early investigations suggested that geldanamycin was a tyrosine kinase inhibitor, this turned out to be an indirect consequence of protein kinases being an HSP90 client [44]. Subsequent biochemical [45] and crystallographic [46] studies established that geldanamycin binds the ATP pocket in the N-terminal domain of HSP90. These findings stimulated the development of many synthetic analogs based upon geldanamycin's ansamycin pharmacophore [47–49] (Fig. 3), as well as the search for other scaffolds that inhibit HSP90, some of which are in clinical trials for the treatment of cancer [50,51].

As there is a substantial literature on small molecules that target HSP90 [52–54], we will comment on only a few examples. Of the numerous small molecules that have been found to bind HSP90, many of these prevent folding activity through the interactions in ATP pocket. In addition to the natural product radicicol [55] (and its derivatives [56–58]), other classes of small molecules that target the HSP90 N-terminal ATP-binding site include the synthetic heterocycles including purine ATP mimetics [59–63], pyrazoles [64–67], and isoxazoles [68,69], among other scaffolds [51,70–74]. The HSP90 ATP pocket has proven to be quite amenable to small molecule binding, and many inhibitors bind this region with high affinity (comparable to geldanamycin's nanomolar binding constant).

Although the HSP90 ATP pocket has received the most attention, a complimentary approach to targeting this chaperone is via the C-terminus. The coumarin antibiotic novobiocin [75] was the first small molecule observed to bind the HSP90 C-terminus, as assessed by the ability of an immobilized derivative to selectively interact with this domain [76–79]. These observations elicited efforts to identify other C-terminal HSP90 interactors, many of which are based upon the novobiocin scaffold (Fig. 3). In contrast to inhibitors that bind the N-terminus, novobiocin derivatives that target the C-terminal domain slow the growth of cancer cells and promote degradation of HSP90 clients without inducing the heat shock response, which is an important and somewhat unexpected distinction [80]. Other non-ATP-competitive HSP90 inhibitors are known, but most are non-selective, which limits their *in vivo* use; examples include epigallocatechin gallate [81] (inhibits several non-chaperone targets [82–86]), cisplatin [87] (damages DNA [88]), and silybin [89] (inhibits P-glycoprotein [90] and cytochrome P450 [91]).

A particularly interesting class of small molecules is capable of modulating co-chaperone access to the EEVD motif in the HSP90 C-terminus. The macrocyclic peptide "compound 2" [92] (Fig. 3) was discovered in a structure–activity relationship study on the Sansalvamide A pharmacophore and was shown to allosterically prevent binding of HSP90 co-chaperones IP6K2, FKBP38, FKBP52, and HOP in a biochemical assay using purified proteins [93]. In a separate study, a high-throughput screen that used an *in vitro* assay to monitor the HSP90–TPR2A protein–protein interaction revealed a small molecule, C9, which has a 7-azapteridine core, that directly binds several TPR-containing co-chaperones [94,95]. C9 directly binds the TPR2A domain of HOP, as assessed by fluorescence polarization and isothermal titration calorimetry, presumably via the peptide groove where the co-chaperone–chaperone protein–protein interaction occurs. The feasibility of targeting HSP90 co-chaperones is further supported by the discovery that the tetranortriterpenoid natural product, gedunin [96], mediates apoptotic cancer cell death through binding p23 [97].

Application of small molecule HSP90 modulators

Since the early observation that geldanamycin has cytotoxic activity in cancer cell lines, significant effort has been expended to develop HSP90 inhibitors for the treatment of cancer [51], but these efforts have yielded limited success. Despite many clinical trials, there is still not a single HSP90 inhibitor approved by the Food and Drug Administration, which is largely due to the toxicity of candidate compounds. Although the early clinical trials may sustain the initial concerns raised with respect to pharmacological modulation of chaperones, many of the clinical candidates exhibit only modest selectivity among HSP90 isoforms [98], and perhaps modulators with greater isoform fidelity may prove less toxic. Additionally, because HSP90 family members are expressed in each subcellular compartment, it could be argued that increased regulatory control may come from the development of isoform-selective inhibitors.

The development of isoform-selective HSP90 inhibitors is challenging because of the high degree of structural similarity among the four human paralogs; however, recent reports suggest progress on paralog-specific inhibitors [99,100]. Some of these paralog-specific inhibitors exhibit different phenotypic outcomes relative to their non-selective counterparts, for example, that GRP94 is disproportionately involved in the chaperoning of the HER2 protein in SKBr3 breast cancer cell lines relative to the other HSP90 isoforms [101]. Likewise, inhibitors optimized for HSP90 α/β specificity displayed lower toxicity than pan inhibitors when characterized in a cell-based model that monitored mutant Huntingtin clearance [102].

While conceptually promising, the treatment of neurodegenerative diseases using HSP90 inhibitors has been complicated by activation of the heat shock response and the fact that inhibition of HSP90 disfavors association and stabilization of the oligomerization-prone clients, which in turn promotes degradation [103]. This highlights an important consideration of HSP90 inhibitors, that general inhibition of HSP90 client interaction will have both positive and negative effects on cell protective mechanisms because of its central role in many cellular processes. Development of HSP90 inhibitors with differential effects on clients, however, could have selective effects on diseased tissues.

Altogether, the application of HSP90 inhibitors toward modification of the PN in cancer has hinted that a highly conserved chaperone system may be targeted in living cells, but this must be carefully controlled. Preliminary indications suggest that small molecule HSP90 modulators could have broad

benefits in disease. Targeting the ATP-binding site has successfully afforded many potent HSP90 inhibitors, but selectivity is usually only modest in large part due to high level of structural similarity in this region. The targeting of areas outside the ATP pocket is promising for increasing the selectivity of HSP90 modulators and, in some cases, provides divergent phenotypic profiles relative to their ATP-competitive counterparts [104]; therefore, even though these sites are more difficult to target, further development is warranted. Additionally, the targeting of co-chaperone interactions remains a largely undeveloped approach for reasons similar to the C-terminal binders, but this may provide a way to finely adjust HSP90 function.

HSP70

Function

HSP70 corresponds to another highly conserved and abundant chaperone that functions in the context of co-chaperones (i.e., J-domain, Bag, and HSP110) to regulate multiple diverse folding processes, including disaggregation, transport, and clearance of proteins. The human HSP70 family includes at least 17 members, represented by HSC70 [105,106] that is expressed constitutively and HSP70s [107,108] that are inducibly regulated by stress conditions. HSP70s are localized to all major subcellular compartments including HSC70 and HSP70 in the cytosol and nucleus, BiP (GRP78) in the endoplasmic reticulum, [109,110], and GRP75 in the mitochondria [111].

HSP70 employs cycles of holding and folding with a diverse array of clients through transient interactions with hydrophobic motifs that are typically confined to the core of a properly folded protein. Consequently, a prominent class of HSP70 substrates is nascent polypeptides [112,113] whose folding is promoted via the ribosome-associated complex [114]. Non-native and misfolded mature clients rely on HSP70 to guide refolding or redirect to the degradative machineries (through both lysosomal [115] and proteasomal [116] pathways); moreover, the appearance of

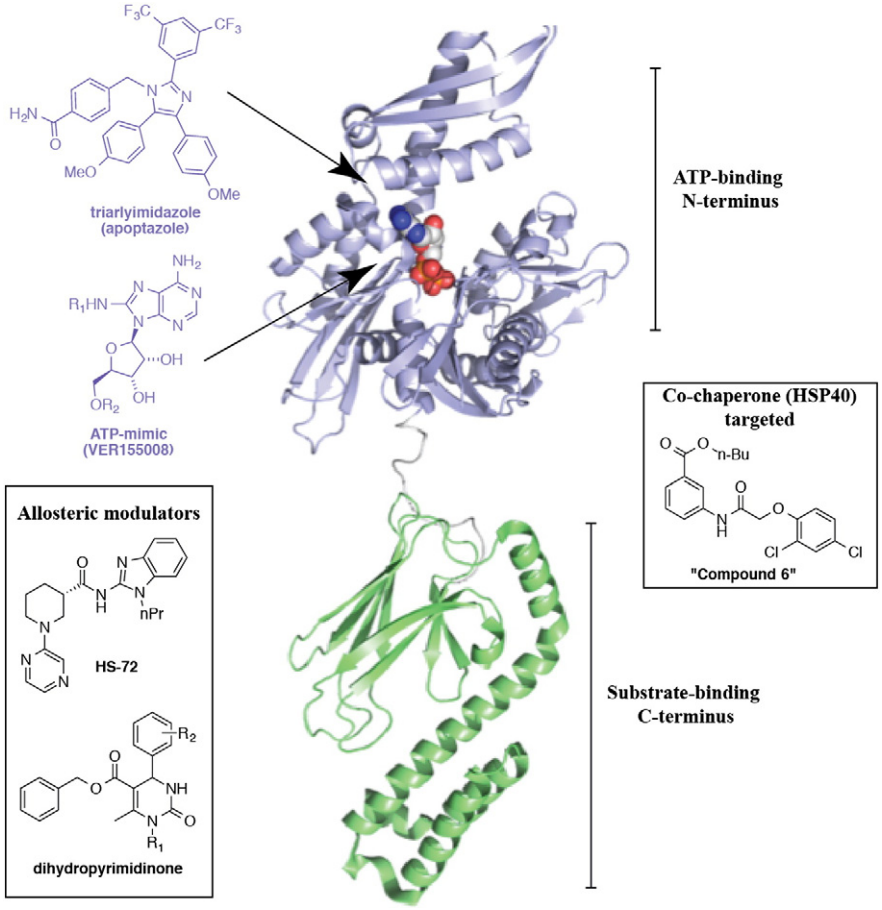


Fig. 4. HSP70 and its pharmacological modulators. The structure of DnaK, the bacterial homolog of HSP70 in ribbon presentation, bound to ADP. The ATP- and substrate-binding domains are labeled and color-coded. ADP is represented in sphere form. The image was generated with PyMOL using PDB structure 2KHO. Representative chemical scaffolds for HSP70 modulators are included, and arrows indicate binding sites.

aggregates by metastable and aggregation-prone species is suppressed through promoting solubility through chaperone interactions [117]. In addition to interactions with a multitude of clients throughout the process of protein biogenesis, the HSC70 isoform also acts to disassemble clathrin triskelia in clathrin-mediated endocytosis and affords a key regulatory step in receptor recycling and endocytic trafficking [118].

Structure

HSP70 substrate interactions occur in the C-terminal domain and are dependent upon ATP hydrolysis, which occurs in the N-terminal domain (Fig. 4). The ATP-bound form has low (micromolar) affinity for its substrates, which allows for rapid exchange, whereas the ADP-bound form has higher affinity for its substrates and exchange rates are retarded [119]. Several hydrolysis cycles can be necessary for complete folding to a native state. *In vitro*, HSP70 has very low ATPase activity [120], which disfavors the binding and dissociation events that are necessary for the folding cycle. The ATPase activity of HSP70 is amplified through association with HSP40/DnaJ proteins and through nucleotide exchange factors, such as Bag-domain-containing proteins, which complete the cycle through reloading the chaperone with a new ATP molecule.

Co-chaperones

Functions of HSP40 include directing non-native clients to the HSP70 machine and acting as an HSP70 co-chaperone to promote hydrolysis of ATP to ADP. HSP40 proteins are J-domain proteins, which are generally divided into three classes. The type I and type II classes are defined by a multidomain structure composed of an N-terminal J-domain, a Gly/Phe-rich region, and a C-terminal domain; the type I class additionally contains a cysteine-rich region (zinc-finger domain) insert in the C-terminal domain. The type III classes include proteins that have a J-domain but do not fall into the type I or type II categories. The J-domain is responsible for interactions with HSP70 [121,122], whereas the C-terminus, which varies widely among members of the J-domain family, has chaperone activity and can act as a dimerization interface [123,124].

Roles in pathology and disease

Given the fundamental role of HSP70 in protein biogenesis and quality control, the aberrant regulation and function of HSP70 has been implicated in many diseases. For example, HSP70 expression is highly elevated in essentially all tumor types and is proposed to be strongly predictive of patient prognosis [125]. HSP70 can contribute to tumorigenesis by inhibition of apoptosis via both caspase-dependent and caspase-independent pathways [126,127], as well as escape of senescence [128]. Additionally, HSP70 has also been proposed to contribute to the development of drug resistance in cancer; for example, chronic myeloid leukemia patients with resistance to the protein kinase inhibitor imatinib consistently display higher HSP70 expression relative to imatinib-responsive patients [129].

Likewise, the HSP70 machine is additionally entwined in the etiology of many neurodegenerative protein-misfolding diseases including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and Huntington disease. The cytotoxicity of both Aβ and tau is influenced by HSP70 because it facilitates disaggregation [130], degradation, and elimination of Aβ [131,132]; inhibits hyperphosphorylation of tau [133]; and prevents aggregation and promotes degradation of abnormal tau [133,134]. Elevated expression of HSP70 has also been shown to alleviate polyglutamine-related toxicity in *Drosophila* models [135,136]. Furthermore, dopaminergic neuron loss associated with α-synuclein toxicity is also mitigated in a *Drosophila* model for Parkinson's disease [137].

Pharmacological targeting of HSP70

Relative to HSP90, efforts to pharmacologically modulate HSP70 are less well developed. There are crucial biochemical and structural differences between these chaperones that could impact their pharmacology. First, HSP70 binds ATP up to 300× more tightly than HSP90 [138]. Second, there is a major divergence in the shape of the ATP-binding sites of HSP90 and HSP70, with the HSP70's site being more solvent exposed, which naturally disfavors small molecule binding [138]. However, the success of targeting the N-terminal ATPase site of HSP90 has nonetheless fueled the exploration of an analogous approach to HSP70 modulation.

Among the early examples of ATP-competitive HSP70 inhibitors is apoptozole, which was discovered as an inducer of apoptosis in a cell-based phenotypic screen, that monitored both the presence of phosphatidylserine on the cell membrane and membrane permeability [139]. Through the use of affinity chromatography and liquid chromatography–mass spectrometry, the action of apoptozole was attributed to binding the ATP pocket of HSC70, and surface plasmon resonance analysis indicated a nanomolar dissociation constant. Using a cell-based model, it was shown that apoptozole influences the membrane presence of mutant CFTR, through a decrease in HSP70-dependent degradation of mutant CFTR by the ubiquitin-proteasome system [140].

Another achievement in ATP-competitive HSP70 inhibition was the development of VER-155008 (Fig. 4), an ATP mimic that binds HSP70 with a mid-nanomolar

in vitro dissociation constant, as assessed by a fluorescence polarization assay [141]. Although VER-155008 is poorly selective among the HSP70 isoforms, a promising subsequent structure–activity relationship study identified one derivative of the parent scaffold that displayed some selectivity for HSP70 over GRP78, an isoform with its own respective role in cancer [142], through exploiting a single amino acid difference in the ATP-binding site (Thr *versus* Ile) [143]. Given that the HSP70 isoform distribution is not uniform, in terms of both cellular compartmentalization and tissue distribution, it would be advantageous to have selective inhibitors for the individual isoforms, and this is an encouraging step toward achieving this in the highly conserved ATP pocket.

An orthogonal route to HSP70 modulation, which may yield small molecules with improved selectivity, is through targeting areas outside of the ATP pocket because these regions have lower sequence conservation [144]. A recent example of this approach is an allosteric inhibitor (HS-72; Fig. 4) of ATP binding for the inducible HSP70 isoform, which was identified using a fluorescence-linked enzyme chemoproteomic approach [145]. HS-72 was shown to have high selectivity for inducible HSP70 over other HSP70 isoforms including HSC70, GRP78, and GRP75. Another report unveiled a class of irreversible inhibitors that bind an allosteric pocket in the N-terminal domain and show higher selectivity for HSP70 over other proteins based upon the ability of an immobilized analog to specifically pull down HSP70 from a SKBr3 cell extract [146].

The HSP40–HSP70 interface represents another site outside of the ATP pocket that has been successfully targeted, particularly in the analogous prokaryotic complex. A set of small molecules with a core dihydropyrimidinone scaffold were identified using an *in vitro* assay and were found to perturb DnaJ binding to DnaK (the bacterial homologs of HSP40 and HSP70) and influence ATPase activity through distributed allosteric effects [147–151]. Small changes in the dihydropyrimidinone scaffold appear to toggle inhibition to activation, which bolsters the hypothesis that targeting such sites may be used to fine-tune chaperone function [151,152]. These dihydropyrimidinones are improved structural analogs of the immunosuppressant HSP70 modulator 15-deoxyspergualin [153] (which has limitations as a HSP70-selective probe due to interactions with HSP90 [154] and possibly other non-chaperone targets [155,156]). A related high-throughput screen additionally revealed small molecules that bind DnaK and allosterically modulate its interaction with the GrpE nucleotide exchange factor [157].

Direct targeting of the HSP40 co-chaperones provides yet another approach to pharmacologically modulate HSP70 activity. However, there is only one example of a small molecule that directly binds HSP40 (Fig. 4). The inhibitor, “compound 6”, was found as part of a small molecule screen using an assay that monitors HSP70 luciferase refolding [158]. Subsequent characterization of “compound 6” using a thermal denaturation assay revealed that the observed effect was through direct binding of the HSP40 co-chaperone. Further development of this class of inhibitors could be another step forward in small molecule modulation of chaperone function; however, this may be challenging given the numerous potential combinations of HSP70–HSP40 interactions.

Application of HSP70 modulators

Altogether, the relatively small collection of potent and selective HSP70 modulators limits our knowledge with respect to the scope and utility of this strategy. Some HSP70 inhibitors have displayed promising preliminary results in the treatment of cancer (i.e., VER-155008 [159], MKT-077 [160], and PES [161]), but toxicity and off-target effects remain an issue. An improved analog of the rhodacyanine HSP70 inhibitor MKT-077 (YM-1) has a promising toxicity profile in cell-based experiments [162] and aided in overcoming resistance in treating tamoxifen resistant MCF7 cells, but the clinical relevance of this compound will require further investigation.

Although pursued to a lesser degree, application of HSP70 modulators is producing exciting preliminary results in models for neurodegenerative diseases [163]. There are two general ways to think about pharmacological modulation within the context of these diseases. First, increasing activity through induction of HSP70 expression appears beneficial for mitigating aggregation in both cell-based and metazoan models [6]. A second approach argues that, in the context of directly targeting chaperones (the focus of this review), specific inhibition of certain aspects of HSP70 activity may be beneficial; for example, small molecule inhibitors that effectively lock HSP70 in the ADP-bound conformation stimulate substrate binding and increase CHIP-dependent ubiquitination and promote polyglutamine-expanded androgen receptor clearance in a cell-based model for spinobulbar muscular atrophy [136]. Clearly, the understanding of how HSP70 contributes to these diseases is not yet complete, and its roles in both substrate stabilization and triaging require further elucidation.

Although an impressive starting toolbox of chemical probes has been developed for HSP70, the chemical biology of this chaperone remains less refined than HSP90. Most of the available HSP70 modulators are inhibitors of only moderate potency and mainly target the ATP pocket. The specificity of current modulators is generally modest or unknown, although promising preliminary studies indicate that isoform selectivity is achievable with further

medicinal chemistry optimization. Very few small molecules that directly activate HSP70 are known, but promisingly, selective activation seems feasible based on early experiments with 15-deoxyspergualin, which increases HSC70 activity but has no effect on BiP [164]. Targeting areas outside of the HSP70 ATP pocket has been met with some success, but there is a paucity of inhibitors that bind the C-terminus. Examples of small molecules that bind the C-terminus have been reported, such as PES [165], but they appear to be reactive and may have off-pathway targets in *in vivo* settings.

HSP60/Chaperonins

Function and structure

The chaperonins represent a third major class of chaperones that engage protein clients by encapsulation within a self-contained folding chamber, to fold post-translational intermediates to native states [166], assist in oligomeric protein assembly [167], and import protein substrates into mitochondria [168]. Much has been learned of the structure of chaperonins from the study of the bacterial homolog, GroEL [169], which exists as an ~800-kDa complex that has a cylindrical shape composed of two rings that respectively contain seven subunits [170,171] (Fig. 5). Non-native substrates are accommodated in the central cavity of the chaperonin, which has dimensions of 6 nm in diameter and 7 nm in height [172]. Upon entry of a non-native substrate into the chamber, the subsequent folding process is associated with major conformational changes in the central cavity driven by ATP hydrolysis [173]. Chaperonins correspond to two major classes based upon folding mechanism, the type I chaperonins (GroEL, mitochondrial HSP60, and the Rubisco subunit binding protein) require a separate co-chaperonin (i.e., GroES or HSP10) to

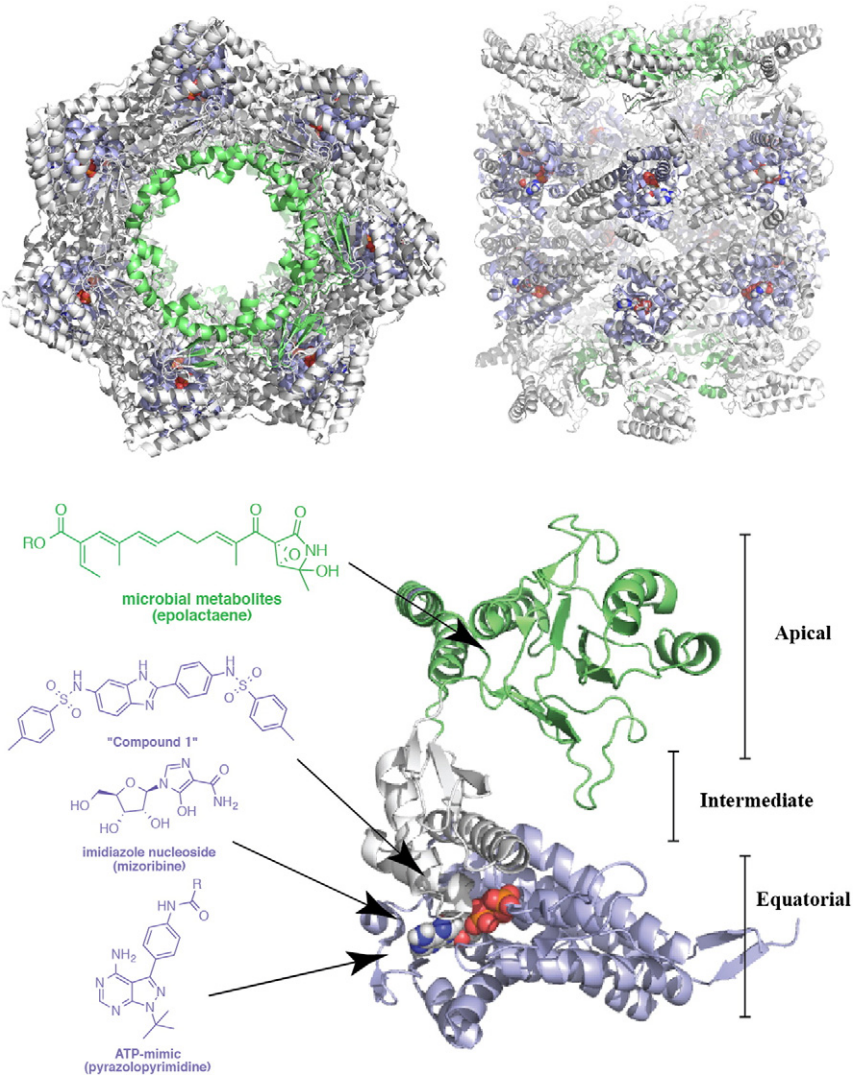


Fig. 5. The chaperonin family and its pharmacological modulators. The structure of GroEL, the bacterial homolog of HSP60 in ribbon presentation. ATP is represented in sphere form. The image was generated with PyMOL using PDB structure 4AB2. The top-left portion of the panel displays an overhead view that highlights the substrate-binding cavity. The top-right portion of the panel displays a side view, which more clearly shows the ATP-binding site. The structure of an individual subunit of GroEL is shown in the bottom right. Representative chemical scaffolds for chaperonin modulators are included, and arrows indicate binding sites.

seal the folding chamber. GroES is expressed in the cytosol of bacteria and in organelles of mitochondria [174] and chloroplasts [167]. In contrast, the type II chaperonins have a built-in lid and are expressed in archaebacteria [175] and the eukaryotic cytosol [176].

Roles in pathology and disease

Human chaperonins have been implicated in motor neuron diseases, including hereditary spastic paraplegia, in which a mutant form of mitochondrial HSP60 with reduced ATPase activity is incapable of forming a productive complex with HSP10, as assessed in an *Escherichia coli* model [177,178]. McKusick-Kaufman syndrome is associated with mutations within a putative subunit of the eukaryotic cytosolic chaperonin CCT [179], and the related Bardet-Biedl syndrome is also associated with deregulation of the same putative chaperonin protein [180]. Mutations in CCT are also correlated with hereditary sensory neuropathy in rat models [181]. Cancer cell survival additionally relies upon HSP60 activity [182], and overexpression is associated with evasion of apoptosis through down-regulation of a p53-promoted pathway [183], loss of senescence that is potentially due to a role in cell cycle regulation [184], and uncontrolled proliferation [185].

Pharmacological targeting of chaperonins

Small molecule modulators of chaperonins, as a whole, have been elusive. Early efforts directed at targeting the chaperonin ATPase site have been unproductive, and only a few inhibitors, including mizoribine [186] and pyrazolopyrimidines [187] (Fig. 5), were reported to bind this region with modest affinity. However, a recent high-throughput screen identified several promising new ATP-competitive leads for GroEL, including “compound 1” [188], which potently inhibits GroEL/GroES-mediated refolding in a biochemical assay. In a separate high-throughput screen that was designed to identify pharmacological activators of HSF1, a small molecule hit, HSF1A, was found to bind CCT through pull-down experiments [189,190], and further investigation using this small molecule demonstrated that CCT directly binds HSF1 to inhibit transcription [190].

Examples of small molecules that bind outside of the chaperonin ATP-binding site are sparse but exist. Epolactaene [191] inhibits GroEL through electrophilic reaction with Cys442, β-lactams are proposed to interact with a loop in the N-terminal region based upon docking simulations [192], and the sesquiterpene natural product suvanine [193] acts as a non-ATP-competitive chaperonin inhibitor, but its mechanism of action has not been fully characterized. The low availability of small molecules that target areas outside of the chaperonin ATPase site, such as the protein-folding chamber, makes pursuit of such pharmacological modulators attractive because of the conceptually unique mechanism of action.

Application of chaperonin modulators

Altogether, the chaperonins have been less tractable to small molecule modulators for reasons that are not entirely clear, a likely therapeutic application will be as antibacterials, which may help address the growing concern of resistance. Chaperonin inhibition may also be used for cancer treatment [194] as efforts develop for inhibitors of the human homologs.

Tabular Summary

As a resource, we have included a table with a representative sample of the pharmacological chaperone modulators that have been discussed within this review (Table 1). An emphasis has been placed on biochemical affinity because of the inherent variability of potency assessment in cell-based experiments. Chaperone modulators without adequate biochemical affinity data were excluded.

Prospectives

The integral role of molecular chaperones in the PN makes them an intriguing pharmacological target, and since the serendipitous discovery of geldanamycin, there has been a growing interest in the potential therapeutic value of small molecule chaperone modulators. Currently, there are 22 open clinical studies related to HSP90 inhibitors in the United States[†], and there is much optimism regarding this approach based upon ongoing clinical studies [195]. However, despite this optimism, toxicity remains a significant issue, and concerns regarding the highly conserved and ubiquitous nature of these proteostasis constituents still loom.

Perhaps proceeding with a renewed focus on selectivity, a feature that has largely been ignored (or underreported) in the development of chaperone modulators may circumvent these toxicity issues. Development of probes with high fidelity for a given chaperone will allow for fine adjustment of the PN and the protein-folding landscape and will help elucidate the individual functions of the chaperone machines. The field may possibly benefit from the development of standardized chaperone selectivity panels, as found in the case of validated pharmaceutical targets such as protein kinases (i.e., KINOMEscan [196], KiNativ [197], and KinaseSeeker [198]), which may allow for a more focused pursuit of the most clinically relevant chaperones.

Table 1. Summary of selected pharmacological chaperone modulators discussed within this review

Target class	Name	Pharmacophore	Binding site	*In vitro* potency (nM)
HSP90	Geldanamycin	Benzoquinone ansamycin	ATP	K_D = 780[a]
HSP90	17-AAG	Benzoquinone ansamycin	ATP	IC_{50} = 6[b]
HSP90	Radicicol	Resorcinol	ATP	K_D = 3[a]
HSP90	PU3	ATP mimic	ATP	EC_{50} = 13,000[c]
HSP90	BIIB021	ATP mimic	ATP	IC_{50} = 1.7[d]
HSP90	HSP990	Other	ATP	IC_{50} = 0.6[e]
TPR	C9	Other	Protein–protein interaction	K_D = 16,000[f]
TPR	"compound 2"	Sansalvamide A derivative	Protein–protein interaction	K_D = 3,600[g]
HSP70	VER-155008	ATP mimic	ATP	K_D = 300[h]
HSP70	Apoptozole	Triarylimidazole	ATP	K_D = 210[i]
HSP70	116-4G	Dihydropyrimidinone	Allosteric	IC_{50} = 120,000[j]
HSP40	"compound 6"	Other	Protein–protein interaction	IC_{50} = 130[k]
chaperonin	Mizoribine	Other	ATP	K_D = 600[l]
chaperonin	"compound 1"	Other	ATP	IC_{50} = 110[m]

[a] Binding affinity for isolated N-domain [55].
[b] Inhibition of HSP90 from BT474 breast carcinoma cell lysate [199].
[c] Competition for HSP90 against immobilized geldanamycin [200].
[d] Competition for HSP90 against FITC-labeled geldanamycin [201].
[e] AlphaScreen binding assay [202].
[f] Isothermal titration calorimetry [95].
[g] Competitive binding assay [92].
[h] Competition assay for fluorescein-labeled ATP [141].
[i] Surface plasmon resonance binding assay [139].
[j] ATPase assay (malachite green) [149].
[k] Luciferase refolding assay [158].
[l] Surface plasmon resonance binding assay [186].
[m] dMDH refolding assay [188].

We postulate that selectivity may be difficult to achieve using the prevailing approach of targeting the highly conserved ATPase site of the chaperone machines. Non-ATP-competitive chaperone modulators offer higher selectivity but additionally display phenotypic activity that diverges from their ATP-competitive counterparts, such as the C-terminal binding HSP90 inhibitors and allosteric modulators of HSP70. While we appreciate the difficulty of pharmacologically targeting these unconventional sites, we expect to see further elaboration of these approaches based upon these preliminary observations.

Looking forward, in addition to streamlining how chaperones are targeted, it will also be important to continue to broaden how they are used. We would like to point out that all of the clinical trials based upon chaperone modulation are exclusively for the treatment of cancer, and with increasing evidence that chaperones are valuable targets for several neurodegenerative diseases, hopefully clinical evaluation of this approach will begin in the near future. Furthermore, most chemical probes targeting molecular chaperones are inhibitors, which limit the capacity to pharmacologically modulate the PN via a targeted approach. This disparity also has clinical relevance given that small molecules that directly bind molecular chaperones and increase their associated activities may have great utility in the treatment of disease.

Acknowledgements

This work was supported by the National Institutes of Health (National Institute on Aging, National Institute of General Medical Sciences, and National Institute of Mental Health), the Chicago Biomedical Consortium, the Ellison Medical Foundation, and the Daniel F. and Ada L. Rice Foundation. We would like to thank Dr. Johnathan Labbadia for thoughtful comments on the manuscript.

Received 3 March 2015;
Received in revised form 9 May 2015;
Accepted 13 May 2015
Available online 21 May 2015

Keywords:
protein folding;
aggregation;
heat shock protein;
small molecule modulators;
pharmacology

†clinicaltrials.gov.

Abbreviations used:
CFTR, cystic fibrosis transmembrane conductance regulator; PN, proteostasis network; TPR, tetratricopeptide repeat;.

References

[1] Powers ET, Morimoto RI, Dillin A, Kelly JW, Balch WE. Biological and chemical approaches to diseases of proteostasis deficiency. Annu Rev Biochem 2009;78:959–91.

[2] Åkerfelt M, Morimoto RI, Sistonen L. Heat shock factors: integrators of cell stress, development and lifespan. Nat Rev Mol Cell Biol 2010;11:545–55.

[3] Brehme M, Voisine C, Rolland T, Wachi S, Soper James H, Zhu Y, et al. A chaperome subnetwork safeguards proteostasis in aging and neurodegenerative disease. Cell Rep 2014;9:1135–50.

[4] Arkin Michelle R, Tang Y, Wells James A. Small-molecule inhibitors of protein–protein interactions: progressing toward the reality. Chem Biol 2014;21:1102–14.

[5] Thompson AD, Dugan A, Gestwicki JE, Mapp AK. Fine-tuning multiprotein complexes using small molecules. ACS Chem Biol 2012;7:1311–20.

[6] Calamini B, Morimoto RI. Protein homeostasis as a therapeutic target for diseases of protein conformation. Curr Top Med Chem 2012;12:2623–40.

[7] Brugge JS, Erikson E, Erikson RL. The specific interaction of the Rous sarcoma virus transforming protein, pp60src, with two cellular proteins. Cell 1981;25:363–72.

[8] Lipsich LA, Cutt JR, Brugge JS. Association of the transforming proteins of Rous, Fujinami, and Y73 avian sarcoma viruses with the same two cellular proteins. Mol Cell Biol 1982;2:875–80.

[9] Taipale M, Krykbaeva I, Koeva M, Kayatekin C, Westover KD, Karras GI, et al. Quantitative analysis of HSP90-client interactions reveals principles of substrate recognition. Cell 2012;150:987–1001.

[10] Joab I, Radanyi C, Renoir M, Buchou T, Catelli MG, Binart N, et al. Common non-hormone binding component in non-transformed chick oviduct receptors of four steroid hormones. Nature 1984;308:850–3.

[11] Schuh S, Yonemoto W, Brugge J, Bauer VJ, Riehl RM, Sullivan WP, et al. A 90,000-dalton binding-protein common to both steroid-receptors and the Rous-sarcoma virus transforming protein, PP60V-SRC. J Biol Chem 1985;260:4292–6.

[12] Sanchez ER, Toft DO, Schlesinger MJ, Pratt WB. Evidence that the 90-kDa phosphoprotein associated with the untransformed L-cell glucocorticoid receptor is a murine heat shock protein. J Biol Chem 1985;260:12398–401.

[13] Minet E, Mottet D, Michel G, Roland I, Raes M, Remacle J, et al. Hypoxia-induced activation of HIF-1: role of HIF-1alpha-Hsp90 interaction. FEBS Lett 1999;460:251–6.

[14] Sato N, Yamamoto T, Sekine Y, Yumioka T, Junicho A, Fuse H, et al. Involvement of heat-shock protein 90 in the interleukin-6-mediated signaling pathway through STAT3. Biochem Biophys Res Commun 2003;300:847–52.

[15] Sepehrnia B, Paz IB, Dasgupta G, Momand J. Heat shock protein 84 forms a complex with mutant p53 protein predominantly within a cytoplasmic compartment of the cell. J Biol Chem 1996;271:15084–90.

[16] Zou J, Guo Y, Guettouche T, Smith DF, Voellmy R. Repression of heat shock transcription factor HSF1 activation by HSP90 (HSP90 complex) that forms a stress-sensitive complex with HSF1. Cell 1998;94:471–80.

[17] Pratt WB, Morishima Y, Murphy M, Harrell M. Chaperoning of glucocorticoid receptors. Handb Exp Pharmacol 2006:111–38.

[18] Owen BAL, Sullivan WP, Felts SJ, Toft DO. Regulation of heat shock protein 90 ATPase activity by sequences in the carboxyl terminus. J Biol Chem 2002;277:7086–91.

[19] Pearl LH, Prodromou C. Structure and mechanism of the Hsp90 molecular chaperone machinery. Annu Rev Biochem 2006;75:271–94.

[20] Hainzl O, Lapina MC, Buchner J, Richter K. The charged linker region is an important regulator of Hsp90 function. J Biol Chem 2009;284:22559–67.

[21] Lotz GP, Lin H, Harst A, Obermann WMJ. Aha1 binds to the middle domain of Hsp90, contributes to client protein activation, and stimulates the ATPase activity of the molecular chaperone. J Biol Chem 2003;278:17228–35.

[22] Csermely P, Kajtar J, Hollosi M, Jalsovszky G, Holly S, Kahn CR, et al. ATP induces a conformational change of the 90-kDa heat shock protein (hsp90). J Biol Chem 1993;268:1901–7.

[23] Grenert JP, Sullivan WP, Fadden P, Haystead TA, Clark J, Mimnaugh E, et al. The amino-terminal domain of heat shock protein 90 (hsp90) that binds geldanamycin is an ATP/ADP switch domain that regulates hsp90 conformation. J Biol Chem 1997;272:23843–50.

[24] Sullivan W, Stensgard B, Caucutt G, Bartha B, McMahon N, Alnemri ES, et al. Nucleotides and two functional states of hsp90. J Biol Chem 1997;272:8007–12.

[25] Siligardi G, Panaretou B, Meyer P, Singh S, Woolfson DN, Piper PW, et al. Regulation of Hsp90 ATPase activity by the co-chaperone Cdc37p/p50cdc37. J Biol Chem 2002;277:20151–9.

[26] Prodromou C, Siligardi G, O'Brien R, Woolfson DN, Regan L, Panaretou B, et al. Regulation of Hsp90 ATPase activity by tetratricopeptide repeat (TPR)-domain co-chaperones. EMBO J 1999;18:754–62.

[27] McLaughlin SH, Smith HW, Jackson SE. Stimulation of the weak ATPase activity of human hsp90 by a client protein. J Mol Biol 2002;315:787–98.

[28] Kosano H, Stensgard B, Charlesworth MC, McMahon N, Toft D. The assembly of progesterone receptor-hsp90 complexes using purified proteins. J Biol Chem 1998;273:32973–9.

[29] Miyata Y, Nishida E. Evaluating CK2 activity with the antibody specific for the CK2-phosphorylated form of a kinase-targeting cochaperone Cdc37. Mol Cell Biochem 2008;316:127–34.

[30] Daniel S, Söti C, Csermely P, Bradley G, Blatch G. Hop: an Hsp70/Hsp90 co-chaperone that functions within and beyond Hsp70/Hsp90 protein folding pathways. Networking of chaperones by co-chaperones. New York: Springer; 2007 26–37.

[31] Chen S, Prapapanich V, Rimerman RA, Honore B, Smith DF. Interactions of p60, a mediator of progesterone receptor assembly, with heat shock proteins hsp90 and hsp70. Mol Endocrinol 1996;10:682–93.

[32] Scheufler C, Brinker A, Bourenkov G, Pegoraro S, Moroder L, Bartunik H, et al. Structure of TPR domain–peptide complexes: critical elements in the assembly of the Hsp70–Hsp90 multichaperone machine. Cell 2000;101:199–210.

[33] Whitesell L, Lindquist SL. HSP90 and the chaperoning of cancer. Nat Rev Cancer 2005;5:761–72.

[34] Richter K, Buchner J. Hsp90: chaperoning signal transduction. J Cell Physiol 2001;188:281–90.

[35] Rodina A, Vilenchik M, Moulick K, Aguirre J, Kim J, Chiang A, et al. Selective compounds define Hsp90 as a major inhibitor of apoptosis in small-cell lung cancer. Nat Chem Biol 2007;3:498–507.

[36] Koga F, Kihara K, Neckers L. Inhibition of cancer invasion and metastasis by targeting the molecular chaperone heat-shock protein 90. Anticancer Res 2009;29:797–807.

[37] Pick E, Kluger Y, Giltnane JM, Moeder C, Camp RL, Rimm DL, et al. High HSP90 expression is associated with decreased survival in breast cancer. Cancer Res 2007;67: 2932–7.
[38] Youker RT, Walsh P, Beilharz T, Lithgow T, Brodsky JL. Distinct roles for the Hsp40 and Hsp90 molecular chaperones during cystic fibrosis transmembrane conductance regulator degradation in yeast. Mol Biol Cell 2004;15: 4787–97.
[39] Wang X, Venable J, LaPointe P, Hutt DM, Koulov AV, Coppinger J, et al. Hsp90 cochaperone Aha1 downregulation rescues misfolding of CFTR in cystic fibrosis. Cell 2006; 127:803–15.
[40] Pratt WB, Gestwicki JE, Osawa Y, Lieberman AP. Targeting Hsp90/Hsp70-based protein quality control for treatment of adult onset neurodegenerative diseases. Annu Rev Pharmacol Toxicol 2015;55:353–71.
[41] Höhfeld J, Cyr DM, Patterson C. From the cradle to the grave: molecular chaperones that may choose between folding and degradation. EMBO Rep 2001;2:885–90.
[42] DeBoer C, Meulman PA, Wnuk RJ, Peterson DH. Geldanamycin, a new antibiotic. J Antibiot 1970;23:442–7.
[43] Supko J, Hickman R, Grever M, Malspeis L. Preclinical pharmacologic evaluation of geldanamycin as an antitumor agent. Cancer Chemother Pharmacol 1995;36:305–15.
[44] Neckers L, Schulte TW, Mimnaugh E. Geldanamycin as a potential anti-cancer agent: its molecular target and biochemical activity. Invest New Drugs 1999;17:361–73.
[45] Whitesell L, Mimnaugh EG, De Costa B, Myers CE, Neckers LM. Inhibition of heat shock protein HSP90-pp60v-src heteroprotein complex formation by benzoquinone ansamycins: essential role for stress proteins in oncogenic transformation. Proc Natl Acad Sci 1994;91:8324–8.
[46] Stebbins CE, Russo AA, Schneider C, Rosen N, Hartl FU, Pavletich NP. Crystal structure of an Hsp90-geldanamycin complex: targeting of a protein chaperone by an antitumor agent. Cell 1997;89:239–50.
[47] Schnur RC, Corman ML, Gallaschun RJ, Cooper BA, Dee MF, Doty JL, et al. erbB-2 oncogene inhibition by geldanamycin derivatives: synthesis, mechanism of action, and structure–activity relationships. J Med Chem 1995;38: 3813–20.
[48] Rastelli G, Tian Z-Q, Wang Z, Myles D, Liu Y. Structure-based design of 7-carbamate analogs of geldanamycin. Bioorg Med Chem Lett 2005;15:5016–21.
[49] Schnur RC, Corman ML, Gallaschun RJ, Cooper BA, Dee MF, Doty JL, et al. Inhibition of the oncogene product p185erbB-2 *in vitro* and *in vivo* by geldanamycin and dihydrogeldanamycin derivatives. J Med Chem 1995;38: 3806–12.
[50] Biamonte MA, Van de Water R, Arndt JW, Scannevin RH, Perret D, Lee W-C. Heat shock protein 90: inhibitors in clinical trials. J Med Chem 2009;53:3–17.
[51] Jhaveri K, Taldone T, Modi S, Chiosis G. Advances in the clinical development of heat shock protein 90 (Hsp90) inhibitors in cancers. Biochim Biophys Acta–Mol Cell Res 2012;1823:742–55.
[52] Neckers L, Workman P. Hsp90 molecular chaperone inhibitors: are we there yet? Clin Cancer Res 2012;18:64–76.
[53] Solit DB, Chiosis G. Development and application of Hsp90 inhibitors. Drug Discov Today 2008;13:38–43.
[54] Taldone T, Sun W, Chiosis G. Discovery and development of heat shock protein 90 inhibitors. Bioorg Med Chem 2009; 17:2225–35.
[55] Roe SM, Prodromou C, O'Brien R, Ladbury JE, Piper PW, Pearl LH. Structural basis for inhibition of the Hsp90 molecular chaperone by the antitumor antibiotics radicicol and geldanamycin. J Med Chem 1999;42:260–6.
[56] Agatsuma T, Ogawa H, Akasaka K, Asai A, Yamashita Y, Mizukami T, et al. Halohydrin and oxime derivatives of radicicol: synthesis and antitumor activities. Bioorg Med Chem 2002;10:3445–54.
[57] Ikuina Y, Amishiro N, Miyata M, Narumi H, Ogawa H, Akiyama T, et al. Synthesis and antitumor activity of novel *O*-carbamoylmethyloxime derivatives of radicicol. J Med Chem 2003;46:2534–41.
[58] Yamamoto K, Garbaccio RM, Stachel SJ, Solit DB, Chiosis G, Rosen N, et al. Total synthesis as a resource in the discovery of potentially valuable antitumor agents: cycloproparadicicol. Angew Chem Int Ed Engl 2003;42:1280–4.
[59] Chiosis G, Timaul MN, Lucas B, Munster PN, Zheng FF, Sepp-Lorenzino L, et al. A small molecule designed to bind to the adenine nucleotide pocket of Hsp90 causes Her2 degradation and the growth arrest and differentiation of breast cancer cells. Chem Biol 2001;8:289–99.
[60] Wright L, Barril X, Dymock B, Sheridan L, Surgenor A, Beswick M, et al. Structure–activity relationships in purine-based inhibitor binding to HSP90 isoforms. Chem Biol 2004; 11:775–85.
[61] Llauger L, He H, Kim J, Aguirre J, Rosen N, Peters U, et al. Evaluation of 8-arylsulfanyl, 8-arylsulfoxyl, and 8-arylsulfonyl adenine derivatives as inhibitors of the heat shock protein 90. J Med Chem 2005;48:2892–905.
[62] Zhang L, Fan J, Vu K, Hong K, Le Brazidec J-Y, Shi J, et al. 7′-Substituted benzothiazolothio- and pyridinothiazolothiopurines as potent heat shock protein 90 inhibitors. J Med Chem 2006;49:5352–62.
[63] Kasibhatla SR, Hong K, Biamonte MA, Busch DJ, Karjian PL, Sensintaffar JL, et al. Rationally designed high-affinity 2-amino-6-halopurine heat shock protein 90 inhibitors that exhibit potent antitumor activity. J Med Chem 2007;50: 2767–78.
[64] Cheung K-MJ, Matthews TP, James K, Rowlands MG, Boxall KJ, Sharp SY, et al. The identification, synthesis, protein crystal structure and *in vitro* biochemical evaluation of a new 3,4-diarylpyrazole class of Hsp90 inhibitors. Bioorg Med Chem Lett 2005;15:3338–43.
[65] Dymock BW, Barril X, Brough PA, Cansfield JE, Massey A, McDonald E, et al. Novel, potent small-molecule inhibitors of the molecular chaperone Hsp90 discovered through structure-based design. J Med Chem 2005;48:4212–5.
[66] Brough PA, Barril X, Beswick M, Dymock BW, Drysdale MJ, Wright L, et al. 3-(5-chloro-2,4-dihydroxyphenyl)-Pyrazole-4-carboxamides as inhibitors of the Hsp90 molecular chaperone. Bioorg Med Chem Lett 2005;15:5197–201.
[67] Kreusch A, Han S, Brinker A, Zhou V, Choi H-s, He Y, et al. Crystal structures of human HSP90α-complexed with dihydroxyphenylpyrazoles. Bioorg Med Chem Lett 2005; 15:1475–8.
[68] Sharp SY, Prodromou C, Boxall K, Powers MV, Holmes JL, Box G, et al. Inhibition of the heat shock protein 90 molecular chaperone *in vitro* and *in vivo* by novel, synthetic, potent resorcinylic pyrazole/isoxazole amide analogues. Mol Cancer Ther 2007;6:1198–211.
[69] Brough PA, Aherne W, Barril X, Borgognoni J, Boxall K, Cansfield JE, et al. 4,5-Diarylisoxazole Hsp90 chaperone inhibitors: potential therapeutic agents for the treatment of cancer. J Med Chem 2007;51:196–218.

[70] Barril X, Brough P, Drysdale M, Hubbard RE, Massey A, Surgenor A, et al. Structure-based discovery of a new class of Hsp90 inhibitors. Bioorg Med Chem Lett 2005;15: 5187–91.
[71] Park H, Kim Y-J, Hahn J-S. A novel class of Hsp90 inhibitors isolated by structure-based virtual screening. Bioorg Med Chem Lett 2007;17:6345–9.
[72] Huth JR, Park C, Petros AM, Kunzer AR, Wendt MD, Wang X, et al. Discovery and design of novel HSP90 inhibitors using multiple fragment-based design strategies. Chem Biol Drug Des 2007;70:1–12.
[73] Jego G, Hazoumé A, Seigneuric R, Garrido C. Targeting heat shock proteins in cancer. Cancer Lett 2013;332: 275–85.
[74] Patel HJ, Modi S, Chiosis G, Taldone T. Advances in the discovery and development of heat-shock protein 90 inhibitors for cancer treatment. Expert Opin Drug Discov 2011;6:559–87.
[75] Hoeksema H, Johnson JL, Hinman JW. Structural studies on streptonivicin,1 a new antibiotic. J Am Chem Soc 1955; 77:6710–1.
[76] Marcu MG, Schulte TW, Neckers L. Novobiocin and related coumarins and depletion of heat shock protein 90-dependent signaling proteins. J Natl Cancer Inst 2000;92:242–8.
[77] Burlison JA, Neckers L, Smith AB, Maxwell A, Blagg BSJ. Novobiocin: redesigning a DNA gyrase inhibitor for selective inhibition of Hsp90. J Am Chem Soc 2006;128:15529–36.
[78] Burlison JA, Avila C, Vielhauer G, Lubbers DJ, Holzbeierlein J, Blagg BSJ. Development of novobiocin analogues that manifest anti-proliferative activity against several cancer cell lines. J Org Chem 2008;73:2130–7.
[79] Donnelly AC, Mays JR, Burlison JA, Nelson JT, Vielhauer G, Holzbeierlein J, et al. The design, synthesis, and evaluation of coumarin ring derivatives of the novobiocin scaffold that exhibit antiproliferative activity. J Org Chem 2008;73:8901–20.
[80] Shelton SN, Shawgo ME, Matthews SB, Lu Y, Donnelly AC, Szabla K, et al. KU135, a novel novobiocin-derived C-terminal inhibitor of the 90-kDa heat shock protein, exerts potent antiproliferative effects in human leukemic cells. Mol Pharmacol 2009;76:1314–22.
[81] Yin Z, Henry EC, Gasiewicz TA. (–)-Epigallocatechin-3-gallate is a novel Hsp90 inhibitor. Biochemistry 2009;48: 336–45.
[82] Choi K-C, Jung MG, Lee Y-H, Yoon JC, Kwon SH, Kang H-B, et al. Epigallocatechin-3-gallate, a histone acetyltransferase inhibitor, inhibits EBV-induced B lymphocyte transformation via suppression of RelA acetylation. Cancer Res 2009;69: 583–92.
[83] Lee WJ, Shim J-Y, Zhu BT. Mechanisms for the inhibition of DNA methyltransferases by tea catechins and bioflavonoids. Mol Pharmacol 2005;68:1018–30.
[84] Li C, Allen A, Kwagh J, Doliba NM, Qin W, Najafi H, et al. Green tea polyphenols modulate insulin secretion by inhibiting glutamate dehydrogenase. J Biol Chem 2006; 281:10214–21.
[85] Suzuki K, Yahara S, Hashimoto F, Uyeda M. Inhibitory activities of −epigallocatechin-3-*O*-gallate against topoisomerases I and II. Biol Pharm Bull 2001;24:1088–90.
[86] Bertoldi M, Gonsalvi M, Borri Voltattorni C. Green tea polyphenols: novel irreversible inhibitors of Dopa decarboxylase. Biochem Biophys Res Commun 2001;284:90–3.
[87] Soti C, Racz A, Csermely P. A nucleotide-dependent molecular switch controls ATP binding at the C-terminal domain of Hsp90. N-terminal nucleotide binding unmasks a C-terminal binding pocket. J Biol Chem 2002;277:7066–75.
[88] Huang Y, Li L. DNA crosslinking damage and cancer—a tale of friend and foe. Transl Cancer Res 2013;2:144–54.
[89] Zhao H, Brandt GE, Galam L, Matts RL, Blagg BS. Identification and initial SAR of silybin: an Hsp90 inhibitor. Bioorg Med Chem Lett 2011;21:2659–64.
[90] Zhou S, Lim LY, Chowbay B. Herbal modulation of P-glycoprotein. Drug Metab Rev 2004;36:57–104.
[91] Wu J-W, Lin L-C, Tsai T-H. Drug–drug interactions of silymarin on the perspective of pharmacokinetics. J Ethnopharmacol 2009;121:185–93.
[92] Ardi VC, Alexander LD, Johnson VA, McAlpine SR. Macrocycles that inhibit the binding between heat shock protein 90 and TPR-containing proteins. ACS Chem Biol 2011;6:1357–66.
[93] Sellers RP, Alexander LD, Johnson VA, Lin C-C, Savage J, Corral R, et al. Design and synthesis of Hsp90 inhibitors: exploring the SAR of Sansalvamide A derivatives. Bioorg Med Chem 2010;18:6822–56.
[94] Yi Fang, Zhu Pingjun, Southall N, Inglese J, Austin CP, Zheng Wei, et al. An AlphaScreenTM-based high-throughput screen to identify inhibitors of Hsp90-cochaperone interaction. J Biomol Screen 2009;14:273–81.
[95] Yi F, Regan L. A novel class of small molecule inhibitors of Hsp90. ACS Chem Biol 2008;3:645–54.
[96] Brandt GEL, Schmidt MD, Prisinzano TE, Blagg BSJ. Gedunin, a novel Hsp90 inhibitor: semisynthesis of derivatives and preliminary structure–activity relationships. J Med Chem 2008;51:6495–502.
[97] Patwardhan CA, Fauq A, Peterson LB, Miller C, Blagg BSJ, Chadli A. Gedunin inactivates the co-chaperone p23 protein causing cancer cell death by apoptosis. J Biol Chem 2013; 288:7313–25.
[98] Taldone T, Patel PD, Patel M, Patel HJ, Evans CE, Rodina A, et al. Experimental and structural testing module to analyze paralogue-specificity and affinity in the Hsp90 inhibitors series. J Med Chem 2013;56:6803–18.
[99] Soldano KL, Jivan A, Nicchitta CV, Gewirth DT. Structure of the N-terminal domain of GRP94: basis for ligand specificity and regulation. J Biol Chem 2003;278:48330–8.
[100] Rosser MFN, Nicchitta CV. Ligand interactions in the adenosine nucleotide-binding domain of the Hsp90 chaperone, GRP94: I. Evidence for allosteric regulation of ligand binding. J Biol Chem 2000;275:22798–805.
[101] Patel PD, Yan P, Seidler PM, Patel HJ, Sun W, Yang C, et al. Paralog-selective Hsp90 inhibitors define tumor-specific regulation of HER2. Nat Chem Biol 2013;9:677–84.
[102] Ernst JT, Neubert T, Liu M, Sperry S, Zuccola H, Turnbull A, et al. Identification of novel HSP90α/β isoform selective inhibitors using structure-based drug design. demonstration of potential utility in treating CNS disorders such as Huntington's disease. J Med Chem 2014;57:3382–400.
[103] Waza M, Adachi H, Katsuno M, Minamiyama M, Tanaka F, Doyu M, et al. Modulation of Hsp90 function in neurodegenerative disorders: a molecular-targeted therapy against disease-causing protein. J Mol Med 2006;84:635–46.
[104] Wang Y, McAlpine SR. N-terminal and C-terminal modulation of Hsp90 produce dissimilar phenotypes. Chem Commun 2015;51:1410–3.
[105] Tavaria M, Gabriele T, Anderson RL, Mirault ME, Baker E, Sutherland G, et al. Localization of the gene encoding the human heat-shock cognate protein, HSP73, to chromosome-11. Genomics 1995;29:266–8.

[106] Dworniczak B, Mirault ME. Structure and expression of a human gene coding for a 71 kd heat shock "cognate" protein. Nucleic Acids Res 1987;15:5181–97.
[107] Wu B, Hunt C, Morimoto R. Structure and expression of the human-gene encoding major heat-shock protein HSP70. Mol Cell Biol 1985;5:330–41.
[108] Milner CM, Campbell RD. Structure and expression of the 3 MHC-linked HSP70 genes. Immunogenetics 1990;32: 242–51.
[109] Hendershot LM, Valentine VA, Lee AS, Morris SW, Shapiro DN. Localization of the gene encoding human BIP/GRP78, the endoplasmic-reticulum cognate of the HSP70 family, to chromosome-9Q34. Genomics 1994;20:281–4.
[110] Ting J, Lee AS. Human-gene encoding the 78,000-dalton glucose-regulated protein and its pseudogene—structure, conservation, and regulation. DNA-J Mol Cell Biol 1988;7: 275–86.
[111] Bhattacharyya T, Karnezis AN, Murphy SP, Hoang T, Freeman BC, Phillips B, et al. Cloning and subcellular localization of human mitochondrial HSP70. J Biol Chem 1995;270:1705–10.
[112] Schaffitzel E, Rudiger S, Bukau B, Deuerling E. Functional dissection of trigger factor and DnaK: interactions with nascent polypeptides and thermally denatured proteins. Biol Chem 2001;382:1235–43.
[113] Frydman J. Folding of newly translated proteins *in vivo*: the role of molecular chaperones. Annu Rev Biochem 2001;70: 603–47.
[114] Preissler S, Deuerling E. Ribosome-associated chaperones as key players in proteostasis. Trends Biochem Sci 2012; 37:274–83.
[115] Chiang H, Terlecky, Plant C, Dice J. A role for a 70-kilodalton heat shock protein in lysosomal degradation of intracellular proteins. Science 1989;246:382–5.
[116] Bercovich B, Stancovski I, Mayer A, Blumenfeld N, Laszlo A, Schwartz AL, et al. Ubiquitin-dependent degradation of certain protein substrates *in vitro* requires the molecular chaperone Hsc70. J Biol Chem 1997;272:9002–10.
[117] Kobayashi Y, Kume A, Li M, Doyu M, Hata M, Ohtsuka K, et al. Chaperones Hsp70 and Hsp40 suppress aggregate formation and apoptosis in cultured neuronal cells expressing truncated androgen receptor protein with expanded polyglutamine tract. J Biol Chem 2000;275:8772–8.
[118] Yu A, Shibata Y, Shah B, Calamini B, Lo DC, Morimoto RI. Protein aggregation can inhibit clathrin-mediated endocytosis by chaperone competition. Proc Natl Acad Sci USA 2014;111:E1481–90.
[119] Mayer MP, Bukau B. Hsp70 chaperones: cellular functions and molecular mechanism. Cell Mol Life Sci 2005;62: 670–84.
[120] Liberek K, Marszalek J, Ang D, Georgopoulos C, Zylicz M. *Escherichia coli* DnaJ and GrpE heat shock proteins jointly stimulate ATPase activity of DnaK. Proc Natl Acad Sci USA 1991;88:2874–8.
[121] Wall D, Zylicz M, Georgopoulos C. The NH2-terminal 108 amino-acids of the *Escherichia coli* DNAJ proteins stimulate the ATPase activity of DNAK and are sufficient for lambda-replication. J Biol Chem 1994;269:5446–51.
[122] Corsi AK, Schekman R. The lumenal domain of Sec63p stimulates the ATPase activity of BiP and mediates BiP recruitment to the translocon in Saccharomyces cerevisiae. J Cell Biol 1997;137:1483–93.
[123] Shi YY, Hong XG, Wang CC. The C-terminal (331-376) sequence of *Escherichia coli* DnaJ is essential for dimerization and chaperone activity—a small angle X-ray scattering study in solution. J Biol Chem 2005;280: 22761–8.
[124] Sha BD, Lee S, Cyr DM. The crystal structure of the peptide-binding fragment from the yeast Hsp40 protein Sis1. Struct Fold Des 2000;8:799–807.
[125] Ciocca DR, Calderwood SK. Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications. Cell Stress Chaperones 2005;10:86–103.
[126] Gabai VL, Zamulaeva IV, Mosin AF, Makarova YM, Mosina VA, Budagova KR, et al. Resistance of Ehrlich tumor cells to apoptosis can be due to accumulation of heat shock proteins. FEBS Lett 1995;375:21–6.
[127] Gabai VL, Meriin AB, Mosser DD, Caron AW, Rits S, Shifrin VI, et al. Hsp70 prevents activation of stress kinases. A novel pathway of cellular thermotolerance. J Biol Chem 1997;272:18033–7.
[128] Gabai VL, Yaglom JA, Waldman T, Sherman MY. Heat shock protein Hsp72 controls oncogene-induced senescence pathways in cancer cells. Mol Cell Biol 2009;29: 559–69.
[129] Pocaly M, Lagarde V, Etienne G, Ribeil JA, Claverol S, Bonneu M, et al. Overexpression of the heat-shock protein 70 is associated to imatinib resistance in chronic myeloid leukemia. Leukemia 2006;21:93–101.
[130] Liberek K, Lewandowska A, Ziętkiewicz S. Chaperones in control of protein disaggregation. EMBO J 2008;27:328–35.
[131] Magrané J, Smith RC, Walsh K, Querfurth HW. Heat shock protein 70 participates in the neuroprotective response to intracellularly expressed β-amyloid in neurons. J Neurosci 2004;24:1700–6.
[132] Veereshwarayya V, Kumar P, Rosen KM, Mestril R, Querfurth HW. Differential effects of mitochondrial heat shock protein 60 and related molecular chaperones to prevent intracellular β-amyloid-induced inhibition of complex IV and limit apoptosis. J Biol Chem 2006;281: 29468–78.
[133] Dou F, Netzer WJ, Tanemura K, Li F, Hartl FU, Takashima A, et al. Chaperones increase association of tau protein with microtubules. Proc Natl Acad Sci 2003;100:721–6.
[134] Petrucelli L, Dickson D, Kehoe K, Taylor J, Snyder H, Grover A, et al. CHIP and Hsp70 regulate tau ubiquitination, degradation and aggregation. Hum Mol Genet 2004;13: 703–14.
[135] Warrick JM, Chan HY, Gray-Board GL, Chai Y, Paulson HL, Bonini NM. Suppression of polyglutamine-mediated neurodegeneration in *Drosophila* by the molecular chaperone HSP70. Nat Genet 1999;23:425–8.
[136] Wang AM, Miyata Y, Klinedinst S, Peng H-M, Chua JP, Komiyama T, et al. Activation of Hsp70 reduces neurotoxicity by promoting polyglutamine protein degradation. Nat Chem Biol 2013;9:112–8.
[137] Auluck PK, Chan HYE, Trojanowski JQ, Lee VMY, Bonini NM. Chaperone suppression of alpha-synuclein toxicity in a *Drosophila* model for Parkinson's disease. Science 2002; 295:865–8.
[138] Massey AJ. ATPases as drug targets: insights from heat shock proteins 70 and 90. J Med Chem 2010;53:7280–6.
[139] Williams DR, Ko S-K, Park S, Lee M-R, Shin I. An apoptosis-inducing small molecule that binds to heat shock protein 70. Angew Chem Int Ed 2008;47:7466–9.
[140] Cho HJ, Gee HY, Baek K-H, Ko S-K, Park J-M, Lee H, et al. A small molecule that binds to an ATPase domain of Hsc70 promotes membrane trafficking of mutant cystic fibrosis

transmembrane conductance regulator. J Am Chem Soc 2011;133:20267–76.
[141] Williamson DS, Borgognoni J, Clay A, Daniels Z, Dokurno P, Drysdale MJ, et al. Novel adenosine-derived inhibitors of 70 kDa heat shock protein, discovered through structure-based design. J Med Chem 2009;52:1510–3.
[142] Li J, Lee AS. Stress induction of GRP78/BiP and its role in cancer. Curr Mol Med 2006;6:45–54.
[143] Macias AT, Williamson DS, Allen N, Borgognoni J, Clay A, Daniels Z, et al. Adenosine-derived inhibitors of 78 kDa glucose regulated protein (Grp78) ATPase: insights into isoform selectivity. J Med Chem 2011;54:4034–41.
[144] Brocchieri L, de Macario EC, Macario AJL. hsp70 genes in the human genome: conservation and differentiation patterns predict a wide array of overlapping and specialized functions. BMC Evol Biol 2008;8:19.
[145] Howe Matthew K, Bodoor K, Carlson David A, Hughes Philip F, Alwarawrah Y, Loiselle David R, et al. Identification of an allosteric small-molecule inhibitor selective for the inducible form of heat shock protein 70. Chem Biol 2014;21:1648–59.
[146] Kang Y, Taldone T, Patel HJ, Patel PD, Rodina A, Gozman A, et al. Heat shock protein 70 inhibitors. 1. 2,5′-Thiodipyrimidine and 5-(phenylthio)pyrimidine acrylamides as irreversible binders to an allosteric site on heat shock protein 70. J Med Chem 2014;57:1188–207.
[147] Wisen S, Bertelsen EB, Thompson AD, Patury S, Ung P, Chang L, et al. Binding of a small molecule at a protein–protein interface regulates the chaperone activity of hsp70-hsp40. ACS Chem Biol 2010;5:611–22.
[148] Wisén S, Gestwicki JE. Identification of small molecules that modify the protein folding activity of heat shock protein 70. Anal Biochem 2008;374:371–7.
[149] Chang L, Bertelsen EB, Wisen S, Larsen EM, Zuiderweg ER, Gestwicki JE. High-throughput screen for small molecules that modulate the ATPase activity of the molecular chaperone DnaK. Anal Biochem 2008;372:167–76.
[150] Wisen S, Androsavich J, Evans CG, Chang L, Gestwicki JE. Chemical modulators of heat shock protein 70 (Hsp70) by sequential, microwave-accelerated reactions on solid phase. Bioorg Med Chem Lett 2008;18:60–5.
[151] Fewell SW, Smith CM, Lyon MA, Dumitrescu TP, Wipf P, Day BW, et al. Small molecule modulators of endogenous and co-chaperone-stimulated Hsp70 ATPase activity. J Biol Chem 2004;279:51131–40.
[152] Fewell SW, Day BW, Brodsky JL. Identification of an inhibitor of hsc70-mediated protein translocation and ATP hydrolysis. J Biol Chem 2001;276:910–4.
[153] Nadler S, Tepper M, Schacter B, Mazzucco C. Interaction of the immunosuppressant deoxyspergualin with a member of the Hsp70 family of heat shock proteins. Science 1992;258:484–6.
[154] Nadeau K, Nadler SG, Saulnier M, Walsh CT, Tepper MA. Quantitation of the interaction of the immunosuppressant deoxyspergualin and analogs with Hsc70 and Hsp90. Biochemistry 1994;33:2561–7.
[155] Kawada M, Masuda T, Ishizuka M, Takeuchi T. 15-Deoxyspergualin inhibits Akt kinase activation and phosphatidylcholine synthesis. J Biol Chem 2002;277:27765–71.
[156] Nishimura K, Ohki Y, Fukuchi-Shimogori T, Sakata K, Saiga K, Beppu T, et al. Inhibition of cell growth through inactivation of eukaryotic translation initiation factor 5A (eIF5A) by deoxyspergualin. Biochem J 2002;363:761–8.
[157] Cesa LC, Patury S, Komiyama T, Ahmad A, Zuiderweg ERP, Gestwicki JE. Inhibitors of difficult protein–protein interactions identified by high-throughput screening of multiprotein complexes. ACS Chem Biol 2013;8:1988–97.
[158] Cassel JA, Ilyin S, McDonnell ME, Reitz AB. Novel inhibitors of heat shock protein Hsp70-mediated luciferase refolding that bind to DnaJ. Bioorg Med Chem 2012;20:3609–14.
[159] Massey A, Williamson D, Browne H, Murray J, Dokurno P, Shaw T, et al. A novel, small molecule inhibitor of Hsc70/Hsp70 potentiates Hsp90 inhibitor induced apoptosis in HCT116 colon carcinoma cells. Cancer Chemother Pharmacol 2010;66:535–45.
[160] Koya K, Li Y, Wang H, Ukai T, Tatsuta N, Kawakami M, et al. MKT-077, a novel rhodacyanine dye in clinical trials, exhibits anticarcinoma activity in preclinical studies based on selective mitochondrial accumulation. Cancer Res 1996;56:538–43.
[161] Leu JI, Pimkina J, Frank A, Murphy ME, George DL. A small molecule inhibitor of inducible heat shock protein 70. Mol Cell 2009;36:15–27.
[162] Koren J, Miyata Y, Kiray J, O'Leary JC, Nguyen L, Guo J, et al. Rhodacyanine derivative selectively targets cancer cells and overcomes tamoxifen resistance. PLoS One 2012;7:e35566.
[163] Miyata Y, Koren J, Kiray J, Dickey CA, Gestwicki JE. Molecular chaperones and regulation of tau quality control: strategies for drug discovery in tauopathies. Future Med Chem 2011;3:1523–37.
[164] Brodsky JL. Selectivity of the molecular chaperone-specific immunosuppressive agent 15-deoxyspergualin: modulation of Hsc70 ATPase activity without compromising DnaJ chaperone interactions. Biochem Pharmacol 1999;57:877–80.
[165] Schlecht R, Scholz SR, Dahmen H, Wegener A, Sirrenberg C, Musil D, et al. Functional analysis of Hsp70 inhibitors. PLoS One 2013;8:e78443.
[166] Kusmierczyk AR, Martin J. Chaperonins—keeping a lid on folding proteins. FEBS Lett 2001;505:343–7.
[167] Hemmingsen SM, Woolford C, van der Vies SM, Tilly K, Dennis DT, Georgopoulos CP, et al. Homologous plant and bacterial proteins chaperone oligomeric protein assembly. Nature 1988;333:330–4.
[168] Cheng MY, Hartl FU, Martin J, Pollock RA, Kalousek F, Neuper W, et al. Mitochondrial heat-shock protein hsp60 is essential for assembly of proteins imported into yeast mitochondria. Nature 1989;337:620–5.
[169] Braig K, Otwinowski Z, Hegde R, Boisvert DC, Joachimiak A, Horwich AL, et al. The crystal structure of the bacterial chaperonin GroEL at 2.8 Å. Nature 1994;371:578–86.
[170] Hendrix RW. Purification and properties of groE, a host protein involved in bacteriophage assembly. J Mol Biol 1979;129:375–92.
[171] Hohn T, Hohn B, Engel A, Wurtz M, Smith PR. Isolation and characterization of the host protein groE involved in bacteriophage lambda assembly. J Mol Biol 1979;129:359–73.
[172] Braig K, Simon M, Furuya F, Hainfeld JF, Horwich AL. A polypeptide bound by the chaperonin groEL is localized within a central cavity. Proc Natl Acad Sci USA 1993;90:3978–82.
[173] Fenton WA, Horwich AL. Chaperonin-mediated protein folding: fate of substrate polypeptide. Q Rev Biophys 2003;36:229–56.
[174] Reading DS, Hallberg RL, Myers AM. Characterization of the yeast HSP60 gene coding for a mitochondrial assembly factor. Nature 1989;337:655–9.

[175] Trent JD, Nimmesgern E, Wall JS, Hartl FU, Horwich AL. A molecular chaperone from a thermophilic archaebacterium is related to the eukaryotic protein t-complex polypeptide-1. Nature 1991;354:490–3.
[176] Frydman J, Nimmesgern E, Erdjument-Bromage H, Wall JS, Tempst P, Hartl FU. Function in protein folding of TRiC, a cytosolic ring complex containing TCP-1 and structurally related subunits. EMBO J 1992;11:4767–78.
[177] Bross P, Naundrup S, Hansen J, Nielsen MN, Christensen JH, Kruhoffer M, et al. The Hsp60-(p.V98I) mutation associated with hereditary spastic paraplegia SPG13 compromises chaperonin function both *in vitro* and *in vivo*. J Biol Chem 2008;283:15694–700.
[178] Hansen JJ, Durr A, Cournu-Rebeix I, Georgopoulos C, Ang D, Nielsen MN, et al. Hereditary spastic paraplegia SPG13 is associated with a mutation in the gene encoding the mitochondrial chaperonin Hsp60. Am J Hum Genet 2002; 70:1328–32.
[179] Stone DL, Slavotinek A, Bouffard GG, Banerjee-Basu S, Baxevanis AD, Barr M, et al. Mutation of a gene encoding a putative chaperonin causes McKusick-Kaufman syndrome. Nat Genet 2000;25:79–82.
[180] Slavotinek AM, Stone EM, Mykytyn K, Heckenlively JR, Green JS, Heon E, et al. Mutations in MKKS cause Bardet-Biedl syndrome. Nat Genet 2000;26:15–6.
[181] Lee MJ, Stephenson DA, Groves MJ, Sweeney MG, Davis MB, An SF, et al. Hereditary sensory neuropathy is caused by a mutation in the delta subunit of the cytosolic chaperonin-containing t-complex peptide-1 (Cct4) gene. Hum Mol Genet 2003;12:1917–25.
[182] Cappello F, Conway de Macario E, Marasà L, Zummo G, Macario AJL. Hsp60 expression, new locations, functions, and perspectives for cancer diagnosis and therapy. Cancer Biol Ther 2008;7:801–9.
[183] Ghosh JC, Dohi T, Kang BH, Altieri DC. Hsp60 regulation of tumor cell apoptosis. J Biol Chem 2008;283:5188–94.
[184] Di Felice V, Ardizzone N, Marciano V, Bartolotta T, Cappello F, Farina F, et al. Senescence-associated HSP60 expression in normal human skin fibroblasts. Anat Rec A Discov Mol Cell Evol Biol 2005;284:446–53.
[185] Cappello F, Zummo G. HSP60 expression during carcinogenesis: a molecular "Proteus" of carcinogenesis? Cell Stress Chaperones 2005;10:263–4.
[186] Itoh H, Komatsuda A, Wakui H, Miura AB, Tashima Y. Mammalian HSP60 is a major target for an immunosuppressant mizoribine. J Biol Chem 1999;274:35147–51.
[187] Chapman E, Farr GW, Furtak K, Horwich AL. A small molecule inhibitor selective for a variant ATP-binding site of the chaperonin GroEL. Bioorg Med Chem Lett 2009;19:811–3.
[188] Johnson SM, Sharif O, Mak PA, Wang HT, Engels IH, Brinker A, et al. A biochemical screen for GroEL/GroES inhibitors. Bioorg Med Chem Lett 2014;24:786–9.
[189] Neef DW, Turski ML, Thiele DJ. Modulation of heat shock transcription factor 1 as a therapeutic target for small molecule intervention in neurodegenerative disease. PLoS Biol 2010;8:e1000291.
[190] Neef Daniel W, Jaeger Alex M, Gomez-Pastor R, Willmund F, Frydman J, Thiele Dennis J. A direct regulatory interaction between chaperonin TRiC and stress-responsive transcription factor HSF1. Cell Rep 2014;9:955–66.
[191] Nagumo Y, Kakeya H, Shoji M, Hayashi Y, Dohmae N, Osada H. Epolactaene binds human Hsp60 Cys(442) resulting in the inhibition of chaperone activity. Biochem J 2005;387:835–40.
[192] Bergeron LM, Shis DL, Gomez L, Clark DS. Small molecule inhibition of a Group II chaperonin: pinpointing a loop region within the equatorial domain as necessary for protein refolding. Arch Biochem Biophys 2009;481:45–51.
[193] Cassiano C, Monti MC, Festa C, Zampella A, Riccio R, Casapullo A. Chemical proteomics reveals heat shock protein 60 to be the main cellular target of the marine bioactive sesterterpene suvanine. ChemBioChem 2012;13: 1953–8.
[194] Cappello F, Marino Gammazza A, Palumbo Piccionello A, Campanella C, Pace A, Conway de Macario E, et al. Hsp60 chaperonopathies and chaperonotherapy: targets and agents. Expert Opin Ther Targets 2013;18:185–208.
[195] Garcia-Carbonero R, Carnero A, Paz-Ares L. Inhibition of HSP90 molecular chaperones: moving into the clinic. Lancet Oncol 2013;14:e358–69.
[196] Davis MI, Hunt JP, Herrgard S, Ciceri P, Wodicka LM, Pallares G, et al. Comprehensive analysis of kinase inhibitor selectivity. Nat Biotechnol 2011;29:1046–51.
[197] Patricelli MP, Nomanbhoy TK, Wu J, Brown H, Zhou D, Zhang J, et al. *In situ* kinase profiling reveals functionally relevant properties of native kinases. Chem Biol 2011;18: 699–710.
[198] Jester BW, Cox KJ, Gaj A, Shomin CD, Porter JR, Ghosh I. A coiled-coil enabled split-luciferase three-hybrid system: applied toward profiling inhibitors of protein kinases. J Am Chem Soc 2010;132:11727–35.
[199] Kamal A, Thao L, Sensintaffar J, Zhang L, Boehm MF, Fritz LC, et al. A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors. Nature 2003;425: 407–10.
[200] Chiosis G, Lucas B, Shtil A, Huezo H, Rosen N. Development of a purine-scaffold novel class of Hsp90 binders that inhibit the proliferation of cancer cells and induce the degradation of Her2 tyrosine kinase. Bioorg Med Chem 2002;10:3555–64.
[201] Lundgren K, Zhang H, Brekken J, Huser N, Powell RE, Timple N, et al. BIIB021, an orally available, fully synthetic small-molecule inhibitor of the heat shock protein Hsp90. Mol Cancer Ther 2009;8:921–9.
[202] Menezes DL, Taverna P, Jensen MR, Abrams T, Stuart D, Yu GK, et al. The novel oral Hsp90 inhibitor NVP-HSP990 exhibits potent and broad-spectrum antitumor activities *in vitro* and *in vivo*. Mol Cancer Ther 2012;11:730–9.