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Therapeutic and diagnostic implications of Hsp90 activation

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The molecular chaperone heat-shock protein 90 (Hsp90) is involved in the stabilization and conformational maturation of many signaling proteins that are deregulated in cancers. Hsp90 inhibition results in the proteasomal degradation of these client proteins and leads to potent antitumor activity. The Hsp90 inhibitor 17-allylaminogeldanamycin (17-AAG) is presently in clinical trials. Recent work has identified the role of Hsp90 in multiple signal transduction pathways and revealed that the molecular mechanism of tumor selectivity by Hsp90 inhibitors is the result of an activated, high-affinity conformation of Hsp90 in tumors. This review discusses these recent advances in the understanding of tumor Hsp90 for the treatment and diagnosis of cancer. In addition, the role of Hsp90 in non-oncological diseases will also be discussed.

Hsp90 is a ubiquitous molecular chaperone protein that is involved in the folding, activation and assembly of many proteins, including key mediators of signal transduction, cell-cycle control and transcriptional regulation [1,2]. Hsp90 client proteins include transmembrane tyrosine kinases [HER-2/neu, epidermal growth factor receptor (EGFR), MET and insulin-like growth factor-1 receptor (IGF-1R)], metastable signaling proteins (Akt, Raf-1 and IKK), mutated signaling proteins (p53, Kit, Flt3 and v-src), chimeric signaling proteins (NPM–ALK, Bcr–Abl), steroid receptors (androgen, estrogen and progesterone receptors) and cell-cycle regulators (cdk4, cdk6) [1–13]. Hsp90 is regulated by co-chaperone proteins, which participate in an ordered series of dynamic multi-protein complexes that are linked to the conformationally coupled ATPase cycle of Hsp90 [2,14,15]. Client proteins bind to Hsp90 in an intermediate complex, containing the co-chaperone proteins Hsp70, Hsp40, Hip and Hop. Upon ATP binding and hydrolysis, Hsp90 forms a mature complex, containing p23, p50cdc37 and immunophilins (IP), that catalyzes the conformational maturation of the Hsp90 client proteins (Figure 1). The naturally occurring ansamycin antibiotic geldanamycin binds to a conserved binding pocket in the N-terminal ATP-binding domain of Hsp90 [16–18], inhibiting ATP binding and ATP-dependent Hsp90 chaperone activity [19–21] and directing the proteasomal degradation of Hsp90 client proteins [22,23]. Geldanamycin displays potent anti-tumor activity *in vitro* but is too hepatotoxic for clinical use [24].

The less toxic geldanamycin derivative 17-allylaminogeldanamycin (17-AAG) also binds to Hsp90 [3], exerts a potent antitumor activity in preclinical models [12,25] and is currently in clinical trials [26]. Why Hsp90 modulators preferentially targeted tumor cells, even though Hsp90 is abundantly expressed in both normal and tumor cells, was unknown. Furthermore, it was unclear why 17-AAG binds to recombinantly isolated Hsp90 protein with micromolar affinity but commonly kills tumor cell lines at nanomolar concentrations [3,27]. Recent data have revealed that the therapeutic selectivity of Hsp90 inhibitors results from the presence of a high-affinity activated form of Hsp90 in tumors, which is in a multi-chaperone complex with high ATPase activity, whereas the Hsp90 in normal tissues is in an inactive, uncomplexed form with low affinity [28]. In this review, we will discuss the role of Hsp90 in multiple signal transduction pathways and the diagnostic and therapeutic implications of the molecular mechanism of tumor selectivity of Hsp90 inhibitors. The structural implications of Hsp90 activation by the formation of multi-chaperone complexes will also be discussed, as will the potential use of Hsp90-inhibitor drugs in non-oncological diseases.

Hsp90 inhibition affects multiple signaling pathways

Hsp90 influences the activity and stability of many of the client proteins that function as key regulators in cellular growth, differentiation and apoptotic pathways. More than 100 known Hsp90 clients regulate multiple signal transduction pathways that are persistently activated in human cancers. Inactivation of the Ras–Raf-1–Mek–ERK and phosphatidyl inositol-3 kinase–Akt pathways by Hsp90 inhibitors causes the downregulation of cyclin D1 and the functional inactivation of Cdk4, both of which are important for the G1–S cell cycle transition (Figure 2). Growth factor receptors that that are sensitive to Hsp90-mediated degradation include the receptors for HER-2, IGF-1, EGF and platelet-derived growth factor (PDGF). Hsp90 inhibitors inactivate multiple kinases, such as Raf-1, Akt and cdk4 [5,6,29]. Furthermore, inactivation of Akt by Hsp90 inhibitors leads to reduced BAD (Bcl-XL/Bcl-1-associated death promoter) phosphorylation, which decreases the activity of the anti-apoptotic proteins Bcl2

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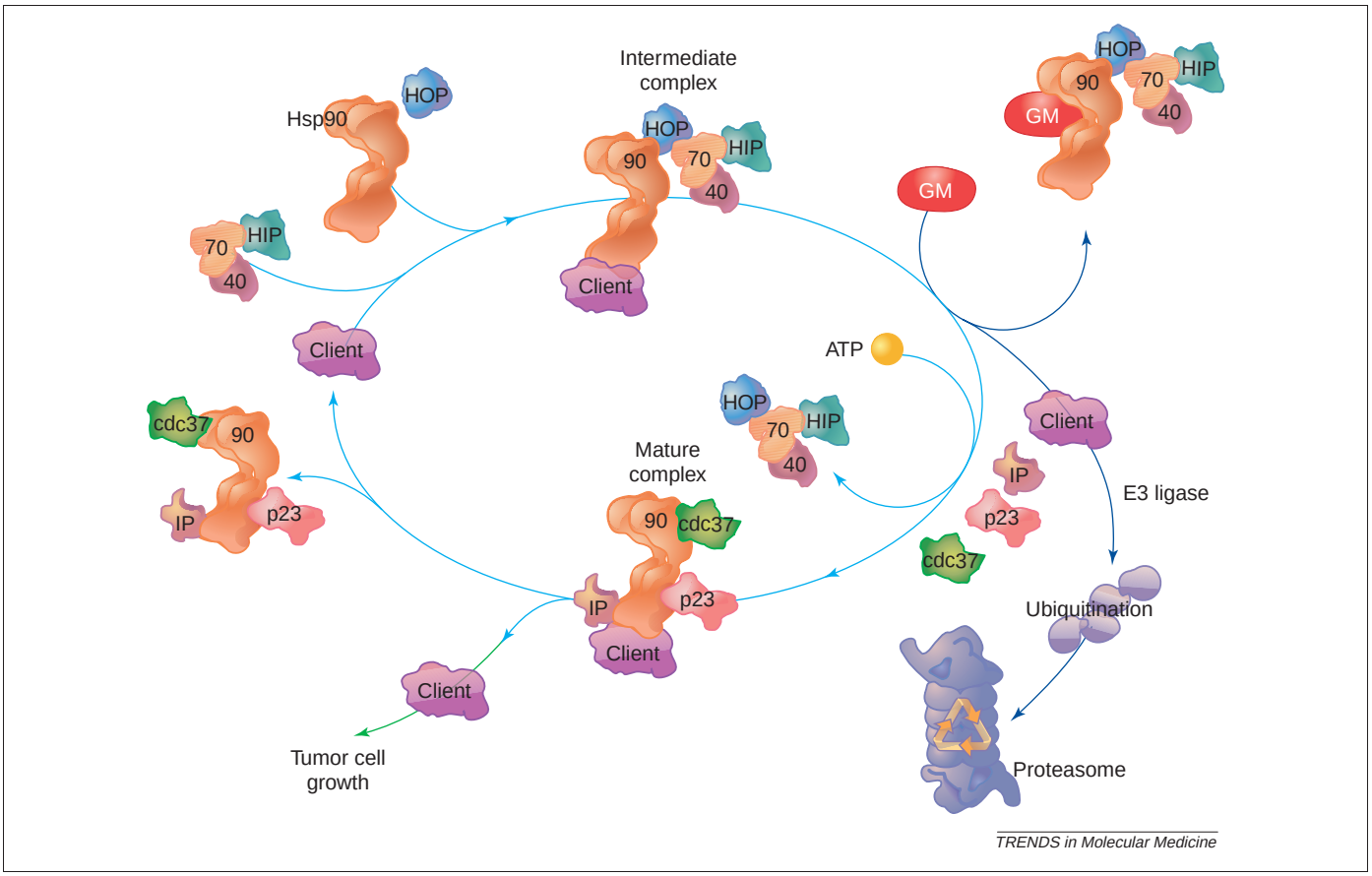

Figure 1. The Hsp90 chaperoning cycle. The Hsp90 chaperoning cycle is a dynamic process in which client proteins bind to Hsp90 in an intermediate complex containing the co-chaperones Hsp70, Hsp40, Hip and Hop. Upon ATP binding and hydrolysis, Hsp90 forms a mature complex, containing p23, p50/cdc37 and immunophilins (IP), which catalyzes the conformational maturation of Hsp90 client proteins. Hsp90-inhibitor drugs, such as geldanamycin (GM), bind to the N-terminal ATP-binding pocket of Hsp90 and inhibit ATP binding and hydrolysis, thereby locking Hsp90 in the intermediate complex. The client protein is subsequently ubiquitinated (possibly by a E3 ubiquitin ligase) and targeted to the proteasome for degradation.

and Bcl-X, thereby inducing apoptosis by cytochrome C release [30]. Hsp90 inhibitors also inactivate steroid receptors, such as the androgen receptor and estrogen receptor, which, when bound to their cognate ligands, translocate to the nucleus and act as transcription factors [2]. Therefore, Hsp90 inhibitors simultaneously destabilize many oncoproteins in multiple signaling pathways, suggesting that the inhibition of Hsp90 could be particularly beneficial in attacking late-stage cancer cells, which can easily circumvent the blockade of a single target or pathway.

Because of the wide array of Hsp90 client proteins, Hsp90 inhibitors can be used to target diverse cancers in which an Hsp90 client protein is necessary for cancer proliferation, survival or progression. In particular, HER-2 is one of the client proteins that is most sensitive to Hsp90 inhibitors [4], and promising preclinical data in HER-2-overexpressing breast- and prostate-cancer models suggest that 17-AAG could be used to treat such tumors [5,12]. The discovery that the Bcr–Abl fusion protein, which is the sole causative factor in chronic myelogenous leukemia (CML), is an Hsp90 client [31] led to studies showing that both geldanamycin and 17-AAG induced apoptosis in CML-derived cell lines [32]. Furthermore, CML cells that have become resistant to the Bcr–Abl inhibitor Gleevec remain sensitive to 17-AAG, suggesting this drug could be beneficial to Gleevec-resistant patients [33]. In addition, there are data indicating that other leukemias and lymphomas that are dependent on chimeric or mutated proteins, such as NPM–ALK [9], Flt3(ITD) [10] or c-Kit [11], can be targeted by Hsp90-inhibitor drugs. Therefore, Hsp90 inhibitors could potentially affect a broad range of cancers, and various ongoing clinical trials using 17-AAG aim to determine whether this is true [1,26].

A high-affinity conformation of tumor Hsp90 confers drug selectivity

Why do Hsp90 inhibitor drugs selectively kill cancer cells, even though Hsp90 is widely expressed in normal tissues? A recent study reported that the Hsp90 in tumor cells is maintained in an activated conformation by the formation of multi-chaperone complexes that have increased ATPase activity and 100-fold greater binding affinity for 17-AAG compared with the uncomplexed, latent form of Hsp90 that is present in normal cells [28]. Interestingly, Hsp90 from tumor cells that are overexpressing the Hsp90 client-protein HER-2 exhibited the highest binding affinity for 17-AAG, which is in accordance with a recent report that HER-2 overexpression in tumor cells confers increased sensitivity to geldanamycin [34]. The increased binding affinity of tumor Hsp90 for 17-AAG also explains the remarkable ability of Hsp90-binding drugs to accumulate selectively in tumors compared with normal tissues *in vivo* [28,35]. Furthermore, these results suggest that

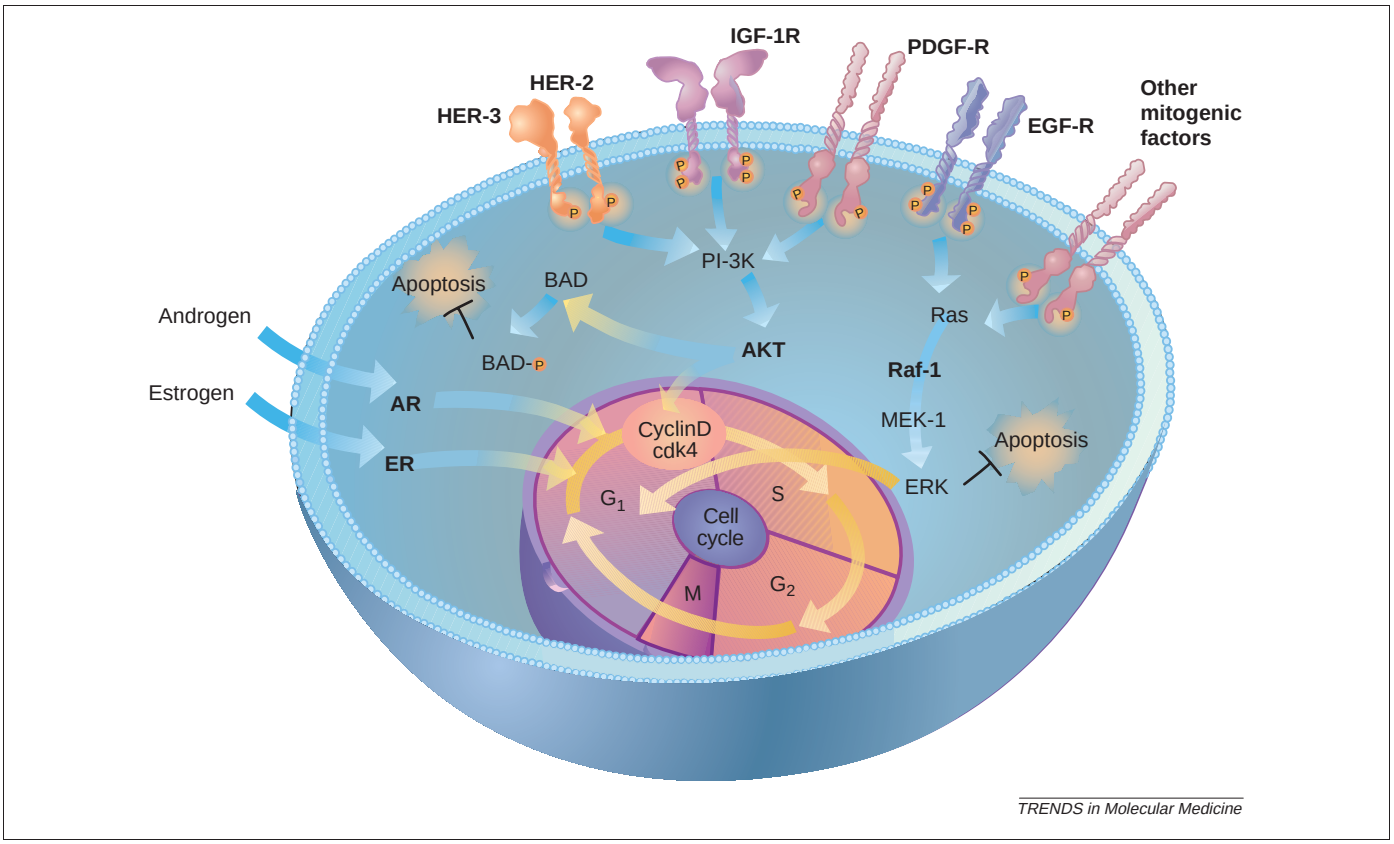

Figure 2. Hsp90 client proteins regulate multiple signal transduction pathways that are deregulated in cancers. Hsp90 client proteins (shown in bold) include key components of the mitogenic signaling pathway that drives cell-cycle progression, as well as survival signal transduction pathways that inhibit apoptosis. Hsp90 client proteins include growth factor receptors (HER-2, IGF-1R, EGF-R and PDGF-R), signaling kinases (Akt and Raf-1), cell-cycle regulators (cdk4) and nuclear steroid receptors (AR and ER). Abbreviations: AR, androgen receptor; EGF-R, epidermal growth factor receptor; ER, estrogen receptor; IGF-1R, insulin-like growth factor-1 receptor; PDGF-R, platelet-derived growth factor receptor.

Hsp90 usage, as indicated by binding-affinity, is a true predictor of the Hsp90 dependence of tumor cells. It has been reported that Hsp90 levels are elevated in many cancer tissues [36–38] but it is probable that it is the activity of Hsp90 that is more closely linked to cancer progression, at least during the early stages. As malignant progression proceeds, the complete usage of tumor Hsp90 within the cell might provide a selection pressure leading to the further upregulation of Hsp90 that is observed in many advanced tumors [37–40].

A model for Hsp90-dependent malignant progression has been proposed in which, as tumor cells gradually accumulate mutant and overexpressed signaling proteins, Hsp90 becomes engaged in the active chaperoning and stabilization of oncoproteins and adopts a high-affinity form that is induced by bound co-chaperone proteins (Figure 3) [28]. By contrast, the Hsp90 in normal cells appears to be largely unused and remains in an uncomplexed form that has low-affinity binding for Hsp90 inhibitors. This model would also predict, based on current knowledge, that proteotoxic stresses in normal cells would convert inactive Hsp90 into complexed Hsp90 with higher binding affinity. Transcription of Hsp90 is regulated by the heat-shock transcription factor HSF-1, which is thought to be regulated through its sequestration in an inactive state by cytosolic Hsp90 [41]. Under conditions of proteotoxic stress (e.g. heat), Hsp90 is recruited to refold partially denatured proteins, liberating HSF-1 from sequestering complexes and enabling it to trimerize and translocate to the nucleus, where it transactivates Hsp90 and other heat-shock genes [42]. Assuming that the stress-induced chaperoning activity of Hsp90 is ATP-dependent, this transition should be accompanied by a sharp increase in Hsp90 usage and a commensurate increase in the affinity of the cellular Hsp90 pool for inhibitors. Similarly, it is interesting to speculate whether the *de novo*-induced Hsp90 will be of high or low affinity for Hsp90-binding drugs. Because 17-AAG and the other active agents that bind the N-terminal ATP site of Hsp90 also cause the dissociation of HSF-1 complexes and a robust heat-shock response [41], it is possible that the time window for the cytotoxic activity of Hsp90 inhibitors could be limited by the development of a drug-induced heat-shock response [43]. If the induced Hsp90 is uncomplexed and has low affinity, it will compete poorly with the pre-existing high-affinity Hsp90 complexes (containing client proteins) for binding to 17-AAG. Alternatively, repopulation of the cytosol with active high-affinity Hsp90 complexes might be expected to dampen ongoing antitumor responses. The fact that several co-chaperone genes are targets for HSF-1, and are coordinately induced during the heat-shock response [42], indicates that the latter possibility is more likely.

Structural implications of Hsp90 activation

The crystal structures of the N-terminal domain of Hsp90 that is bound to either geldanamycin, radicicol, ATP or

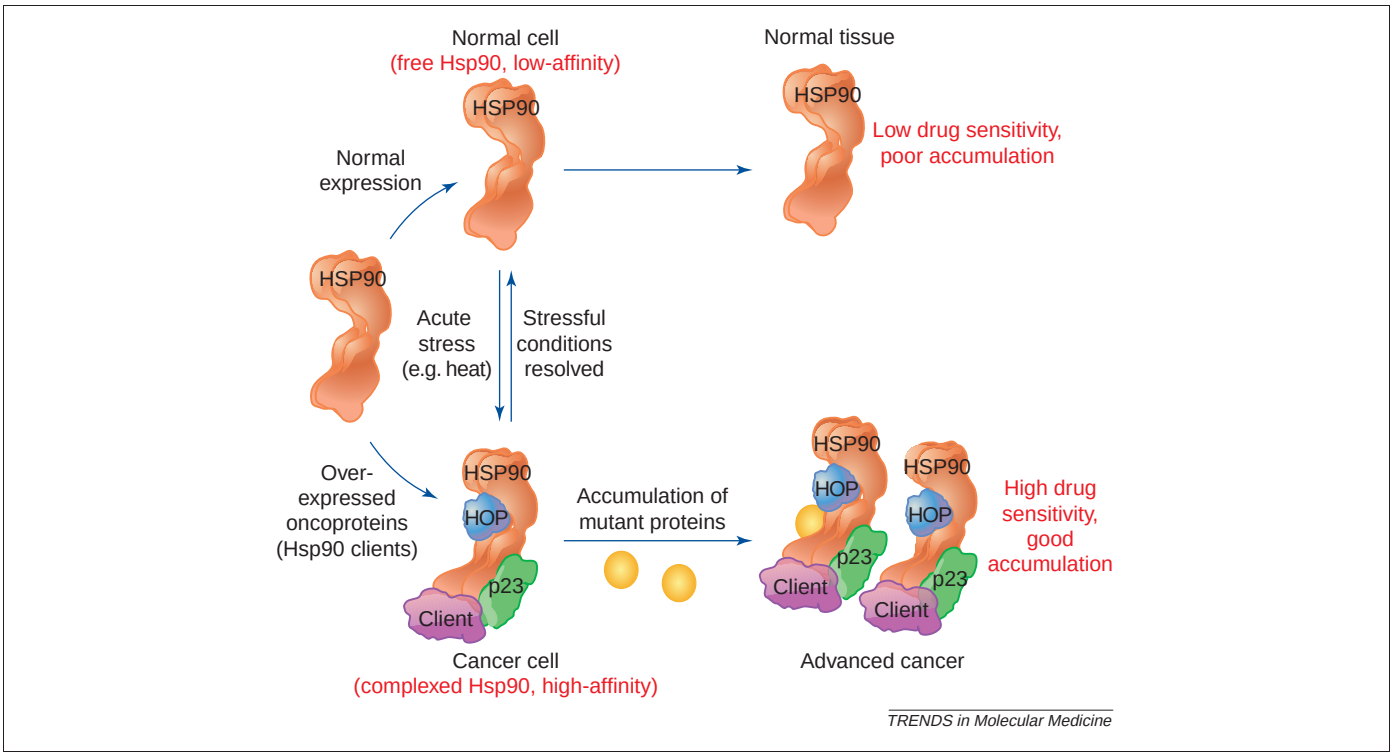

Figure 3. Model for tumor selectivity of Hsp90 inhibitors and Hsp90-dependent malignant progression. Hsp90 in normal cells exists in an uncomplexed form that has low-affinity for Hsp90 inhibitor drugs, which accumulate poorly in normal tissues, and normal cells exhibit poor drug sensitivity. By contrast, the Hsp90 in cancer cells is involved in the active chaperoning of overexpressed oncoproteins and exists in a complexed form with co-chaperone proteins (p23 and Hop are not in the same complex). Complexed Hsp90 in cancer cells exhibits high-affinity binding to Hsp90 inhibitor drugs, which accumulate in tumor tissues, and tumor cells exhibit good drug sensitivity. This model predicts that the accumulation of mutant proteins in advanced cancer would further increase Hsp90 usage and make tumor cells more Hsp90 dependent. Furthermore, this model suggests that the high-affinity change of Hsp90 can be driven by the overexpression of oncoproteins, as well as by stressful conditions in normal cells (e.g. heat).

ADP (Figure 4) have been solved [17,18,44,45]. Key amino acids within the N-terminal binding pocket of Hsp90 form crucial interactions with these Hsp90-inhibitor compounds [44]. It is important to note, however, that all of the structural information is based on free, uncomplexed

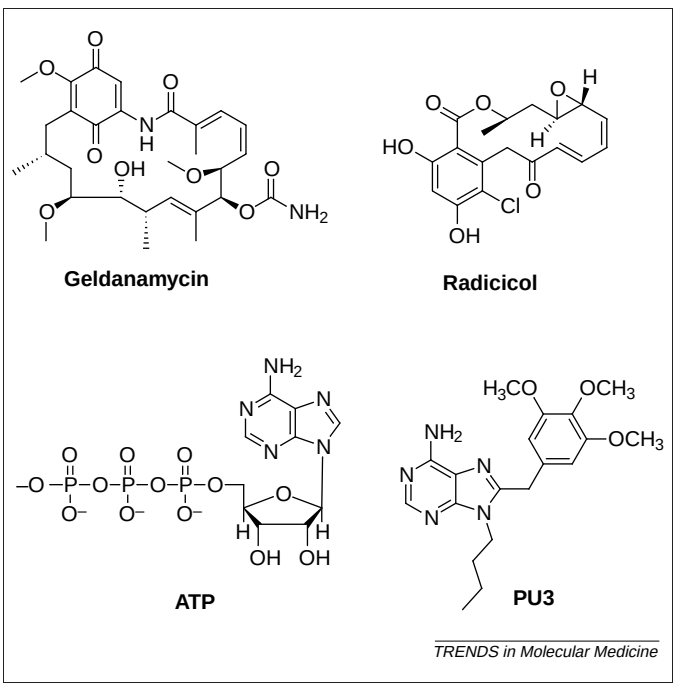

Figure 4. Hsp90 inhibitors. Chemical structures of geldanamycin, radicicol, ATP and PU3.

Hsp90. Because the active form of Hsp90 comprises a multi-chaperone protein complex [28], which imparts structural changes on the ATP-binding site (as evidenced by the dramatic increase in binding activity for Hsp90 inhibitors), the precise binding configuration of Hsp90 inhibitors to the activated form of Hsp90 remains to be determined. Based upon the crystal structure of ATP that is bound to Hsp90, analogues such as PU3 (Figure 4), which exhibit a C-shaped or compact conformation, have been synthesized [27,46] and the crystal structure of PU3 with recombinant Hsp90 has been solved [47]. These small-molecule Hsp90-inhibitor compounds also exhibit many of the biological properties of a classical Hsp90 inhibitor [46], but it remains to be determined whether these compounds also bind to complexed Hsp90 with a higher affinity, as do the geldanamycin derivatives.

The difference in the binding activities of complexed versus free Hsp90 might be explained by the conformational differences of geldanamycin in the liganded and unliganded forms. Similar to ATP, geldanamycin and its derivatives adopt a compact, C-shaped conformation when bound to Hsp90 that is unlike the extended planar structure of unbound geldanamycin [17,18]. This flexibility in conformation probably enables geldanamycin to adopt the precise conformation that is necessary for binding to Hsp90 complexed with co-chaperones, resulting in exceptional selectivity for complexed Hsp90 compared with free Hsp90. An intriguing possibility that has been suggested is that either the multi-chaperone-bound Hsp90, or other components of the multi-chaperone

complex, are responsible for catalyzing the conformational change in geldanamycin which enables it to bind with high-affinity to Hsp90 [48]. Alternatively, the Hsp90 that is complexed with co-chaperone proteins might cause a conformational change in the nucleotide binding pocket of Hsp90, such that geldanamycin derivatives bind with a higher affinity. Recently, the crystal structure of another geldanamycin derivative, 17-allylamino-17-demethoxy-geldanamycin (17-DMAG), with human Hsp90 has been solved, and molecular modeling studies suggested that 17-DMAG, when constrained to a *cis*-amide bond in the ground state, would increase binding affinity for Hsp90 [49]. These latter changes might occur more easily when Hsp90 is complexed with co-chaperone proteins and thus modulate the higher binding affinity of complexed Hsp90.

The co-chaperone proteins Hsp70, Hsp40, p23 and Hop were found to modulate Hsp90 activation by increasing 17-AAG binding affinity by 50-fold and Hsp90 ATPase activity by 32-fold [28]. This set of co-chaperones had been previously used for *in vitro* reconstitution experiments, where they were needed for Hsp90-chaperoning activity of the progesterone receptor [15]. Previous data showed that Hop strongly inhibits and p23 weakly inhibits Hsp90 ATPase, but those experiments were performed using yeast Hsp90 [50]. By contrast, human Hsp90 is a much weaker ATPase than is yeast Hsp90, and Hop has little effect on the basal ATPase rate of Hsp90 but inhibits the client-protein-stimulated ATPase rate, whereas p23 exhibits a small suppression [51]. These results suggest that the formation of Hsp90 multi-chaperone complexes is a dynamic process that regulates Hsp90 activity. It will be interesting to determine the effects of two other co-chaperone proteins on the affinity of Hsp90 for inhibitors such as 17-AAG: cdc37, which is important for chaperoning kinases [52], and Aha1, which increases Hsp90 ATPase activity 12-fold [53]. Unlike most co-chaperones, which bind to the C-terminus of Hsp90, the crystal structure of cdc37 with Hsp90 revealed that cdc37 binds to the N-terminus of Hsp90 and prevents the transactivating interaction of the N-terminal domains of Hsp90, thus inhibiting Hsp90 ATPase activity [52]. The central region of Hsp90 is involved in the direct interaction with Aha1, and it has been suggested that Aha1 increases Hsp90 ATPase activity by stabilizing interactions between the central and N-terminal domains of Hsp90 [54]. Therefore, future studies will be aimed at better understanding the dynamic role of co-chaperone proteins in modulating Hsp90 activation by increasing the binding affinity for Hsp90 inhibitors.

Diagnostic applications of Hsp90 assays

The profound differences in Hsp90 activity between normal and malignant cells were revealed using three experimental approaches: a competitive-binding assay using Hsp90 extracted from cell lysates, co-immunoprecipitation of Hsp90-binding co-chaperone proteins and Hsp90 ATPase activity [28]. The magnitude of the divergence (100-fold, in terms of Hsp90 binding affinity and percentage of free versus complexed Hsp90) and the close correlation between binding affinity and cell-killing potency ($R^2 = 0.92$) suggest that these analyses could have significant diagnostic value. The lysate-binding assay can be performed with a quantity of malignant tissue that could be obtained from a routine blood sample (for leukemias) or a fine-needle aspirate (other tumors), raising hopes that the current method, or a streamlined version of it, could be used to guide patient selection for the clinical development of Hsp90 inhibitors. This type of assay could have advantages over existing molecular diagnostics such as HercepTest™ [55] because, in addition to measuring the levels of Hsp90 in the sample, it indicates the extent to which the tumor is using (and is dependent upon) Hsp90.

Clearly, a simpler methodology, such as an immunoassay, to determine Hsp90 usage in clinical samples would be preferable. The Hsp90 antibody AC88 recognizes uncomplexed Hsp90 [28,41] and might be a useful tool to measure decreased Hsp90 usage, but a better reagent would be an antibody that specifically recognizes the high-affinity conformation of Hsp90. Such an antibody might recognize an epitope that is produced by the close juxtaposition of Hsp90 and a co-chaperone protein, or an activated conformation-specific epitope on Hsp90 itself. A conformation-specific antibody, 9G10, has been reported for Grp94 [56], the endoplasmic reticulum homolog of Hsp90, and it remains to be seen if a conformation-specific Hsp90 antibody can be developed.

Hsp90 inhibitors in non-oncological diseases

Hsp90 inhibitors might have therapeutic potential in other conditions where diseased cells are dependent on increased Hsp90 activity. Because cellular Hsp90 is recruited in cells that are undergoing viral infection or mediating autoimmune responses, Hsp90 inhibition might be useful for treating these diseases. Furthermore, Hsp90 inhibitors can also upregulate the heat-shock proteins that provide protective functions in central nervous system (CNS) disorders and cardiovascular diseases [57–59]. It is possible that increased Hsp90 usage in these diseases could provide a selective therapeutic target for the high-affinity binding of Hsp90 inhibitors within the diseased tissue. In most cases, it is unlikely that the Hsp90-related cellular changes in diseased cells will be as profound as those in highly malignant cells, but it will be interesting to determine whether Hsp90 activity might represent a useful independent diagnostic and/or prognostic marker in these non-oncological diseases.

Virology

Viral infections are stressful for the infected cell, and this is reflected by an increased expression of heat-shock proteins, including Hsp90. The virus essentially 'steals' the host Hsp90 to facilitate its own assembly and replication, and studies have demonstrated that geldanamycin can block the viral life-cycle of hepatitis B virus (HBV), hepatitis C virus (HCV) and herpes simplex virus type 1 (HSV-1) [60–62]. Hsp90 is an essential host factor for HBV replication and interacts with the viral reverse transcriptase [60], and it is also necessary for the activity of NS2–3 protease of HCV [61]. Furthermore, a recent study showed that HSV-1 replication *in vitro* was significantly inhibited by geldanamcyin with an IC_{50} of

93 nM, whereas geldanamycin inhibited the cellular growth of uninfected cells with an IC_{50} of 350 μM, suggesting good therapeutic selectivity [62]. The same study also demonstrated that geldanamycin exhibited broad-spectrum activity against a range of viruses with an IC_{50} of 0.5–4 μM: HIV-1 and SARS coronavirus exhibited the highest sensitivity [62]. The mechanism of HIV-1 inhibition might be explained by the ability of geldanamycin to block Tat activation of HIV-1, through its effects on the host cofactor cdk9 [63]. Therefore, these studies suggest that Hsp90 inhibitors could be used in multiple viral diseases, and it remains to be determined whether the Hsp90 in virally infected cells exhibits high-affinity binding to Hsp90 inhibitors and could provide therapeutic selectivity between the virally infected and uninfected cells.

Autoimmune disease

Autoimmune diseases are the result of inappropriate and persistent lymphocyte activation in an aberrant response to self determinants. Destructive symptoms of autoimmunity might arise by the action of T cells or from B-cell-derived antibody and complement fixation. T and B lymphocytes use similar signal transduction pathways in their mitogenic and activation programs as do tumor cells, suggesting that Hsp90 modulators might be potent inhibitors of lymphocyte function. Yorgin *et al.* showed the connection between the inhibition of T-cell activation and the killing of activated T cells by geldanamycin with the degradation of the non-receptor tyrosine kinase $p56^{lck}$ [64]. Geldanamycin also depletes Raf-1 (an inhibitor-sensitive Hsp90 client) from rat splenocytes and consequently blocks rat T-cell proliferation in response to repeated exposures to antigen [65]. Furthermore, Hsp90 inhibitors also block the Raf–ERK-mediated proliferation of murine B cells that are stimulated through the B-cell antigen receptor [66]. Taken together, these studies suggest that antibody-mediated autoimmune disease could be doubly affected by Hsp90 inhibition of both the T-cell and B-cell compartments.

CNS disorders and cardiovascular diseases

Ischemia is a stress phenomenon that accompanies most cerebrovascular and cardiovascular disease states. Heat-shock proteins are upregulated during the stress response and provide neuroprotection and cardioprotection against ischemic damage. Geldanamycin, via the HSF-1 pathway, also causes the upregulation of heat-shock proteins, including Hsp27, Hsp70 and Hsp90. These have a protective role in stress-induced ischemic damage, exerting potent anti-apoptotic activity by inhibiting the assembly of the caspase 9-Apaf-1-cytochrome C apoptosome [67]. Studies have demonstrated that geldanamycin is an effective post-treatment neuroprotective agent in a cell-culture model of oxidative toxicity [58] and also protects the brain from focal ischemia in an *in vivo* rat model [59], providing strong evidence that the Hsp90 inhibitors could be used as a neuroprotective agents. Geldanamycin has also been shown to induce a heat-shock response in myogenic cells and to confer protection against ischemic stress [57], suggesting that Hsp90 inhibitors offer a pharmacological method for increasing the level of heat-shock proteins in cardiac tissue, thus protecting the heart against ischemic injury. In addition to ischemic injury, there is evidence that Hsp90 inhibitors could be utilized in protein-aggregation diseases of the CNS, such as Huntington's disease (HD), because nanomolar concentrations of geldanamycin activated a heat-shock response and inhibited HD protein aggregation in a cell-culture model for HD [68]. Furthermore, α-synuclein-mutant flies fed on the drug were completely protected from neuronal loss in a *Drosophila* model of Parkinson's disease [69]. Collectively, these studies suggest that Hsp90 inhibitors could be used to modulate the heat-shock response and provide protective function in damaged cells in the brain and heart.

Concluding remarks

Inhibiting a protein that regulates multiple signal transduction pathways in cancer cells is an attractive approach for cancer therapy. Because Hsp90 is involved in the conformational maturation of various oncogenic signaling proteins, it is emerging as a prime target for anticancer drugs. The dependence of cancer cells on the activated form of Hsp90 suggests that the multi-chaperone Hsp90 complex could be a unique cancer target, and provides a mechanism for why 17-AAG and other Hsp90 inhibitors selectively destroy cancer cells but not normal cells. Hsp90 might serve an analogous role in the somatic evolution of tumors as in Darwinian evolution [70], rescuing potentially misfolded mutant proteins to prevent the mutation becoming lethal to the cell and thus enabling these oncoproteins to support tumor-cell proliferation, survival and malignancy. Further work will be required to determine how closely Hsp90 usage mirrors disease progression in cancer and other conditions, but increased Hsp90 usage in cancer cells might be useful as a diagnostic tool to stratify patients in clinical trials of Hsp90 inhibitors or as an independent staging criterion in a range of diseases.

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References

1 Isaacs, J.S. *et al.* (2003) Heat shock protein 90 as a molecular target for cancer therapeutics. *Cancer Cell* 3, 213–217
2 Pratt, W.B. and Toft, D.O. (2003) Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. *Exp. Biol. Med. (Maywood)* 228, 111–133
3 Schulte, T.W. and Neckers, L.M. (1998) The benzoquinone ansamycin 17-allylamino-17-demethoxygeldanamycin binds to HSP90 and shares important biologic activities with geldanamycin. *Cancer Chemother. Pharmacol.* 42, 273–279
4 Xu, W. *et al.* (2001) Sensitivity of mature Erbb2 to geldanamycin is conferred by its kinase domain and is mediated by the chaperone protein Hsp90. *J. Biol. Chem.* 276, 3702–3708
5 Basso, A.D. *et al.* (2002) Ansamycin antibiotics inhibit Akt activation and cyclin D expression in breast cancer cells that overexpress HER2. *Oncogene* 21, 1159–1166
6 Schulte, T.W. *et al.* (1995) Disruption of the Raf-1–Hsp90 molecular complex results in destabilization of Raf-1 and loss of Raf-1–Ras association. *J. Biol. Chem.* 270, 24585–24588
7 An, W.G. *et al.* (2000) The heat shock protein 90 antagonist geldanamycin alters chaperone association with p210bcr–abl and

v-src proteins before their degradation by the proteasome. *Cell Growth Differ.* 11, 355–360
8 Blagosklonny, M.V. *et al.* (1995) Geldanamycin selectively destabilizes and conformationally alters mutated p53. *Oncogene* 11, 933–939
9 Bonvini, P. *et al.* (2002) Nucleophosmin-anaplastic lymphoma kinase (NPM-ALK), a novel Hsp90–client tyrosine kinase: down-regulation of NPM-ALK expression and tyrosine phosphorylation in ALK^+ $CD30^+$ lymphoma cells by the Hsp90 antagonist 17-allylamino,17-demethoxygeldanamycin. *Cancer Res.* 62, 1559–1566
10 Yao, Q. *et al.* (2003) FLT3 expressing leukemias are selectively sensitive to inhibitors of the molecular chaperone heat shock protein 90 through destabilization of signal transduction-associated kinases. *Clin. Cancer Res.* 9, 4483–4493
11 Fumo, G. *et al.* (2004) 17-allylamino-17-demethoxygeldanamycin (17-AAG) is effective in down-regulating mutated, constitutively activated KIT protein in human mast cells. *Blood* 103, 1078–1084
12 Solit, D.B. *et al.* (2002) 17-Allylamino-17-demethoxygeldanamycin induces the degradation of androgen receptor and HER-2/neu and inhibits the growth of prostate cancer xenografts. *Clin. Cancer Res.* 8, 986–993
13 Whitesell, L. and Cook, P. (1996) Stable and specific binding of heat shock protein 90 by geldanamycin disrupts glucocorticoid receptor function in intact cells. *Mol. Endocrinol.* 10, 705–712
14 Smith, D.F. *et al.* (1990) Reconstitution of progesterone receptor with heat shock proteins. *Mol. Endocrinol.* 4, 1704–1711
15 Kosano, H. *et al.* (1998) The assembly of progesterone receptor–hsp90 complexes using purified proteins. *J. Biol. Chem.* 273, 32973–32979
16 Whitesell, L. *et al.* (1994) 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. U. S. A.* 91, 8324–8328
17 Prodromou, C. *et al.* (1997) Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. *Cell* 90, 65–75
18 Stebbins, C.E. *et al.* (1997) Crystal structure of an Hsp90–geldanamycin complex: targeting of a protein chaperone by an antitumor agent. *Cell* 89, 239–250
19 Obermann, W.M. *et al.* (1998) *In vivo* function of Hsp90 is dependent on ATP binding and ATP hydrolysis. *J. Cell Biol.* 143, 901–910
20 Panaretou, B. *et al.* (1998) ATP binding and hydrolysis are essential to the function of the Hsp90 molecular chaperone *in vivo*. *EMBO J.* 17, 4829–4836
21 Grenert, J.P. *et al.* (1999) The importance of ATP binding and hydrolysis by hsp90 in formation and function of protein heterocomplexes. *J. Biol. Chem.* 274, 17525–17533
22 Miller, P. *et al.* (1994) Depletion of the erbB-2 gene product p185 by benzoquinoid ansamycins. *Cancer Res.* 54, 2724–2730
23 Mimnaugh, E.G. *et al.* (1996) Polyubiquitination and proteasomal degradation of the p185c–erbB-2 receptor protein-tyrosine kinase induced by geldanamycin. *J. Biol. Chem.* 271, 22796–22801
24 Supko, J.G. *et al.* (1995) Preclinical pharmacologic evaluation of geldanamycin as an antitumor agent. *Cancer Chemother. Pharmacol.* 36, 305–315
25 Solit, D.B. *et al.* (2003) Inhibition of heat shock protein 90 function down-regulates Akt kinase and sensitizes tumors to Taxol. *Cancer Res.* 63, 2139–2144
26 Sausville, E.A. *et al.* (2003) Clinical development of 17-allylamino, 17-demethoxygeldanamycin. *Curr. Cancer Drug Targets* 3, 377–383
27 Chiosis, G. *et al.* (2001) 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.* 8, 289–299
28 Kamal, A. *et al.* (2003) A high-affinity conformation of Hsp90 confers tumor selectivity on Hsp90 inhibitors. *Nature* 425, 407–410
29 Stepanova, L. *et al.* (1996) Mammalian p50Cdc37 is a protein kinase-targeting subunit of Hsp90 that binds and stabilizes Cdk4. *Genes Dev.* 10, 1491–1502
30 Datta, S.R. *et al.* (1999) Cellular survival: a play in three Akts. *Genes Dev.* 13, 2905–2927
31 Blagosklonny, M.V. *et al.* (2001) The Hsp90 inhibitor geldanamycin selectively sensitizes Bcr–Abl-expressing leukemia cells to cytotoxic chemotherapy. *Leukemia* 15, 1537–1543
32 Nimmanapalli, R. *et al.* (2001) Geldanamycin and its analogue 17-allylamino-17-demethoxygeldanamycin lowers Bcr–Abl levels and induces apoptosis and differentiation of Bcr–Abl-positive human leukemic blasts. *Cancer Res.* 61, 1799–1804
33 Gorre, M.E. *et al.* (2002) BCR–ABL point mutants isolated from patients with imatinib mesylate-resistant chronic myeloid leukemia remain sensitive to inhibitors of the BCR–ABL chaperone heat shock protein 90. *Blood* 100, 3041–3044
34 Smith, V. *et al.* (2002) ErbB2 overexpression in an ovarian cancer cell line confers sensitivity to the HSP90 inhibitor geldanamycin. *Anticancer Res.* 22, 1993–1999
35 Xu, L. *et al.* (2003) Physiologically-based pharmacokinetics and molecular pharmacodynamics of 17-(allylamino)-17-demethoxygeldanamycin and its active metabolite in tumor-bearing mice. *J. Pharmacokinet. Pharmacodyn.* 30, 185–219
36 Ferrarini, M. *et al.* (1992) Unusual expression and localization of heat-shock proteins in human tumor cells. *Int. J. Cancer* 51, 613–619
37 Gress, T.M. *et al.* (1994) Differential expression of heat shock proteins in pancreatic carcinoma. *Cancer Res.* 54, 547–551
38 Yano, M. *et al.* (1996) Expression and roles of heat shock proteins in human breast cancer. *Jpn. J. Cancer Res.* 87, 908–915
39 Mileo, A.M. *et al.* (1990) Selective over-expression of mRNA coding for 90 KDa stress-protein in human ovarian cancer. *Anticancer Res.* 10, 903–906
40 Cardillo, M.R. *et al.* (2000) Heat shock protein-90, IL-6 and IL-10 in bladder cancer. *Anticancer Res.* 20, 4579–4583
41 Zou, J. *et al.* (1998) Repression of heat shock transcription factor HSF1 activation by HSP90 (HSP90 complex) that forms a stress-sensitive complex with HSF1. *Cell* 94, 471–480
42 Morimoto, R.I. (1998) Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. *Genes Dev.* 12, 3788–3796
43 Bagatell, R. *et al.* (2000) Induction of a heat shock factor 1-dependent stress response alters the cytotoxic activity of hsp90-binding agents. *Clin. Cancer Res.* 6, 3312–3318
44 Roe, S.M. *et al.* (1999) Structural basis for inhibition of the Hsp90 molecular chaperone by the antitumor antibiotics radicicol and geldanamycin. *J. Med. Chem.* 42, 260–266
45 Chene, P. (2002) ATPases as drug targets: learning from their structure. *Nat. Rev. Drug Discov.* 1, 665–673
46 Chiosis, G. *et al.* (2002) 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.* 10, 3555–3564
47 Dymock, B. *et al.* (2004) Adenine derived inhibitors of the molecular chaperone HSP90–SAR explained through multiple X-ray structures. *Bioorg. Med. Chem. Lett.* 14, 325–328
48 Neckers, L. and Lee, Y.S. (2003) Cancer: the rules of attraction. *Nature* 425, 357–359
49 Jez, J.M. *et al.* (2003) Crystal Structure and Molecular Modeling of 17-DMAG in Complex with Human Hsp90. *Chem. Biol.* 10, 361–368
50 Prodromou, C. *et al.* (1999) Regulation of Hsp90 ATPase activity by tetratricopeptide repeat (TPR)- domain co-chaperones. *EMBO J.* 18, 754–762
51 McLaughlin, S.H. *et al.* (2002) Stimulation of the weak ATPase activity of human hsp90 by a client protein. *J. Mol. Biol.* 315, 787–798
52 Roe, S.M. *et al.* (2004) The Mechanism of Hsp90 regulation by the protein kinase-specific cochaperone p50(cdc37). *Cell* 116, 87–98
53 Panaretou, B. *et al.* (2002) Activation of the ATPase activity of hsp90 by the stress-regulated cochaperone aha1. *Mol. Cell* 10, 1307–1318
54 Meyer, P. *et al.* (2003) Structural and functional analysis of the middle segment of hsp90: implications for ATP hydrolysis and client protein and cochaperone interactions. *Mol. Cell* 11, 647–658
55 Hatanaka, Y. *et al.* (2001) Quantitative immunohistochemical evaluation of HER2/neu expression with HercepTest™ in breast carcinoma by image analysis. *Pathol. Int.* 51, 33–36
56 Vogen, S. *et al.* (2002) Radicicol-sensitive peptide binding to the N-terminal portion of GRP94. *J. Biol. Chem.* 277, 40742–40750
57 Conde, A.G. *et al.* (1997) Induction of heat shock proteins by tyrosine kinase inhibitors in rat cardiomyocytes and myogenic cells confers protection against simulated ischemia. *J. Mol. Cell. Cardiol.* 29, 1927–1938

58 Xiao, N. *et al.* (1999) Geldanamycin provides posttreatment protection against glutamate- induced oxidative toxicity in a mouse hippocampal cell line. *J. Neurochem.* 72, 95–101
59 Lu, A. *et al.* (2002) Geldanamycin induces heat shock proteins in brain and protects against focal cerebral ischemia. *J. Neurochem.* 81, 355–364
60 Hu, J. and Seeger, C. (1996) Hsp90 is required for the activity of a hepatitis B virus reverse transcriptase. *Proc. Natl. Acad. Sci. U. S. A.* 93, 1060–1064
61 Waxman, L. *et al.* (2001) Host cell factor requirement for hepatitis C virus enzyme maturation. *Proc. Natl. Acad. Sci. U. S. A.* 98, 13931–13935
62 Li, Y.H. *et al.* (2004) Geldanamycin, a ligand of heat shock protein 90, inhibits the replication of herpes simplex virus type 1 *in vitro*. *Antimicrob. Agents Chemother.* 48, 867–872
63 O'Keeffe, B. *et al.* (2000) Requirement for a kinase-specific chaperone pathway in the production of a Cdk9/cyclin T1 heterodimer responsible for P-TEFb-mediated tat stimulation of HIV-1 transcription. *J. Biol. Chem.* 275, 279–287
64 Yorgin, P.D. *et al.* (2000) Effects of geldanamycin, a heat-shock protein 90-binding agent, on T cell function and T cell nonreceptor protein tyrosine kinases. *J. Immunol.* 164, 2915–2923
65 Sugita, T. *et al.* (1999) Immunosuppressive effects of the heat shock protein 90-binding antibiotic geldanamycin. *Biochem. Mol. Biol. Int.* 47, 587–595
66 Piatelli, M.J. *et al.* (2002) Requirement for a hsp90 chaperone-dependent MEK1/2-ERK pathway for B cell antigen receptor-induced cyclin D2 expression in mature B lymphocytes. *J. Biol. Chem.* 277, 12144–12150
67 Beere, H.M. and Green, D.R. (2001) Stress management – heat shock protein-70 and the regulation of apoptosis. *Trends Cell Biol.* 11, 6–10
68 Sittler, A. *et al.* (2001) Geldanamycin activates a heat shock response and inhibits huntingtin aggregation in a cell culture model of Huntington's disease. *Hum. Mol. Genet.* 10, 1307–1315
69 Auluck, P.K. and Bonini, N.M. (2002) Pharmacological prevention of Parkinson disease in Drosophila. *Nat. Med.* 8, 1185–1186
70 Rutherford, S.L. and Lindquist, S. (1998) Hsp90 as a capacitor for morphological evolution. *Nature* 396, 336–342