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Review

Inhibition of Hsp90: a new strategy for inhibiting protein kinases

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Abstract

The 90-kDa heat shock protein (Hsp90) is a ubiquitous, evolutionarily highly conserved, molecular chaperone in the eukaryotic cytosol. Hsp90, together with a number of other chaperones, promotes the conformational maturation of a large variety of protein kinases. Inhibition of Hsp90 function results in the collapse of the metastable conformation of most of these kinases and leads to their proteolytic elimination by the proteasome. Numerous natural and synthetic Hsp90 inhibitors have been developed in recent years. Some of these inhibitors are also involved in sensitizing tumor cells to pro-apoptotic insults, hence serve as anti-cancer drugs. Here we review these novel protein kinase inhibitors and their emerging role in various cellular processes, apart from their inhibition of Hsp90 protein function. We focus not only on Hsp90-tumor progression, but also on cytoarchitecture, as the higher levels of cellular organization need constant remodeling, where the role of Hsp90 requires investigation. Our last major aspect deals with protein oxidation, since several Hsp90 inhibitors exert pro-oxidant effects.

Keywords: Chaperone; Cisplatin; Hsp90; Heat shock protein; Geldanamycin; Protein kinase inhibitor

1. Introduction: chaperones and their roles in health and disease

Heat shock proteins (Hsp-s) are highly conserved ubiquitous proteins among species. They are inducible by a variety of stressors but their constitutive expression under non-stressful conditions shows their important role in the maintenance of cellular homeostasis. Hsp-s are involved in maintaining appropriate folding and conformation of other proteins, hence most of them can also be referred to "molecular chaperones". They also help to transport proteins from one compartment to another inside the cell, and present damaged proteins to proteasomal degradation [1–4]. Hsp-s are also believed to play a role in antigen-presentation and to serve as "danger signals" to help the immune system recognize dead or damaged cells [5–7].

The accumulation of Hsp-s is seen not only in stressful conditions, but also in many pathophysiological conditions and tumors. This accumulation helps in cell recovery by refolding partially damaged functional proteins and also by increasing the association of cell survival factors and stabilizing them. Many types of tumors are associated with high expression of multiple Hsp-s compared to the normal parental cells [8,9]. In some instances, the differential expression of Hsp-s specifies the grade and type of tumor [10–15]. Conditions like Alzheimer's disease, prion disease, and Huntington disease, where the accumulation of misfolded proteins is the major cause of neurodegenerative disorders [16–20], require therapies, which induce Hsp overexpression.

There are multiple Hsp-s which are classified according to their molecular weight to the following major Hsp families: Hsp100, Hsp90, Hsp70, Hsp60 and the large family of small Hsp-s [21,22]. Despite the fact that the structure and function of these proteins vary between and

Abbreviations: 17AAG, 17-allylamino-17-demethoxy-geldanamycin; Cdk, cyclin-dependent kinase; CK II, CK refers to the old name of protein kinase CK II, casein kinase; EGF, epidermal growth factor; ER, endoplasmic reticulum; Grp94, 94-kDa glucose regulated protein, the endoplasmic reticulum homologue of Hsp90; HSF, heat shock factor; Hsp, heat shock protein; Hsp70, the 70-kDa heat shock protein; Hsp90, the 90-kDa heat shock protein; ILGF, insulin-like growth factor; MEK, mitogen activated protein kinase kinase; MOK, mitogen activated protein kinase; MAK, male germ cell associated kinase; NQQ1, NADH quinone oxidoreductase, DT-diaphorase; PDGF, platelet-derived growth factor; PKB, protein kinase B; PU3 and PU24F-Cl, purine-based Hsp90 inhibitors; RTK, receptor tyrosine kinase

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within families, they all work co-operatively at different stages of protein folding [3,23]. Both Hsp90 and Hsp70 are known to be associated with a number of signaling molecules, including v-Src, Raf1, Akt and steroid receptors, suggesting an important role for these proteins in malignant transformation and metastasis development [23,24]. The discovery of some natural products, like geldanamycin and radicicol, with antitumor activity through inhibition of Hsp90 function, opened a new era of therapeutic targeting Hsp90 [25–27].

2. Hsp90: a chaperone with an unusual ATP-binding site, the Bergerat-fold

Hsp90 is one of the most abundant proteins in the eukaryotic cells, comprising 1–2% under non-stress conditions. It is evolutionarily conserved among species, and is proven essential for cell survival. Its contribution to various cellular processes, including signal transduction, protein folding and degradation, and morphological evolution, has been extensively studied. Hsp90 is primarily a cytosolic protein, but a small portion rapidly accumulates in cell nuclei upon stress [28–30]. There are two isoforms for this protein identified as Hsp90-a (inducible form/major form) and Hsp90-b (constitutive form/minor form). Its analogues include Grp94 in the endoplasmic reticulum (ER), and Hsp75/TRAP1 in the mitochondrial matrix [28]. The full functional activity of Hsp90 is displayed in concert with other co-chaperones, playing an important role in the folding of newly synthesized proteins and stabilization and refolding of denatured proteins after stress.

Hsp90 is mainly a constitutive homodimer (aa or bb), but its monomers, heterodimers (ab) and higher oligomers are also present. Its dimerization potential resides mainly at the carboxy-terminal 190 amino acids [31–35]. Hsp90 is a phosphoprotein containing two to three covalently bound phosphate molecules per monomer [36], and the phosphorylation is thought to enhance its function. The monomer of Hsp90 consists of a conserved 25-kDa N-terminal domain and a 55-kDa C-terminal domain linked by a 35-kDa charged linker region. Together with the C-terminal domain, this linker region helps in the dimerization of the protein [37]. Both termini are reported to bind to substrate polypeptides, including client proteins and co-chaperones.

The N-terminal domain of Hsp90 contains an ATP binding site. This site is rather unique including a Bergerat-fold characteristic of bacterial gyrases, topoisomerases and histidine-kinases [23,26,27,37]. The unique ATP binding site allowed the development of specific Hsp90 inhibitors, which we will describe later. Hsp90 is an ATP-dependent chaperone. ATP binding helps dimerization because of a change in Hsp90 conformation. Hsp90 also exhibits ATPase activity that is necessary for its chaperone function [28,29,37]. Recently it was shown that Hsp90 contains a second nucleotide binding site at the C-terminal domain [38–40]. However, the biological significance of this second nucleotide binding site needs to be further elucidated. Both the N- and C-terminal domains have been implicated in binding of substrate polypeptides [30,41]. The tetratricopeptide repeat present in Hsp90 co-chaperones binds to the C-terminal MEEVD motif, and these interactions help in forming the chaperone complex. Apart from its co-chaperones, Hsp90 binds to an array of client proteins, where its co-chaperone specificity varies and depends on the actual client. There is a growing list of Hsp90 client proteins and, interestingly, most of them include molecules involved in signal transduction [23].

Hsp90 forms several discrete sub-complexes, each containing a different set of co-chaperones that function at different steps during the folding process of the client protein [23,42]. Unlike other chaperones, Hsp90 contains two independent chaperone sites that differ in their substrate specificity [30], probably working in the form of a switch between Hsp90 and Hsp70 client protein interactions. The best understood molecular association of Hsp90-multichaperone complexes was elucidated in conjunction with the maturation of steroid receptors. The folding process of steroid receptors and their translocation to the cell nucleus requires Hsp90. Steroid receptors also require molecular chaperones for their ligand binding, transcriptional activation and repression after stimulus [23,43]. Though there are reports that some Hsp90 co-chaperones can work independently of Hsp90 in preventing the aggregation of misfolded protein substrates, the full competence of these co-chaperones in folding-assistance requires Hsp90 [29]. Co-chaperones also help Hsp90-client protein binding [44], interactions between Hsp70 client proteins [45], and docking of cytoskeletal proteins [46,47].

3. Hsp90-dependent signal transduction

Over the years, many different tyrosine and serine/threonine protein kinases have been selected as potential pharmacological targets in antitumor therapies, based either on their overexpression and/or dysfunction in a particular organ or tissue, or through their association in deregulated signal transduction/cell cycle pathways. Our current understanding is that a number of distinct tyrosine kinases play a role in diverse but fundamentally important aspects of tumor progression, such as growth, survival, metastasis and angiogenesis. Hsp90's role is implicated in many kinases from both tyrosine and serine/threonine family members. At the cellular level, the ability of a cell to know whether to grow, divide, differentiate or die depends upon extracellular signals and the ability to respond to these signals in an orchestrated manner. Several molecules like hormones, small peptides, surface proteins from other cells, etc. are involved in initiating these transduction mechanism through tyrosine and serine/threonine kinase cascades.

3.1. Tyrosine kinases

Hsp90 interacts with and stabilizes a growing list of various kinases (Table 1). One of the major receptor tyrosine kinases (RTKs) is ErbB2 (HER2 or *neu*). This kinase either works alone or in homo/hetero-complexes with its homologues. ErbB2 was first identified as an oncogene, hence the down-regulation of ErbB2 signaling emerged as an anti-cancer strategy [48,49]. ErbB2 was shown to be an in vitro substrate for Hsp90 chaperone complex, and inhibition of Hsp90 results in the dissociation of ErbB2 from the Hsp90 chaperone complex [50].

The Src (Rouse sarcoma virus, $p60^{src}$) family of tyrosine kinases was implicated in signal transduction following growth factor stimulation and integrin-mediated cell-substrate adhesion. Both v-Src and c-Src bind to Hsp90, where the chaperone maintains the kinase in an inactive form, and helps in its membrane recruitment, suggesting that the Src activity is governed by Hsp90 [51].

Abelson leukemia virus tyrosine kinase (v-Abl) and its cellular counterpart, c-Abl, share sequence homology with Src members. Hsp90 affects the function and stability of these kinases [52,53]. At the nuclear level the Wee1 tyrosine kinase phosphorylates the mitotic regulator Cdk1 (Cdc2), preventing mitosis during S phase, and delaying it in response to DNA damage or developmental signals during G2 phase [54,55]. Hsp90 is required for the assembly and/or disassembly of the functional Wee1 protein complex [56,57]. One RTK is c-Met, which stimulates invasive growth of carcinoma cells, is tumorigenic, and overexpressed in many solid tumors [58]. c-Met overexpression, as well as activating c-Met mutations, can lead to carcinogenesis. c-Met, on activation by autophosphorylation, can associate with, and activate, multiple signal transducing intermediates, such as Grb2, the p85 subunit of phosphatidyl-inositol-3-kinase (PI-3-kinase), Stat-3, and Gab1 [59]. Though there is no direct association of Hsp90 and the c-Met/HGF pathway, coordinated regulation of c-Met and Hsp90 levels has been reported [60].

Table 1
Protein kinases forming a complex with Hsp90, its endoplasmic homologue, Grp94, or both[a]

Protein kinase	References[b]
Tyrosine kinases	
v-Src, c-Src	[51]
v-Fes, c-Fes, v-Fps, v-Ros, v-Yes	
Lck, v-Fgr, c-Fgr	
v-Abl, c-Abl	[52,53]
Wee1	[56,57]
Sevenless	
c-Met	[58]
p75-v-erbA	
p185erbB2	[50]
Insulin, ILGF, EGF, PDGF receptors	
Serine-threonine kinases	
v-Raf, c-Raf, B-Raf, Gag-Mil, Ste11	[61]
Akt (PKB)	[64]
Kinase suppressor of Ras	
MEK, MOK, MAK, MAK-related kinase	
Cdc2, Cdk4, Cdk6, Cdk9	[65,66,68]
Polo mitotic kinase	
eIF-2-α kinase	[63]
dsRNA-dependent kinase	
eEF-2-α kinase	
Protein kinase CK-II	[62]
Tropomyosin related kinase	

[a] Most of the interacting kinases are stabilized by Hsp90, however, protein kinase CK II is protected only after stress.
[b] When no reference is given, refer to reviews [23,28].

3.2. Serine/threonine kinases

The most important serine/threonine kinase members involved in malignant transformation and tumor progression include the major signaling cascades consisting of the Ras, Raf, MEK, and mitogen-activated protein kinase (MAPK) proteins. Raf-1 is the most extensively studied member of the Raf family. Active Ras in its GTP-bound state binds to the amino-terminal regulatory domain of Raf-1, leading to the recruitment of Raf-1 to the cell membrane. Raf-1 is primarily located in the cytosol, and the cytosolic Raf-1 exists in a complex with Hsp90, where Hsp90 binding to Raf is shown to be required for its activity [61].

Both protein kinase CK II [62] and the heme-regulated eukaryotic initiation factor (eIF-2a) kinase were identified in a complex with Hsp90 [63]. Apart from its tight binding to Hsp90, protein kinase CK II also phosphorylates both isoforms of Hsp90, as well as the ER-resident Grp94 [28]. Hsp90 also binds both co-translationally as well as post-translationally to eIF-2a kinase, and this binding is essential for maintaining the activity of eIF-2a kinase.

Protein kinase B or Akt (Akt/PKB) is a downstream target for phosphatidyl-inositol-3-kinase (PI-3-kinase) involved in the regulation of cell growth. Several studies showed that Akt forms complexes with Hsp90, which enhance cell survival. However, this complex formation requires Cdc37, a co-chaperone of Hsp90 [64].

At the nuclear level the major and initial cell cycle transition regulators, the Cdk4/Cdk6 kinases, were also shown to form complexes with Hsp90. As is characteristic for most Hsp90-kinase complexes, binding of Cdk4/Cdk6 kinases to Hsp90 also involves their association with the co-chaperone, Cdc37 [65,66]. It was recently shown that another cyclin-dependent kinase, Cdk9, acts preferentially as a control of transcription and the balance between differentiation and apoptosis, suggesting that this kinase can serve as a switch between many important cellular processes [67]. Although Hsp70 is a general chaperone for Cdk9, Hsp90 has been shown to play a role in the regulation of Cdk9 via Cdc37 [68].

Apart from protein kinases, Hsp90 interacts with a number of transcription factors and other proteins [23]. As already mentioned, the role of Hsp90 in glucocorticoid receptor (GR) and progesterone receptor (PR) regulation is the best studied

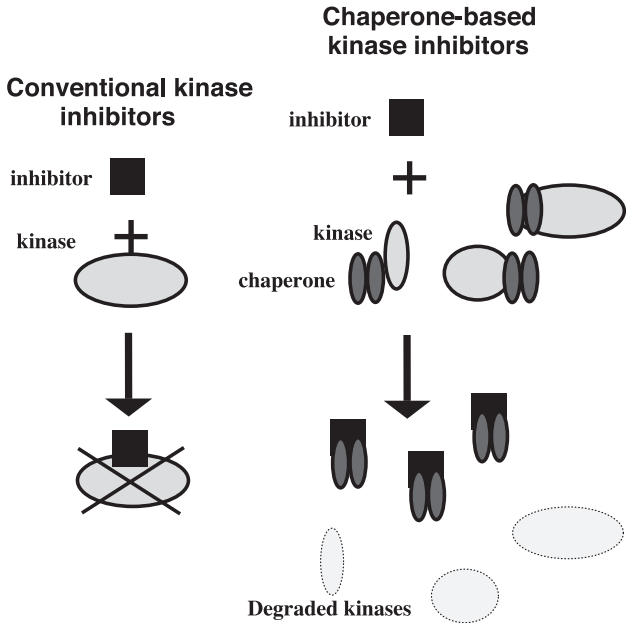

Fig. 1. Differences between "conventional" and chaperone-based inhibition of protein kinases. "Conventional" protein kinase inhibitors interact with the kinase, and directly inhibit its enzyme action. However, most kinases require molecular chaperones to maintain their activation-competent conformation [23,28]. Chaperone-based inhibitors do not interact with the protein kinase itself, but prevent the associated chaperone(s) from maintaining the activation-competent conformation of the kinase. As a result, large amounts of the kinase become degraded by the proteasome. In contrast to most direct kinase inhibitors, which are fairly specific for a given protein kinase, chaperone-based inhibitors diminish the level and consequently the activity of many kinases in parallel. Since the 90-kDa molecular chaperone (Hsp90) has the most specific and most cell-permeable inhibitors, and since this chaperone is the center of the kinase-related chaperone machinery, in most cases, chaperone-based kinase inhibition is achieved by using Hsp90 inhibitors.

among the signaling events related to transcription factors. Hypoxia-inducible factor-1 (HIF-1a) is associated with hypoxia-induced transcription of genes together with the nuclear protein, aryl hydrocarbon receptor nuclear translocator (ARNT). The resulting HIF-1a/ARNT heterodimers interact specifically with the hypoxia-responsive element (HRE), and thereby increase transcription, where Hsp90 modulates the conformation of HIF-1a/ARNT heterodimers [69].

Additionally, Hsp90 binding has been shown to contribute to the accumulation of mutant forms of the tumor suppressor transcription factor, p53. Hsp90 is associated with several cytoskeletal proteins, members of the G-protein family, nitric oxide synthases, the anti-apoptotic protein, Apaf-1, etc. As the list of Hsp90-associated proteins is growing enormously [23], besides protein kinases, only some of the major molecular interactions are mentioned.

3.3. Differences between conventional and chaperone-based protein kinase inhibition

The above examples show that a large number of protein kinases need the help of molecular chaperones to maintain their activation-competent conformation [23,28]. "Conventional" protein kinase inhibitors interact with the kinase, and directly inhibit its enzyme action. However, chaperone-based inhibitors do not interact with the protein kinase itself, but inhibit the ability of the associated chaperone(s) to maintain the activation-competent conformation of the kinase (Fig. 1). As a result, large amounts of the kinase are degraded by the proteasome. In contrast to most direct kinases inhibitors, which are often fairly specific for a given protein kinase, chaperone-based inhibitors diminish the level of many kinases in parallel. Since the 90-kDa molecular chaperone (Hsp90) has the most specific and most cell-permeable inhibitors, and since this chaperone is the center of the kinase-related chaperone machinery, in most cases chaperone-based kinase inhibition is achieved by using Hsp90 inhibitors.

4. Hsp90 inhibitors

In several tumor models the selective inhibition of Hsp90 function causes a selective degradation of important signaling proteins that are involved in cell proliferation, cell cycle regulation, and apoptosis [70]. As a consequence of this, there are several Hsp90-specific drugs developed and some of them are already in clinical trials (Table 2).

4.1. Geldanamycin

The first Hsp90 inhibitor drug was geldanamycin, a natural product isolated from *Streptomyces hygroscopicus* [71]. Though the antitumor effects of geldanamycin were

Table 2
Hsp90 inhibitors[a]

Hsp90 inhibitor[a]	Company and web-site[b]	References
Specific inhibitors[c]		
Geldanamycin and analogues	Conforma Inc. (www.conforma.com); Kosan Bioscience (www.kosan.com)	[71–88]
Radicicol	Kyowa Hakko Kogyo Ltd. (www.kyowa.co.jp)	[89–96]
Purine-scaffold Hsp90 binders, PU3		[81,102,103]
Nonspecific inhibitors		
Cisplatin		[39,101]
Novobiocin		[38,39]
Taxol		[106]

[a] These inhibitors may also inhibit the ER homologue, Grp94; the mitochondrial homologue, Hsp75 and the bacterial homologue, HtpG. In many cases these "cross-reactions" have already been demonstrated.

[b] RiboTargets Co. (www.ribotargets.com) and Telik Co. (www.telik.com) also develop Hsp90 inhibitors.

[c] Probably none of these inhibitors is strictly specific for the Hsp90 chaperone family: many of them induce superoxide production independently of Hsp90 inhibition and radicicol also inhibits citrate lyase.

initially thought to be due to specific tyrosine kinase inhibition [72], later studies revealed that the antitumor potential relies on depletion of oncogenic protein kinases via the proteasome [73]. The major regulatory signaling proteins that are affected by geldanamycin include the proto-oncogene kinases ErbB2, EGF, v-Src, Raf-1 and Cdk4 [23].

Subsequent immunoprecipitation and X-ray crystallographic studies [74] revealed that geldanamycin directly binds to Hsp90, and inhibits the formation of Hsp90-multichaperone complexes resulting in the ubiquitin-mediated degradation of Hsp90 client proteins. Geldanamycin binds to the conserved N-terminal domain of Hsp90, and competes with ATP binding. The geldanamycin–Hsp90 crystal structure also shows that this binding inhibits substrate protein binding [74,75]. Geldanamycin also binds to Grp94, the Hsp90 analogue in the ER [76].

Though geldanamycin shows clear anti-tumor effects, it encountered difficulties in clinical trials due to its high hepatotoxicity in some of the human tumor models [77]. Thus, a search for new classes of Hsp90 inhibitors with lower toxicity began, and was successful in developing the analogue 17AAG (17-allylamino-17-demethoxy-geldanamycin). 17AAG possesses all the Hsp90-related characteristics of geldanamycin [78,79] with lower toxicity [80–82], and could enter Phase I clinical trials. Though 17AAG is metabolized to 17AG [17-amino-17-demethoxy-geldanamycin] by cytochrome P450 CYP3A4, this metabolite was found be stable, and retains both Hsp90 inhibitory and antitumor activities [83]. Both geldanamycin and 17AAG can be metabolized by NADH quinone oxidoreductase 1 (DT-diaphorase, NQO1), which is known to potentiate antitumor activity by stabilizing the tumor suppressor p53. NQQ1 may be a major factor in conferring on 17AAG, as well as its parent compound, the advantage that they specifically accumulate in tumor cells [81,82]. On the other hand, geldanamycin is likely to be a substrate for the P-glycoprotein multidrug resistance efflux transporter, and possibly also the related MRP efflux pump [82].

Several geldanamycin–steroid complexes [84,85] as well as ligand–geldanamycin compounds [86] were also developed, and showed selective effects in certain tumors. Interestingly, the comparison of various tumor and experimental models suggests that the mode of action of Hsp90 inhibitors vary. In some cases, they induce apoptosis [87] and in other cases they induce either differentiation or cytostasis [88].

4.2. Radicicol

Radicicol is a macrocyclic antibiotic isolated from *Monosporium bonorden*. Because of its potential to reverse the malignant phenotype like geldanamycin [89,90], it was initially thought to be a tyrosine kinase inhibitor. However, later studies showed its role in Hsp90 client protein degradation [91]. Radicicol is involved in the degradation of NQQ1, followed by degradation of mutant p53, which is involved in the malignant transformation. The antitumor potential of NQQ1 is due to its involvement in stabilizing the tumor suppressor protein p53 [92] and failure of this results in p53-mediated apoptosis. Radicicol inhibits peptide binding to Grp94 though the peptide and radicicol binding sites are different in Grp94 [93]. However, radicicol lacks antitumor activity in vivo in experimental models because of its instability. The oxime derivatives of radicicol [94] exhibit antitumor activity in vivo as well as in vitro, and hence serve as good anticancer drug candidates. Radicicol binds to the N-terminal domain of Hsp90 with much higher affinity than the structurally different drugs, geldanamycin and 17AAG [95]. Moreover, radicicol reduces hypoxia-induced VEGF expression, which is an efficient way to decrease hypoxia-induced angiogenesis [96].

4.3. Cisplatin

Cisplatin, *cis*-diammine-dichloro-platinum (II), was first identified 125 years ago. Its clinical development started in the 1970s for its effective antitumor activity in a wide variety of tumors [97,98]. Its ability to form DNA-adducts, thereby interfering with the DNA transcription, was thought to be the major cause of its antitumor activity [99]. Its non-DNA targets include phospholipids, especially phosphatidyl serine and RNA [97,98,100]. However, it also interacts with thiol-containing proteins and peptides, such as cytoskeletal proteins [97,98]. Specific binding of cisplatin to Hsp90 was also demonstrated [101]. Later studies showed that cisplatin binds to the C-terminal domain of Hsp90, and specifically interferes with nucleotide binding at this site [39].

4.4. Novobiocin

Novobiocin belongs to the family of coumarin antibiotics, and is known to inhibit bacterial DNA synthesis by direct binding to DNA gyrase. The novobiocin binding site of DNA gyrase is similar to the Hsp90 ATP-binding site [38]. Its structural analogues such as chlorobiocin and coumermycin A1 also bind to Hsp90, resulting in the destabilization and proteolytic degradation of a number of proteins. Novobiocin binds to a previously unrecognized ATP-binding domain in the carboxy terminus of Hsp90, and inhibits its function. However, binding of novobiocin to Hsp90 inhibits geldanamycin binding, suggesting an extensive interaction between the N- and C-terminal domains in regulating Hsp90 chaperone function [39].

4.5. Purine-based inhibitors

Hsp90 contains a conserved N-terminal ATP/ADP binding pocket and nucleotide binding regulates the chaperone function of the protein. Earlier studies with various Hsp90 inhibitors showed that most of them bind directly to the N-terminal ATP/ADP site, resulting in a change of Hsp90 conformation and a consequent interference with its chaperone function [95]. In a recent development, PU3, a purine-

based Hsp90 inhibitor was designed using X-ray crystallographic data [102]. PU3 behaves like geldanamycin in inhibiting Hsp90 client protein degradation, and in possessing a robust antitumor potential. Attempts to modify and improve PU3 led to the development of PU24F-Cl, which binds to the N-terminus of Hsp90 with a 30-fold higher affinity than the parent compound, PU3, thus approximating the binding affinity of 17AAG. PU24F-Cl was found more selective than Hsp90 inhibitors. Its water solubility is also an advantage over geldanamycin and 17AAG [103]. However, PU24F-Cl may not show the specific intracellular accumulation typical for the more hydrophobic geldanamycin analogues [81].

4.6. Taxol

Taxol is a plant-derived antitumor agent. Its antitumor action is ascribed to its ability to block mitosis by binding and stabilizing microtubules [104,105]. Members of the Hsp90 and Hsp70 families were recently identified as targets of taxol [106]. However, taxol-induced cell death is found to be independent of the Raf kinase, which is one of the usual targets of Hsp90 [107].

5. Advantages of Hsp90 inhibitors

In most cases, Hsp90 inhibition has been shown to induce either cytostasis or apoptosis [87,88]. However, there are some reports showing that, at low doses, Hsp90 inhibitors induce cell differentiation [108]. Though the differences in the downstream effects of Hsp90 inhibition leading to these various final outcomes in the fate of the cells are not known, several prominent features of Hsp90 inhibition are associated with all of these effects [109–111]. There is a selectivity of drug-induced effects differentiating by the type and grade of tumor. Clark et al. [112] showed that, with human colon carcinoma cells, 17AAG depletes Raf and Akt through Hsp90 inhibition without affecting other client protein expression. In certain cancers, single administration of an Hsp90 inhibitor, such as 17AAG, was shown more effective for a variety of client protein degradation [113]. Despite this, Hsp90 inhibition results in either differentiation or apoptosis, depending on the cell type [109]. Cell cycle arrest is the crossroads for this decision, since both geldanamycin and 17AAG [110] were found to induce G1 arrest. Small molecular purine inhibitors exhibit a similar behavior [102,103].

These preclinical studies emphasize the important role of Hsp90 inhibitors in clinical applications. Combination therapies, applying low doses of these drugs together with conventional chemotherapeutic agents, seem to be an effective way to target various cancers. For example, in the case of Bcr/Abl-expressing leukemias, a low dose of geldanamycin is sufficient to sensitize these cells to apoptosis in the presence of ineffective concentrations of doxorubicin, through caspase activation [114]. In another example, 17AAG in combination with taxol shows enhanced cytotoxic effects on taxol-resistant ErbB2 overexpressing breast cancer cells [115,116].

6. Redox homeostasis

Oxidative stress is an imbalance between oxidant exposure and anti-oxidative protection within the cellular environment, resulting in a range of responses that depend on the cell type and on the stressor. There is cross-talk between the amount of Hsp-s and the intracellular redox homeostasis. Hsp-s, like a-crystalline, Hsp27 and Hsp70, were extensively studied for their antioxidant properties. These proteins also contribute to maintaining the intracellular redox homeostasis [117,118]. Though the exact involvement of Hsp90 in redox homeostasis regulation is not known, Hsp90 was shown to possess reactive cysteines and was able to reduce cytochrome *c*, suggesting a role for this chaperone in modulating the redox status in resting and apoptotic cells [119].

7. Hsp90 and the cytoarchitecture: Hsp90 inhibition leads to increased lysis of cells after hypoxia or complement attack

The eukaryotic cytoskeleton contains three major components, microfilaments, intermediate filaments, and microtubules. Extensive research in this field points to the importance of Hsp-s in stabilizing the cytoskeleton by direct interaction with cytoskeletal proteins [120,121]. The growing list of Hsp90-interacting cytoskeletal proteins suggests that Hsp90 plays a major role in preserving these structures, hence it is involved in maintaining the cell shape. It was also proposed that apart from these known cytoskeletal interactions of various Hsp-s, Hsp90 is also involved in maintaining a fine cytoplasmic meshwork, called the microtrabecular lattice [28,122]. In accordance with this, Hsp90 inhibition is associated with disturbances in the cytoskeleton and cytoskeletal signaling [123]. Our recent data indicate that inhibition of Hsp90 by various drugs, such as geldanamycin, radicicol or cisplatin, as well as by anti-Hsp90 ribozyme, leads to an increased susceptibility of various cells to cell lysis induced by detergent, hypotonic shock, hypoxia, or complement attack [124,125]. These findings support the idea that Hsp90 is intimately involved in the maintenance of cellular integrity [28,122] and suggest that, among many others, Hsp90 inhibitors exert their antitumor action via the sensitization of tumor cells to various lytic events.

8. Limitations of anti-Hsp90 drugs

Though Hsp90 inhibitors exhibit selective effects in inducing the degradation of Hsp90 client proteins, they

are also associated with other effects unrelated to their binding to Hsp90. Geldanamycin, which contains a quinone group, is known to induce reactive oxygen species, and in general, the cytotoxicity of the ansamycin antibiotics has been attributed to free radical generation [126–129]. Radicicol is also involved in free-radical formation from non-peroxide compounds [130], while cisplatin and novobiocin have multiple targets, which are independent of Hsp90. Obviously, among the multitude of Hsp90 client proteins, there are many which have not pro-, but anti-tumor activities, such as the tumor suppressor LKB1 kinase [131]. Inhibition of Hsp90 leads to the down-regulation of this tumor suppressor kinase, showing a potential disadvantage of Hsp90 inhibitor therapies [131].

9. Conclusions and perspectives

The word ‘‘cancer’’ can be regarded as a gross term for a vast number of many different disease conditions with distinct characteristics and therapeutic requirements. Though the general features of cancer include unrestrained cell proliferation, a great variety of mutations as well as deregulation of numerous genes can cause this. Among the hallmarks of cancer [132], up-regulation of growth signals and evasion of apoptosis are the most important. As most growth regulatory signals depend on Hsp90 for their functional stability, Hsp90 is an ideal molecule to intervene in complex oncogenic pathways. Hence, most drugs targeting Hsp90 are much more beneficial than the selective oncogene pathway inhibitors.

On the other hand, Hsp90 inhibition suffers from the problems of most chemotherapeutic interventions: in principle, the drug affects normal and tumor cells equally. Moreover, Hsp90 inhibition also induces Hsp-s, including Hsp90 itself, by releasing HSF-1 from its Hsp90 inhibitory complex and (in some cases like that with geldanamycin) by inducing reactive oxygen species, which serve as an additional stress leading to Hsp induction. The larger Hsp90 requirement of tumor cells may overcome many of these limitations, but a detailed comparison of the complexity of Hsp90 inhibition in normal cells versus tumor cells is clearly lacking. Another way to circumvent the general effects of Hsp90 inhibitors is to increase their specificity by targeting them to a tumor-specific Hsp90 client protein, which is a clear task for future drug development in the field of Hsp90 inhibitors.

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