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Spotlight on Heat Shock Proteins

The Achilles Heel of ErbB-2/HER2

Regulation by the Hsp90 Chaperone Machine and Potential for Pharmacological Intervention

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ABSTRACT

Signal transduction mediated by ErbB/HER receptor tyrosine kinases is crucial for the development and maintenance of epithelial tissues, and aberrant signaling is frequently associated with malignancies of epithelial origin. This review focuses on the roles played by the Hsp90 chaperone machinery in the regulation of signaling through the ErbB/HER network, and discusses potential therapeutic strategies that disrupt chaperone functions. Hsp90 and its associated cochaperones regulate ErbB signal transduction through multiple mechanisms. The chaperone system controls the stability of the nascent forms of both ErbB-1 (EGF-receptor) and ErbB-2/HER2, while regulation of the mature form is restricted to ErbB-2. Regulation by the Hsp90 complex extends to downstream effectors of ErbB signaling, namely Raf-1, Pdk-1 and Akt/PKB. Disrupting the function of Hsp90 results in the degradation of both the receptors and their effectors, thereby inhibiting tumor cell growth. The importance of an Hsp90-recognition motif located within the kinase domain of ErbB-2 is discussed, as well as a direct role for Hsp90 in regulating tyrosine kinase activity. In light of recent observations, we emphasize the ability of specific tyrosine kinase inhibitors to selectively target ErbB-2 to the chaperone-mediated degradation pathway. ErbB-specific drugs are already used to treat cancers, and clinical trials are underway for additional compounds that intercept ErbB signaling, including drugs that target Hsp90. Hence, the dependence of ErbB-2 upon Hsp90 reveals an Achilles heel, which opens a window of opportunity for combating cancers driven by the ErbB/HER signaling network.

THE ERBB FAMILY OF RECEPTOR TYROSINE KINASES

Transmission of a proper signal following growth factor activation of the ErbB family of receptor tyrosine kinases is dependent on precise regulation. Disregulation of the activity of these receptors can have dire consequences, often leading to cellular transformation and cancer. These receptors act as a layered network, in which the four receptors are activated by ten growth factor ligands of the EGF/neuregulin family, to bring about formation of homo- and hetero- dimers with different signaling capabilities (for a review see ref. 1). The first level of regulation of the network relates to formation of the primary signaling unit, which depends on the proper temporal and spatial coexpression of a ligand and two receptors. The major signaling pathways activated by this signaling unit of the ErbB-receptors include the Ras-Raf1-Mek-Erk, and the PI3K-Pdk1-Akt pathways, both of which culminate in activation of transcription programs, as well as Cyclin-dependent kinases, leading to progression through the cell cycle (Fig. 1). While ErbB-1, ErbB-3 and ErbB-4 each bind multiple ligands, ErbB-2 is a ligand-less receptor that enhances and prolongs signaling upon heterodimerization with other ErbBs.

The signaling of the receptors through these pathways is regulated by the activity of phosphatases for rapid, reversible shutdown of signaling, as well as by multiple mechanisms that regulate the localization, availability, and activity of the downstream effectors (for a review see refs. 2–4). The most potent form of signal attenuation is ligand-induced receptor endocytosis (for a review see ref. 5), which terminates receptor signaling through ubiquitin-dependent lysosomal degradation of active receptors. The purpose of this review is to describe our understanding of the cellular function of Hsp90 in regulation of ErbB receptor tyrosine kinases. As will be discussed, the Hsp90 chaperone machine constitutes an additional layer of regulation of ErbB-receptors, which is amenable to pharmacological intervention, and might be utilized for the therapy of a variety of malignancies and other pathologies.

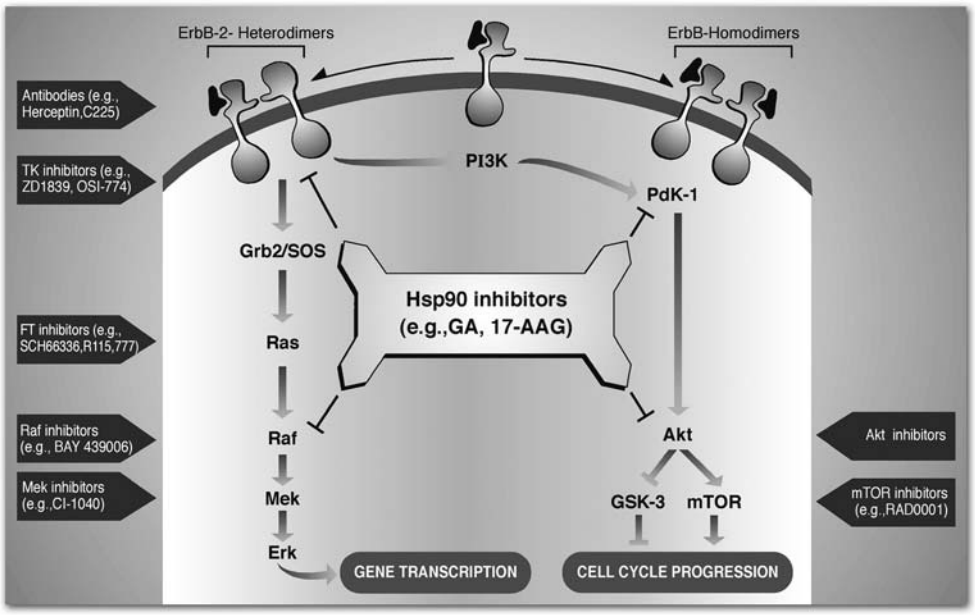

Figure 1. Drugs intercepting ErbB signaling. Ligand binding to monomeric, inactive ErbB receptors is followed by dimer formation and kinase activation. Upon recruitment into heterodimers, the ligand-less coreceptor, ErbB-2/HER2 enhances and prolongs signaling through several pathways, including the indicated mitogen-activated protein kinase (MAPK) pathway and the phosphatidylinositol 3 kinase (PI3K) route leading to activation of the Akt/PKB kinase. Drugs targeted at individual signaling molecules are indicated. Points of intervention by antagonists of Hsp90 are shown, and they include the receptors themselves (primarily ErbB-2), Raf, Pdk-1 and Akt kinases. The abbreviations used are: FT, farnesyltransferase; Pdk-1, phosphoinositide-dependent kinase 1; TK, tyrosine kinase; GSK-3, glycogen synthase kinase 3.

THE ROLE OF ERBB RECEPTORS IN MALIGNANT TRANSFORMATION

Much of the interest in ErbB-receptors has arisen due to the role these receptors play in human malignancies. Hyperactivation of ErbB signaling pathways is implicated in driving the proliferation of many cancer cell types, and is often correlated with poor patient survival (for a review see refs. 1 and 6). Both overexpression and structural alterations of ErbB-1 are frequent in various human malignancies,[7] (for a review see ref. 6). Overexpression of ErbB-1 occurs in 40% of gliomas, and is associated with higher tumor grade, enhanced proliferation and reduced patient survival,[8] (for a review see ref. 6). The most common structural alteration found in ErbB-1 (EGFRvIII) deletes amino-acids 6-273, yielding a protein whose ligand binding is defective, but is constitutively active and displays enhanced tumorogenicity.[9]

Overexpression of ErbB-2 (also called HER2/*neu*) has been detected in up to 30% of breast and ovarian cancers.[10] High frequency of ErbB-2 overexpression has also been noted in other common types of cancers including lung, gastric, and oral cancers.[11] In addition, overexpression of ErbB-2 has been correlated with invasive and poor prognostic features of breast, ovarian, and other human cancers, and it is often associated with shorter patient survival, early relapse, and an increased number of lymph node metastases.[12] Importantly, overexpression of ErbB-2 has been implicated in mediating increased resistance to chemotherapeutic agents (for a review see ref. 13). ErbB-2 is thought to induce chemoresistance by activation of the PI3K/Akt pathway[14,15] (for a review see ref. 13), as well as through upregulation of p21-Cip1.[16] The activity of ErbB-2 most likely depends on the coexpression of other ErbB-receptors, primarily ErbB-3, for formation of potent signaling heterodimers. Thus, when assessing the ErbB status of a given tumor, it is crucial to determine the levels of expression of the different receptors, as these will be pivotal in defining the clinical outcome (for review a see ref. 17). In the context of Hsp90, it is interesting to note that growth factors acting through receptor tyrosine kinases induce elevated expression of Hsp90,[18] and Hsp90 is found overexpressed 2-10 fold in tumor cells.[19]

ERBB RECEPTORS AS CLIENTS OF CHAPERONE-MEDIATED PROTEIN TRIAGE

Regulation of ErbB receptors by the Hsp90-based chaperone system falls into a larger conceptual framework, in which chaperones are viewed as part of the cellular 'triage' machinery (for a review see ref. 20). Triage is defined as the 'sorting and allocation of treatment to patients', while in protein triage the patients are cellular proteins. This triage is activated upon cellular stress, but also serves in maintaining homeostasis of the cell. The triage model proposes that cellular chaperones recognize a surface determinant on a protein, defining it as a client protein, whose conformation necessitates association with chaperones for stabilization. The outcome of chaperone action is the refolding of the client. If this refolding is unsuccessful, the client protein is directed to a degradative fate. Protein triage occurs through the dynamic, ATP-dependent interaction of a limited subset of proteins with chaperones (for a review see ref. 20). Thus, the majority of cellular proteins do not interact with chaperones, and are resistant to the chaperone-associated degradative machinery due to the intrinsic stability of their structure. As will be discussed in the context of ErbB receptors, the core of the triage chaperone complex consists of Hsp90, and degradation occurs through an active function of the chaperones in recruitment of ubiquitin ligases, and perhaps even in coupling to the 26S proteasome.

THE HSP90 CHAPERONE MACHINE

One of the major cellular proteins, conserved in a role essential for life throughout the eukaryotic lineage, is the molecular chaperone Hsp90, which is central in maintaining cellular homeostasis (for a review see ref. 21). Unique in its interaction with substrates in their final stages of folding, the function of the Hsp90 chaperone is in the highly selective maintenance of the properly folded form of a subset of proteins, which are essential for cellular signaling (for a review see ref. 22). Recently, it has been suggested that Hsp90 plays a role as a buffer for protein conformational diversity in the cell, acting as a chaperone that silences the effects of accumulating mutations within cellular proteins, by holding them in the native conformation.[23] This function has been demonstrated to allow the accumulation of a reservoir of genetic diversity that permits the emergence of adaptive changes in the face of selective pressure, suggesting a crucial role for Hsp90 in evolution.[23] The function of Hsp90 depends on the

ATPase activity of the chaperone, as well as the activities of a number of auxiliary proteins, collectively referred to as cochaperones (for a review see ref. 24). A major breakthrough in the research into the activity of Hsp90 was initiated by the discovery that the chaperone is inhibited by compounds of the benzoquinone ansamycin family, geldanamycin (GA) and herbimycin A,[25] which set the stage for identification of the role played by Hsp90 in the regulation of cellular client proteins (for a review see ref. 26).

The interaction of Hsp90 with its client proteins is characterized by low affinity binding, and repeated cycles of association and dissociation.[27] Hsp90 recognizes structural features that are common to unstable proteins, such as unveiled hydrophobic patches, rather than specific sequence motifs. As minor changes in amino-acid sequence can have substantial effects on the conformational stability of a protein, large variability can be seen in the dependence of individual members of very homologous protein families on interactions with Hsp90. Examples are apparent from the families of steroid hormone receptors (for a review see ref. 28), the group of Cyclin-dependent kinases,[29] Src family kinases,[30,31] as well as the ErbB family.[32,33]

As a rule, it appears that oncogenically activated kinase mutants (e.g., v-Src, mutant forms of Lck and Hck, and Bcr-Abl), as well as the more highly active members of a kinase family (as in the case of the ErbB receptors), require chaperoning by Hsp90 for their stability. This phenomenon may be attributed to the possibility that activation of tyrosine kinases releases them from the inflexible, but stable, conformation associated with the inactive state. This release, and the increased conformational flexibility associated with it, are likely accompanied by an increased propensity to unfold into unstable conformations. Thus, intrinsically hyperactive kinases like ErbB-2 would need the buffering capacity offered through association with Hsp90, for maintenance of their active conformation and stability. As will be discussed later, this may allow the chaperone to maintain a related function, in limiting the catalytic activity of its hyperactive clients.

Hsp90 possesses a nucleotide-binding pocket, and although substrate binding is ATP-independent, the release step requires the very slow ATPase activity of Hsp90.[34] According to the prevailing model (for a review see ref. 26), Hsp90 is found in two distinct multi-component complexes, which regulate both the interaction of the client with Hsp90, and its functional outcome for the client. These two complexes define the two arms of the triage pathway: While one arm is responsible for attempted refolding and stabilization of the client, the second arm is responsible for substrate ubiquitylation and degradation. Upon binding of ATP, the chaperone is found associated with p23 and Cdc-37, which promote client stabilization. Conversely, in the ADP-bound form, Hsp90 is found in a complex with Hsp70 and p60-HOP (Hsp70/Hsp90 organizing protein,[35] for a review see ref. 28). The latter cochaperone is an adaptor comprised of three TPR (tetratricopeptide repeats) domains, of which the amino-terminal one has been found to bind a TPR-acceptor site on Hsp70 while the central domain binds a similar TPR-acceptor site on Hsp90, bridging the two chaperones. The complex of Hsp90 with Hsp70 and p60-HOP has been suggested to be associated with client degradation.[26]

The nucleotide-binding pocket of Hsp90 is the target of intervention of Hsp90-binding drugs of the benzoquinone ansamycin family. Binding of the drugs to the ATP-binding pocket of the chaperone results in displacement of ATP, preventing completion of the interaction cycle.[36] This results in the formation of a complex reminiscent of that formed in the presence of ADP, and leads to ubiquitylation and degradation of Hsp90 clients.[37] Interestingly, the ATPase activity of Hsp90 is induced upon interaction with a fully folded client protein, suggesting a mechanism through which the client induces its release from the chaperone upon completion of folding.[38]

HSP90 COCHAPERONES AND THEIR RELEVANCE TO ERBB REGULATION

While our understanding of the mechanism of action of Hsp90 is still rudimentary, it is quite clear that the chaperone is dependent upon the function of its associated cochaperones for its activity. Many of these cochaperones interact with Hsp90 and Hsp70 by binding through their TPR domain to the TPR-acceptor site at the carboxyl-terminus of the chaperones.

Two major systems have been utilized for investigation of the activity of Hsp90 in mammalian cells: an in-vitro system, which enabled the elucidation of the minimal protein components needed for refolding and maintenance of activity of Hsp90 client proteins (mostly steroid hormone receptors), and cellular models, in which the role of the chaperone in regulation of the stability and activity of its client proteins has been examined. In-vitro, Hsp90 functions in the context of a minimal five-component complex (Hsp90, Hsp70, p23, p60-HOP and Hsp40) to bring about the refolding of substrate proteins to a functional state[39] (for a review see ref. 28). The roles of Cdc-37, CHIP and Bag-1 in regulating the stability of substrate proteins have been addressed in cellular models. While the role of CHIP in ubiquitylation and degradation of ErbB-2 has been characterized,[40,41] similar analyses have not been performed with the other two cochaperones. Hence, we will describe the data relating to the activities of Bag-1 and Cdc-37 in relation to other Hsp90 client proteins and extrapolate to ErbB-receptors.

Cdc-37. Originally described as a kinase-specific Hsp90 cochaperone, Cdc-37 has recently been found to associate with additional proteins, such as the androgen receptor[42] and hepadnavirus reverse transcriptase.[43] Cdc-37 was originally implicated in the stabilization of client proteins to which it binds in the context of Hsp90. However, Cdc-37 also exhibits intrinsic chaperone activities, acting partially independently of Hsp90.[44] It has been suggested that Cdc-37 stabilizes client proteins following their interaction with Hsp90[45] (for a review see ref. 46), while one of the key roles of Cdc-37 in the context of Raf-1 is to promote the assembly of a Hsp90-containing heterotrimeric complex crucial for Raf-1 activity.[47] The cell cycle kinases Cdc-28 and Cak-1 are destabilized in a *cdc37* yeast mutant, and as a result, fail to form complexes with Cyclins.[48] Hck catalytic activity is also significantly enhanced by overexpression of Cdc-37, because Cdc-37 can promote its association with Hsp90 and folding into a catalytically active conformation.[49] Yeast Cdc-37 can compensate in-vivo for loss of Hsp90 in maintaining the activity of v-Src.[44] These examples suggest a role for Cdc-37 in maintaining the signaling potential of its client proteins, beyond a role in maintaining protein stability (for a review see ref. 46). Interestingly, Cdc-37 acts as an oncogene, and collaborates with c-Myc and Cyclin D to promote tumorigenesis upon ectopic expression in the mammary gland.[50]

In relation to ErbB receptors, Cdc-37 is found associated with the nascent form of the oncogenic ErbB-1 deletion mutant commonly found in gliomas, namely EGFRvIII,[51] which contains a deletion within the extracellular domain of the receptor, resulting in constitutive activation.[8] In addition, our own observations suggest that Cdc-37

promotes the stabilization of the mature form of ErbB-2 (Citri et al., manuscript in preparation). As the structures of the active state of different kinases are very similar,[52] and Cdc-37 has been found to bind to the amino-terminal lobe of the catalytic domains of several protein kinases[53] (for a review see ref. 46), it is possible that Cdc-37 has evolved to recognize a subset of hyper-active kinases. We speculate that Cdc-37 preferentially interacts with the activated form of kinases, stabilizing the open, active conformation and promoting kinase activity. Interestingly, the interaction of Cdc-37 with nonkinase substrates may also be explained by binding to a similar fold, as the structure of reverse transcriptase appears to be similar to that of kinases.[43]

Bag-1. This cochaperone was originally identified as a Bcl-2-interacting suppressor of apoptosis, but additional cellular functions have been ascribed to it, including stimulation of Raf-1 kinase activity,[54] regulation of steroid hormone receptor activity, and transcriptional regulation (for a review see ref. 55). Bag-1 exists in several translation initiation isoforms, and a family of mammalian paralogues has been identified. All Bag family members maintain a carboxyl-terminal BAG domain, which associates with the ATPase domain of Hsp70, stimulating ADP-ATP exchange and client protein release.[56] Bag-1 associates with the 26S proteasome through an amino-terminal ubiquitin-like (Ubl) domain, as well as through specific ubiquitin moieties appended to Bag-1 by CHIP.[57] It has been suggested that this mechanism may underlie the recruitment of Hsp70 to the proteasome,[58] resulting in trafficking of ubiquitylated client proteins, such as Raf-1, to proteasomal degradation.[59] Bag-1 has recently been identified as key in the coordination of signal transduction following stress, whereby a shift of a Bag-1/Raf-1 complex to a Bag-1/Hsp70 complex resulted in inhibition of signaling by Raf-1.[60] In addition, Bag-1 overexpression is detected in tumor cells, in association with increased cell survival.[61,62] Likewise, Bag-1 is found in complexes with receptor tyrosine kinases, including the receptors for the platelet-derived growth factor (PDGF) and the hepatocyte growth factor (HGF)[63] (for a review see ref. 55).

CHIP. CHIP was originally identified as an Hsp70-binding TPR domain protein, which inhibits the client refolding activity of the chaperone.[64] The domain structure of CHIP, which comprises an N-terminal TPR clamp domain, and a C-terminal E3-ubiquitin ligase U-box domain, implicates it as a chaperone-dependent E3 ubiquitin ligase (for a review see ref. 65). Consistent with its structural landmarks, CHIP has been documented to act as an E3 ubiquitin ligase[68,69] which ubiquitylates chaperone client proteins[67] and denatured model proteins,[70] as well as the chaperone machinery itself.[57,69] CHIP acts in regulation of triage decisions in the context of steroid hormone receptors,[66] the CFTR conductance regulator[67] and Raf-1.[59]

In relation to ErbB-2, CHIP has been characterized to function as an E3 ubiquitin ligase, which is recruited to the receptor upon inhibition of Hsp90. CHIP binds to ErbB-2 in a chaperone-dependent manner, and enhances its ubiquitylation and proteasomal degradation, in a manner dependent on the integrity of the U-box and TPR domains of CHIP.[40,41] Expression of a U-box domain mutant of CHIP enhanced the interaction between ErbB-2 and Hsp70, and blocked dissociation of Hsp90 from the receptor following inhibition of Hsp90. Interestingly, CHIP is dispensable for GA-induced degradation of ErbB-2, suggesting redundancy at the level of the E3 ubiquitin ligase functioning in the degradative arm of triage decisions in the context of ErbB-2.[40] According to a recent model,[71] CHIP competes with p60-HOP for interaction with Hsp70, disrupting the association of Hsp90 with Hsp70. The complex formed by CHIP and Hsp70 includes Bag-1, and functions in ubiquitylation and proteasomal degradation of the client protein. In contrast, in the complex formed by p60-HOP, Hsp70 is associated with Hsp90, and the consequence is a tendency towards a stable conformation of the client protein. Thus, it is suggested that the activity of the chaperone machine is dictated by the identity of the TPR-domain cochaperone associated with Hsp70. As GA and other Hsp90 inhibitors increase the association of client proteins with the Hsp90-p60HOP-Hsp70 complex, these drugs may improve the chances of CHIP to associate with Hsp70, thereby displacing HOP-Hsp90, and determining the fate of the client protein.[26]

REGULATION OF ERBB RECEPTORS BY HSP90

The activity of Hsp90 towards client proteins can be subdivided into three categories: one action is promoting the maturation of nascent clients into an active and stable conformation, while a second role is the continued maintenance of the mature form of the client. A third role of Hsp90 is intertwined with the second, and relates to maintenance of the client protein in a state competent for activation. The function of Hsp90 in the stabilization of client proteins has been extensively reviewed,[21,72] while its role in regulating the function of client proteins is an emerging concept (for a review see ref. 73). This role has been established for the glucocorticoid receptor,[28,74] the RNA-dependent kinase PKR,[75] as well as for a mutant form of Hck.[49,76] In its role in enabling the activity of client proteins, Hsp90 maintains the potential for optimal induced activation, while retaining the basal activity of the client minimal.

Of the four ErbB receptors, both ErbB-1 and ErbB-2 have been established as clients of Hsp90.[32,33,77-79] The interaction with Hsp90, as well as the regulation by Hsp90, differs between the two receptors, although the kinase domain of either receptor is responsible for the recruitment of Hsp90, and these domains share 80% identity in their amino-acid sequences.[78,80] ErbB-1 is dependent upon Hsp90 during maturation in the endoplasmic reticulum (ER), but upon incorporation into the plasma membrane, it loses its dependence upon Hsp90.[32,33,51,78,79] In contrast, and similar to v-Src,[79] ErbB-2 is dependent upon Hsp90 for its stability throughout the whole life span of the receptor, including the maturation process in the ER, and during the residency of the receptor at the plasma membrane.[32,40] These differences are reflected in distinct kinetics of receptor degradation; while ErbB-2 is depleted within 2 hours of Hsp90 inactivation, ErbB-1 is degraded only following much longer kinetics.[32] These dissimilar sensitivities of the kinase domains of mature ErbB-1 and ErbB-2 receptors to inhibition of Hsp90 function correlate with the differential heat sensitivities of the receptors.[81] While the degradation of ErbB-2 upon inactivation of Hsp90 occurs from both the ER and the plasma membrane,[32] degradation of ErbB-1 occurs only from the ER, resulting in reduction in the number of low affinity EGF-binding sites at the cell surface.[78,82] The chaperone-associated degradation of both ErbB-1 and ErbB-2 is proteasome- and ubiquitin-dependent,[77,83] but the mechanism through which a transmembrane receptor undergoes proteasomal degradation is still unclear.

The Chaperone-Mediated Pathway of ErbB-2 Degradation. The data relating to the pathway through which ErbB-receptors undergo ubiquitin-dependent proteasomal degradation following inhibition of Hsp90 is still very fragmentary. The first step involves shuffling of the chaperone complexes associated with the client pro-

tein, such that interaction with Hsp90 becomes more sensitive to detergent, while the interaction with Hsp70 is significantly enhanced (for a review see refs. 26 and 84). We have found that a similar shuffling of chaperones associated with ErbB-2 occurs upon treatment of cells with an irreversible, ErbB-specific tyrosine kinase inhibitor (TKI; CI-1033).[32] The subsequent ubiquitylation of the receptor involves the ubiquitin ligase CHIP, as described above,[40,41] (our unpublished observations). Interestingly, ErbB-2 is found in vesicular structures upon treatment with degradation inducing factors, namely GA and TKIs,[32,85] but how these structures relate to proteasomal degradation is still unclear. Interestingly, data from the Carpenter lab describes a pathway for GA-, curcumin-, and staurosporin-induced degradation of ErbB-2 which involves a caspase-dependent cleavage event within the kinase domain, resulting in the formation of a 135 kDa ectodomain fragment and a fragment of ~50 kDa containing the carboxyl-terminal region. This 50 kDa fragment is further cleaved, in a caspase-dependent manner, to form a ~23 kDa fragment, that is subject to proteasomal degradation.[85-87]

Hsp90 as a Regulator of ErbB-Receptor Activity. It has been reported that Hsp90 inhibition can disrupt the ability of ErbB-2 to form signaling heterodimers upon ligand activation.[88] It is interesting to note that in this report, treatment with low doses of GA significantly enhanced the basal kinase activity of the receptor. This data, in the context of the recent concept of Hsp90 as a regulator of activity, may hint to an additional, yet uncharacterized role of Hsp90 in regulation of the activity of ErbB receptors. In line with this notion, several lines of evidence suggest a direct function for Cdc-37 and Hsp90 in supporting the activation of another kinase, Raf-1. This data stems from genetic studies in Drosophila, demonstrating that Cdc-37 and Hsp90 are essential for the activity of Raf-1,[89] as well as biochemical data demonstrating a role for Cdc-37 and Hsp90 in the activation of Raf-1.[47] In addition, treatment of cells with Hsp90 inhibitors causes degradation of Raf-1 over a long time course, while inducing a transient burst of Raf-1 activity when administered for a short time.[75,90] Similar evidence has been demonstrated for the activity of the RNA-dependent kinase PKR, which becomes active upon short treatment with GA, suggesting that Hsp90 acts to restrain the basal signaling of this kinase.[75] Additional examples for the role of Hsp90 in activation of clients are found in the regulation of cytosolic tyrosine kinases and steroid hormone receptors. The constitutive activity of v-Src in yeast was found to depend upon the activity of Cdc-37 and Hsp90,[31,45] as was the activity of a mutant of Hck.[49,76] Further, Hsp90 masks dimerization and inhibits DNA binding of steroid hormone receptors until chaperone interactions are interrupted, typically as a consequence of hormone binding. Thus, steroid hormone receptors stripped from chaperones are competent for dimerization and DNA binding in the absence of hormone[91] (for a review see ref. 27). Analysis of point mutations in the hsp90 gene revealed that chaperone functions play separable roles in protein accumulation and kinase activation.[74]

While this function of Hsp90 may not hold true for all its client proteins, we predict that in the case of ErbB-2, Hsp90 may undertake a similar role, restraining the intrinsic kinase basal kinase activity of the receptor, while retaining the kinase in a primed conformation, ready for induction following interaction with a ligand-activated heterodimerization partner. In this context, it is interesting to note that the kinase activity of ErbB-1 is induced upon heat shock,[92] ultraviolet irradiation[93] and oxidative stress,[94] all of which may potentially affect the activity of Hsp90 in chaperoning the receptor.

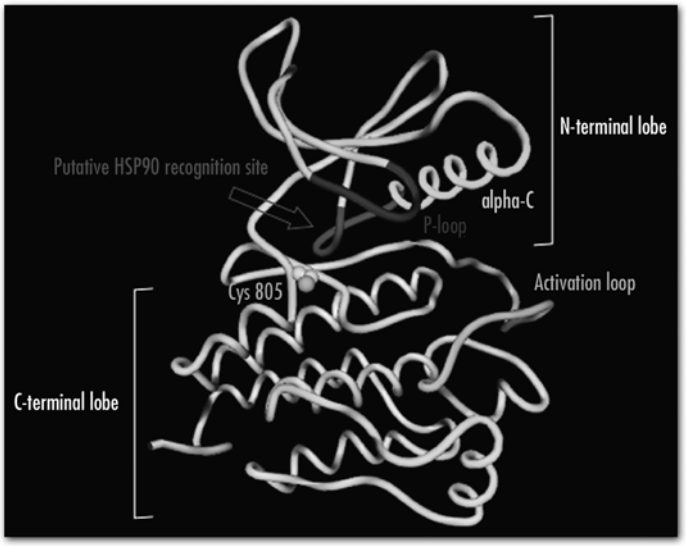

Figure 2. Predicted structure of the kinase domain of ErbB-2, including the putative Hsp90 recognition site. The homology model of the kinase domain of ErbB-2 was generated using the structure of the kinase domain of ErbB-1 (1M14[97]) as a template. The model shows the characteristic bi-lobular kinase structure; the N-lobe contains the nucleotide-binding pocket, and both the activation and the catalytic loops are included in the C-lobe. The glycine-rich loop (P-loop) of the N-lobe is indicated, along with the C-alpha helix and the putative Hsp90 recognition site (residues 776-786; see text). Also indicated is cysteine 805, the target for covalent binding with an irreversible tyrosine kinase inhibitor. The sequence to structure alignment was generated using the Fugue server and the model was built using MODELLER. The quality of the model was assessed by analyzing the Joy formatted alignments of the model and the template structures generated by COMPARER, RAMPAGE and PROCHECK. The figure was generated using SWISS-PDB viewer and rendered using QuickDraw3D.

The Achilles Heel of ErbB-2: An Hsp90 Recognition Site within the Kinase Domain. It is clear that the determinant defining sensitivity of ErbB-2 to Hsp90 blockers is found within the kinase domain of ErbB-2, while kinase activity per se is not necessary for the regulation of the receptor by Hsp90.[32,33,80] For example, mutating the ATP binding site of ErbB-1 conferred sensitivity of the receptor to GA,[32] and mutations affecting the kinase domains of other receptor,[95] as well as cytosolic tyrosine kinases[76,96] enhance sensitivity to GA. Comparing the highly similar sequences of the kinase domains of ErbB-1 and ErbB-2, which remarkably differ in their regulation by Hsp90, Tikhomirov and Carpenter[87] defined a short sequence which differs significantly in charge between the two receptors. Replacing a short sequence of ErbB-2 with that of ErbB-1 resulted in a receptor that retained kinase activity, but became insensitive to GA-induced degradation. Modeling the kinase domain of ErbB-2 based on the published structure of the kinase domain of ErbB-1,[97] it is clear that this region is located on the surface of the receptor, at the end of the Cα helix (Fig. 2), the helix whose movement is most profound upon transition of kinases between active and inactive conformations (for a review see ref. 52). We speculate that Hsp90 binds to this surface region, modulating the flexibility of the hinge between the two domains of the kinase. In such a manner, the interaction of Hsp90 with the kinase domain of ErbB-2 might modulate the kinase activity of the receptor by affecting the kinetics of ATP binding and hydrolysis, substrate binding, or the propensity of the kinase domain to interact with kinase domains of other receptors.

The Cdk family of protein kinases provides one of the clearest examples of allosteric regulation of catalytic activity via the Cα helix. Cyclins bind directly to the Cα helix, reverting the inhibition of kinase activity.[98] The complexes formed by Cdk4 with Hsp90/Cdc-37 are mutually exclusive from complexes formed upon binding to Cyclin D,[29] again suggesting that Hsp90 serves to sequester Cdk molecules in an inactive, but primed conformation, ready for interaction with Cyclin D and activation.

Hsp90 Mediated Regulation of Signaling Downstream of ErbB Receptors. Signaling mediated by ErbB-2, especially when present in the highly potent ErbB-2/ErbB-3 heterodimer, results in activation of multiple signaling pathways, which promote cell survival and proliferation (for a review see ref. 17; Fig. 1). The role of Hsp90 in regulating signaling by this complex network extends beyond regulation of the receptors themselves. It is evident that Hsp90 plays an additional role in regulating the effector proteins activated by ErbB-receptors at several levels downstream of the receptor (for a review see ref. 99). Thus, Ras,[100] Raf-1,[101] MEK,[90] Pdk-1,[102] Akt[103,104] and Cdk4,[29] crucial transducers of ErbB signals, are all Hsp90 client proteins. Both the expression levels of these proteins, as well as their activity, are regulated by Hsp90, and are disrupted upon Hsp90 inhibition by GA.

The dependence of Raf-1 upon Hsp90 for its stabilization and activation has been demonstrated using genetic studies in Drosophila, as well as biochemical studies in mammalian cells (for a review see ref. 73). Mutations in the insect Hsp90 that decrease the interaction with Raf suppress Raf gain-of-function mutations, and reduce the biochemical activity of the kinase.[89,105] Similar results have been demonstrated for Cdc-37, which is essential for formation of the Raf-1/Hsp90 complex, and for Raf-1 activation.[47,89] Inhibition of Hsp90 activity by use of GA and GA-derivatives results in degradation of Raf-1 and disruption of signaling downstream of Raf-1, leading to growth arrest and apoptosis.[100,106,107]

Akt is found in complexes with Cdc37 and Hsp90,[15,103,104] and is dependent upon the interaction with Hsp90 for its activation.[104] Upon interruption with this interaction, Akt undergoes rapid dephosphorylation and inactivation mediated by the phosphatases PP2A[104] or PP1.[108] Furthermore, inactivation of ErbB-2 and Akt by administration of the GA derivative 17-AAG resulted in inhibition of Akt-dependent Cyclin D1 activation,[103] promoting Rb-dependent G_1 arrest, and sensitization of tumors to Taxol-induced apoptosis.[15]

INTERFERENCE WITH THE ACTIVITY OF HSP90 AS A THERAPEUTIC MODALITY

In contrast to the effect of ErbB-2 over-expression in promoting chemoresistance to tumors, high levels of expression of ErbB-2 confer sensitivity to treatment of tumor cells with Hsp90 inhibitors.[14,109] This sensitivity to Hsp90 blockers depends upon coexpression of ErbB-3 and activation of the phosphatidylinositol 3-kinase (PI3K),[110] factors which play a crucial role in the establishment of chemoresistance. Thus, regulation of ErbB-2 by Hsp90 opens a potential therapeutic opportunity for pharmacological intervention. A number of strategies are in different stages of clinical development for assessment of their efficiency in targeting ErbB-receptors in cancers (for review see refs. 1 and 13). Among these are immunological approaches, which include Herceptin/Trastuzumab, a humanized monoclonal antibody to ErbB-2, which is already in clinical use,[111] and low molecular weight tyrosine kinase inhibitors (TKIs), including the already approved Iressa (ZD1839) compound.[112] The approach of disrupting the function of Hsp90 in regulation of ErbB-2 is a novel, potentially advantageous, approach. 17-AAG, a derivative of GA modified at position 17, has completed phase I multi-center clinical trials with hepatotoxicity occurring as the major dose limiting factor (for review see ref. 113). Targeted phase I/II combination trials and phase II single agent trials are being developed, including a trial testing the efficacy of 17-AAG in combination with Taxol in ErbB-2 over-expressing breast and prostate tumors (for review see ref. 84). Additional ansamycins and other compounds capable of targeting the Hsp90/ErbB-2 complex, are in preclinical testing as reviewed below.

Benzoquinone Ansamycins. In the course of screening microbial fermentation products for anticancer activity, it was noted that the benzoquinone ansamycins herbimycin A,[114] GA[115] and macbecin[116] (for review see ref. 27), were able to revert transformation of cells by v-Src,[117] as well as by other oncogenic kinases, including ErbB-2.[118] Using an immobilized GA derivative, it was found that the compound interacts in a highly selective manner with Hsp90 family proteins, and that this interaction disrupts the association of Hsp90 with kinases such as v-Src and ErbB-2, leading to loss of their transforming activity.[25,119] The activity of the benzoquinone ansamycins has been attributed to their interaction with the ATP- binding pocket of the chaperone, resulting in remodeling of chaperone complexes and subsequent degradation of client proteins.[120,121] The 17-allylamino-17-desmethoxy derivative of GA (17-AAG),[122] for which clinical trials are underway, is better tolerated than GA and therefore more efficacious in vivo.[123,124] An interesting approach for enhancing the target specificity of GA was demonstrated by Rosen and colleagues.[125] In this approach, a phosphatidylinositol-3-kinase (PI3K) inhibitor (LY294002) was covalently attached to GA, resulting in effective Hsp90-dependent PI3K inhibition in vitro. This inhibition was found to be more potent than use of the PI3K inhibitor itself, but its efficacy in vivo is still a matter for investigation.

Radicicol and Coumarins. Radicicol is a macrocyclic antibiotic of fungal origin. This compound is structurally distinct from the benzoquinone ansamycins, but has been found to compete with GA for binding to the amino-terminal nucleotide-binding pocket on Hsp90.[126] While GA and radicicol bind to the N-terminal nucleotide-binding pocket of the chaperone, the coumarin antibiotics, such as novobiocin, bind to a pocket in the carboxyl-terminus of the chaperone[127,128] resulting in depletion of Hsp90-dependent signaling proteins.

Additional Compounds. The activity of a number of small molecular weight compounds in promotion of ErbB-2 degradation has been described. These include aspirin,[129] as well as curcumin and staurosporin. Curcumin is a major pigment in turmeric, used as a flavor in curry and as a herbal medicine for treatment of inflammatory diseases. Curcumin has been described as a kinase inhibitor of ErbB receptors,[130] but it has recently been found to disrupt the complex of ErbB-2 with the chaperone Grp94, resulting in ErbB-2 degradation over a relatively long time course.[131] The fragmentation of ErbB-2 induced by curcumin resembles that induced by GA, but diverges in the fact that the degradation of the receptor is retained even in the presence of mutations that disrupt sensitivity to GA.[86,87]

Staurosporin is a potent wide-spectrum protein kinase inhibitor,[132] and has been reported to cause apoptosis in many cell types. Staurosporin induces a pattern of cleavage of ErbB-2 similar to that induced by GA. However, degradation of ErbB-2 induced by

staurosporin utilizes a pathway somewhat distinct from that induced by GA, since it is blocked by inhibitors of caspases and the 26S proteasome in a different manner than GA-induced degradation of the receptor. Further, staurosporin maintains its activity on a GA-resistant mutant of ErbB-2.[86,87] The activity of staurosporin towards ErbB-1 and ErbB-2 is distinct; while staurosporin promotes degradation of ErbB-2, it acts to increase the number of ErbB-1 receptors on the surface of A431 cells.[133]

Finally, a recent report has described the establishment of a microtiter cell-based assay for detection of degradation-inducing factors for ErbB-1 and ErbB-2.[134] It will be interesting to see if screening with this assay will uncover additional compounds that induce degradation of ErbB-2 through the Hsp90 chaperone machine.

Hsp90 Inhibitors with Increased Specificity to ErbB-2. A number of attempts have been made to generate inhibitors of Hsp90 with increased selectivity towards ErbB-2, as this is expected to reduce the toxicity potentially associated with inhibition of an essential, ubiquitous protein such as Hsp90. These approaches include formation of GA dimers,[135] as well as ErbB-2-directed antibody-conjugates of GA.[136] In addition, different approaches are being taken in an effort to produce additional classes of Hsp90 inhibitory drugs[137-140] (for a review see ref. 141).

Targeted Degradation of Hsp90 Client Proteins: The Case of ErbB-2. Inhibition of Hsp90 acts to promote degradation of a large number of crucial cellular proteins, resulting in inhibition of signaling pathways critical for cell survival. Thus, a potential caveat restricting the clinical use of Hsp90 inhibitors, is the possibility of limiting toxicity, due to broad effects on healthy tissues.[113] In contrast, rationally designed, target-based agents are characterized by low toxicity, clinical efficacy and wide therapeutic indices, due to their potential for inducing selective tumor cell cytotoxicity, while sparing normal tissue.[142] Strategies combining the efficacy of chaperone-mediated degradation, with high target selectivity may therefore hold significant promise for cancer therapy.

A mutation within the ATP-binding pocket of ErbB-1 results in recognition of the receptor by Hsp90, which shortens the half-life of the receptor, and promotes sensitivity to degradation induced by GA.[32] These observations imply that Hsp90 senses conformational perturbations occurring in the regions associated with the ATP-binding pocket. Thus, it was reasoned that TKIs that interact with the ATP-binding pocket in a specific and stable manner, could transduce a similar structural perturbation, resulting in enhanced receptor ubiquitylation and degradation. Indeed, this phenomenon was observed with a number of TKIs, the most potent of which, CI-1033, irreversibly alkylates a specific cysteine residue (cysteine 805) within the ATP binding pocket of ErbB-receptors (Fig. 2).[143] The pathway of ubiquitylation and degradation induced by the TKIs recapitulates the pathway induced by Hsp90 inhibitors, and as each compound targets a different component, Hsp90 inhibitors and CI-1033 act in an additive manner in inhibiting ErbB-driven tumor cell growth.[32] The synergy of the compounds potentially allows for combination therapy with enhanced targeting specificity (for a review see refs. 144,145). We have found that the degradation of ErbB-2 induced by CI-1033 is dependent upon the integrity of the TPR and U-box domains of CHIP, similar to the role played by CHIP in degradation induced by GA (our unpublished observations).

Inline with these results, an additional TKI (Quercitin) has been described which inhibits the phosphorylation of ErbB-2, and decreases receptor levels.[146] Interestingly, TKI-induced degradation of an additional oncogenic receptor tyrosine kinase has been recently described.[147] This work describes ubiquitin- and proteasome-dependent degradation of the two Ret oncoproteins, RETMEN2A and RETMEN2B, induced by the kinase inhibitor PP1. A potentially significant finding relates to degradation of the Hsp90 client protein nitric oxide synthase (NOS), by irreversible inhibitors directed against this distinct class of enzymes.[148] Collectively, these lines of evidence predict a wide clinical potential for selective, rather than general targeting of Hsp90 client proteins to degradation.

Insights into the atomic basis of TKI- induced degradation can come from structures of kinases crystallized in the presence of ATP or TKIs[149] (for review see ref. 52). Within these structures, the conserved glycine-rich motif (P-loop; GXGXϕG; ϕ being tyrosine or phenylalanine) binds the phosphates of ATP, and due to the glycine residues, this loop is very flexible in the absence of ATP, allowing the interaction with small molecule inhibitors. Some of the inhibitors may induce a large structural distortion in the P-loop by interaction with the conserved aromatic residue. This distortion may allosterically affect the conformation of the adjacent Cα helix, which could potentially impact on the interaction with Hsp90 (Fig. 2).

PERSPECTIVES

In analogy to the proposed evolutionary role of Hsp90 in buffering the detrimental effects of genetic variation, the chaperone may play a role in the microevolution of tumor cell populations, allowing tumors to depend for their proliferation on mutant, hyperactive kinases. In line with this possibility, the functions of oncogenically mutant protein kinases, such as v-Src and Bcr-Abl, depend on Hsp90. In this context, ErbB-2 may be viewed as a hyperactive tyrosine kinase, whose stability, and perhaps also catalytic activity, is regulated by chaperones. The dependence of certain carcinomas on ErbB-2, which itself is dependent on Hsp90, opens a potential therapeutic window. Specifically, because the Hsp90 recognition site is close to the relatively deep nucleotide-binding pocket of ErbB-2, drugs that modify this groove may mimic the effect of Hsp90 blockers. However, unlike ansamycin blockers, tyrosine kinase inhibitors will act only upon ErbB-2, while sparing other chaperone clients. Similar Achilles heels may exist on other chaperone clients, such as mutant forms of p53. Hence, targeted recruitment of the chaperoning machinery may allow selective destruction of pathogenic proteins by the 26S proteasome.

References

1. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nat Rev Mol Cell Biol 2001; 2:127-37.
2. Fiorini M, Alimandi M, Fiorentino L, Sala G, Segatto O. Negative regulation of receptor tyrosine kinase signals. FEBS Lett 2001; 490:132-41.
3. Ostman A, Bohmer FD. Regulation of receptor tyrosine kinase signaling by protein tyrosine phosphatases. Trends Cell Biol 2001; 11:258-66.
4. Schlessinger J. Cell signaling by receptor tyrosine kinases. Cell 2000; 103:211-25.
5. Waterman H, Yarden Y. Molecular mechanisms underlying endocytosis and sorting of ErbB receptor tyrosine kinases. FEBS Lett 2001; 490:142-52.
6. Klapper LN, Kirschbaum MH, Sela M, Yarden Y. Biochemical and clinical implications of the ErbB/HER signaling network of growth factor receptors. Adv Cancer Res 2000; 77:25-79.
7. Gullick WJ. Prevalence of aberrant expression of the epidermal growth factor receptor in human cancers. Br Med Bull 1991; 47:87-98.
8. Wong AJ, Ruppert JM, Bigner SH, Grzeschik CH, Humphrey PA, Bigner DS, et al. Structural alterations of the epidermal growth factor receptor gene in human gliomas. Proc Natl Acad Sci USA 1992; 89:2965-9.
9. Nishikawa R, Ji XD, Harmon RC, Lazar CS, Gill GN, Cavenee WK, et al. A mutant epidermal growth factor receptor common in human glioma confers enhanced tumorigenicity. Proc Natl Acad Sci USA 1994; 91:7727-31.

10. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 1989; 244:707-12.
11. Wang SC, Hung MC. HER2 overexpression and cancer targeting. Semin Oncol 2001; 28:115-24.
12. Lohrisch C, Piccart M. HER2/neu as a predictive factor in breast cancer. Clin Breast Cancer 2001; 2:129-35; discussion 36-7.
13. Yu D, Hung MC. Overexpression of ErbB2 in cancer and ErbB2-targeting strategies. Oncogene 2000; 19:6115-21.
14. Munster PN, Basso A, Solit D, Norton L, Rosen N. Modulation of Hsp90 function by ansamycins sensitizes breast cancer cells to chemotherapy-induced apoptosis in an RB- and schedule-dependent manner. See: Sausville EA. Combining cytotoxics and 17-allylamino, 17-demethoxygeldanamycin: Sequence and tumor biology matters. Clin Cancer Res 2001; 7:2155-2158. Clin Cancer Res 2001; 7:2228-36.
15. Solit DB, Basso AD, Olshen AB, Scher HI, Rosen N. Inhibition of heat shock protein 90 function down-regulates Akt kinase and sensitizes tumors to Taxol. Cancer Res 2003; 63:2139-44.
16. Yu D, Jing T, Liu B, Yao J, Tan M, McDonnell TJ, et al. Overexpression of ErbB2 blocks Taxol-induced apoptosis by upregulation of p21Cip1, which inhibits p34Cdc2 kinase. Mol Cell 1998; 2:581-91.
17. Citri A, Skaria KB, Yarden Y. The deaf and the dumb: The biology of ErbB-2 and ErbB-3. Exp Cell Res 2003; 284:54-65.
18. Jerome V, Leger J, Devin J, Baulieu EE, Catelli MG. Growth factors acting via tyrosine kinase receptors induce HSP90 alpha gene expression. Growth Factors 1991; 4:317-27.
19. Ferrarini M, Heltai S, Zocchi MR, Rugarli C. Unusual expression and localization of heat-shock proteins in human tumor cells. Int J Cancer 1992; 51:613-9.
20. Gottesman S, Wickner S, Maurizi MR. Protein quality control: Triage by chaperones and proteases. Genes Dev 1997; 11:815-23.
21. Young JC, Moarefi I, Hartl FU. Hsp90: A specialized but essential protein-folding tool. J Cell Biol 2001; 154:267-73.
22. Pratt WB. The role of the hsp90-based chaperone system in signal transduction by nuclear receptors and receptors signaling via MAP kinase. Annu Rev Pharmacol Toxicol 1997; 37:297-326.
23. Rutherford SL, Lindquist S. Hsp90 as a capacitor for morphological evolution. Nature 1998; 396:336-42.
24. Pratt WB. The hsp90-based chaperone system: Involvement in signal transduction from a variety of hormone and growth factor receptors. Proc Soc Exp Biol Med 1998; 217:420-34.
25. 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 USA 1994; 91:8324-8.
26. Neckers L. Hsp90 inhibitors as novel cancer chemotherapeutic agents. Trends Mol Med 2002; 8:S55-61.
27. Smith DF, Whitesell L, Katsanis E. Molecular chaperones: Biology and prospects for pharmacological intervention. Pharmacol Rev 1998; 50:493-514.
28. Pratt WB, Toft DO. Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. Exp Biol Med (Maywood) 2003; 228:111-33.
29. Stepanova L, Leng X, Parker SB, Harper JW. Mammalian p50Cdc37 is a protein kinase-targeting subunit of Hsp90 that binds and stabilizes Cdk4. Genes Dev 1996; 10:1491-502.
30. Bijlmakers MJ, Marsh M. Hsp90 is essential for the synthesis and subsequent membrane association, but not the maintenance, of the Src-kinase p56(lck). Mol Biol Cell 2000; 11:1585-95.
31. Xu Y, Lindquist S. Heat-shock protein hsp90 governs the activity of pp60v-src kinase. Proc Natl Acad Sci USA 1993; 90:7074-8.
32. Citri A, Alroy I, Lavi S, Rubin C, Xu W, Grammatikakis N, et al. Drug-induced ubiquitylation and degradation of ErbB receptor tyrosine kinases: Implications for cancer therapy. Embo J 2002; 21:2407-17.
33. Xu W, Mimnaugh E, Rosser MF, Nicchitta C, Marcu M, Yarden Y, et al. Sensitivity of mature Erbb2 to geldanamycin is conferred by its kinase domain and is mediated by the chaperone protein Hsp90. J Biol Chem 2001; 276:3702-8.
34. Panaretou B, Prodromou C, Roe SM, O'Brien R, Ladbury JE, Piper PW, et al. ATP binding and hydrolysis are essential to the function of the Hsp90 molecular chaperone in vivo. Embo J 1998; 17:4829-36.
35. Smith DF, Sullivan WP, Marion TN, Zaitsu K, Madden B, McCormick DJ, et al. Identification of a 60-kilodalton stress-related protein, p60, which interacts with hsp90 and hsp70. Mol Cell Biol 1993; 13:869-76.
36. Young JC, Hartl FU. Polypeptide release by Hsp90 involves ATP hydrolysis and is enhanced by the cochaperone p23. Embo J 2000; 19:5930-40.
37. Schneider C, Sepp-Lorenzino L, Nimmesgern E, Ouerfelli O, Danishefsky S, Rosen N, et al. Pharmacologic shifting of a balance between protein refolding and degradation mediated by Hsp90. Proc Natl Acad Sci USA 1996; 93:14536-41.
38. 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.
39. 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.
40. Xu W, Marcu M, Yuan X, Mimnaugh E, Patterson C, Neckers L. Chaperone-dependent E3 ubiquitin ligase CHIP mediates a degradative pathway for c-ErbB2/Neu. Proc Natl Acad Sci USA 2002; 99:12847-52.
41. Zhou P, Fernandes N, Dodge IL, Reddi AL, Rao N, Safran H, et al. ErbB2 degradation mediated by the cochaperone protein CHIP. J Biol Chem 2003; 278:13829-37.
42. Rao J, Lee P, Benzeno S, Cardozo C, Albertus J, Robins DM, et al. Functional interaction of human Cdc37 with the androgen receptor but not with the glucocorticoid receptor. J Biol Chem 2001; 276:5814-20.
43. Wang X, Grammatikakis N, Hu J. Role of p50/CDC37 in hepadnavirus assembly and replication. J Biol Chem 2002; 277:24361-7.
44. Kimura Y, Rutherford SL, Miyata Y, Yahara I, Freeman BC, Yue L, et al. Cdc37 is a molecular chaperone with specific functions in signal transduction. Genes Dev 1997; 11:1775-85.
45. Lee P, Rao J, Fliss A, Yang E, Garrett S, Caplan AJ. The Cdc37 protein kinase-binding domain is sufficient for protein kinase activity and cell viability. J Cell Biol 2002; 159:1051-9.
46. Maclean M, Picard D. Cdc37 goes beyond Hsp90 and kinases. Cell Stress Chaperones 2003; 8:114-9.
47. Grammatikakis N, Lin JH, Grammatikakis A, Tsichlis PN, Cochran BH. p50(cdc37) acting in concert with Hsp90 is required for Raf-1 function. Mol Cell Biol 1999; 19:1661-72.
48. Farrell A, Morgan DO. Cdc37 promotes the stability of protein kinases Cdc28 and Cak1. Mol Cell Biol 2000; 20:749-54.
49. Scholz GM, Hartson SD, Cartledge K, Volk L, Matts RL, Dunn AR. The molecular chaperone Hsp90 is required for signal transduction by wild-type Hck and maintenance of its constitutively active counterpart. Cell Growth Differ 2001; 12:409-17.
50. Stepanova L, Finegold M, DeMayo F, Schmidt EV, Harper JW. The oncoprotein kinase chaperone CDC37 functions as an oncogene in mice and collaborates with both c-myc and cyclin D1 in transformation of multiple tissues. Mol Cell Biol 2000; 20:4462-73.
51. Lavictoire SJ, Parolin DA, Klimowicz AC, Kelly JF, Lorimer IA. Interaction of Hsp90 with the nascent form of the mutant epidermal growth factor receptor EGFRvIII. J Biol Chem 2003; 278:5292-9.
52. Huse M, Kuriyan J. The conformational plasticity of protein kinases. Cell 2002; 109:275-82.
53. Mort-Bontemps-Soret M, Facca C, Faye G. Physical interaction of Cdc28 with Cdc37 in Saccharomyces cerevisiae. Mol Genet Genomics 2002; 267:447-58.
54. Wang HG, Takayama S, Rapp UR, Reed JC. Bcl-2 interacting protein, BAG-1, binds to and activates the kinase Raf-1. Proc Natl Acad Sci USA 1996; 93:7063-8.
55. Takayama S, Reed JC. Molecular chaperone targeting and regulation by BAG family proteins. Nat Cell Biol 2001; 3:E237-41.
56. Gassler CS, Wiederkehr T, Brehmer D, Bukau B, Mayer MP. Bag-1M accelerates nucleotide release for human Hsc70 and Hsp70 and can act concentration-dependent as positive and negative cofactor. J Biol Chem 2001; 276:32538-44.
57. Alberti S, Demand J, Esser C, Emmerich N, Schild H, Hohfeld J. Ubiquitylation of BAG-1 suggests a novel regulatory mechanism during the sorting of chaperone substrates to the proteasome. J Biol Chem 2002; 277:45920-7.
58. Luders J, Demand J, Hohfeld J. The ubiquitin-related BAG-1 provides a link between the molecular chaperones Hsc70/Hsp70 and the proteasome. J Biol Chem 2000; 275:4613-7.
59. Demand J, Alberti S, Patterson C, Hohfeld J. Cooperation of a ubiquitin domain protein and an E3 ubiquitin ligase during chaperone/proteasome coupling. Curr Biol 2001; 11:1569-77.
60. Song J, Takeda M, Morimoto RI. Bag1-Hsp70 mediates a physiological stress signalling pathway that regulates Raf-1/ERK and cell growth. Nat Cell Biol 2001; 3:276-82.
61. Tang SC, Shehata N, Chernenko G, Khalifa M, Wang X, Shaheta N. Expression of BAG-1 in invasive breast carcinomas. J Clin Oncol 1999; 17:1710-9.
62. Tang SC. BAG-1, an anti-apoptotic tumour marker. IUBMB Life 2002; 53:99-105.
63. Bardelli A, Longati P, Albero D, Goruppi S, Schneider C, Ponzetto C, et al. HGF receptor associates with the anti-apoptotic protein BAG-1 and prevents cell death. Embo J 1996; 15:6205-12.
64. Ballinger CA, Connell P, Wu Y, Hu Z, Thompson LJ, Yin LY, et al. Identification of CHIP, a novel tetratricopeptide repeat-containing protein that interacts with heat shock proteins and negatively regulates chaperone functions. Mol Cell Biol 1999; 19:4535-45.
65. Murata S, Chiba T, Tanaka K. CHIP: A quality-control E3 ligase collaborating with molecular chaperones. Int J Biochem Cell Biol 2003; 35:572-8.
66. Connell P, Ballinger CA, Jiang J, Wu Y, Thompson LJ, Hohfeld J, et al. The cochaperone CHIP regulates protein triage decisions mediated by heat-shock proteins. Nat Cell Biol 2001; 3:93-6.
67. Meacham GC, Patterson C, Zhang W, Younger JM, Cyr DM. The Hsc70 cochaperone CHIP targets immature CFTR for proteasomal degradation. Nat Cell Biol 2001; 3:100-5.
68. Hatakeyama S, Yada M, Matsumoto M, Ishida N, Nakayama KI. U box proteins as a new family of ubiquitin-protein ligases. J Biol Chem 2001; 276:33111-20.
69. Jiang J, Ballinger CA, Wu Y, Dai Q, Cyr DM, Hohfeld J, et al. CHIP is a U-box-dependent E3 ubiquitin ligase: Identification of Hsc70 as a target for ubiquitylation. J Biol Chem 2001; 276:42938-44.
70. Murata S, Minami Y, Minami M, Chiba T, Tanaka K. CHIP is a chaperone-dependent E3 ligase that ubiquitylates unfolded protein. EMBO Rep 2001; 2:1133-8.
71. Hohfeld 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.

72. Picard D. Heat-shock protein 90, a chaperone for folding and regulation. Cell Mol Life Sci 2002; 59:1640-8.
73. Nollen EA, Morimoto RI. Chaperoning signaling pathways: Molecular chaperones as stress-sensing 'heat shock' proteins. J Cell Sci 2002; 115:2809-16.
74. Nathan DF, Lindquist S. Mutational analysis of Hsp90 function: Interactions with a steroid receptor and a protein kinase. Mol Cell Biol 1995; 15:3917-25.
75. Donze O, Abbas-Terki T, Picard D. The Hsp90 chaperone complex is both a facilitator and a repressor of the dsRNA-dependent kinase PKR. Embo J 2001; 20:3771-80.
76. Scholz G, Hartson SD, Cartledge K, Hall N, Shao J, Dunn AR, et al. p50(Cdc37) can buffer the temperaturesensitive properties of a mutant of Hck. Mol Cell Biol 2000; 20:6984-95.
77. Mimnaugh EG, Chavany C, Neckers L. Polyubiquitination and proteasomal degradation of the p185c-erbB-2 receptor protein-tyrosine kinase induced by geldanamycin. J Biol Chem 1996; 271:22796-801.
78. Murakami Y, Mizuno S, Uehara Y. Accelerated degradation of 160 kDa epidermal growth factor (EGF) receptor precursor by the tyrosine kinase inhibitor herbimycin A in the endoplasmic reticulum of A431 human epidermoid carcinoma cells. Biochem J 1994; 301:63-8.
79. Sakagami M, Morrison P, Welch WJ. Benzoquinoid ansamycins (herbimycin A and geldanamycin) interfere with the maturation of growth factor receptor tyrosine kinases. Cell Stress Chaperones 1999; 4:19-28.
80. Miller P, DiOrio C, Moyer M, Schnur RC, Bruskin A, Cullen W, et al. Depletion of the erbB-2 gene product p185 by benzoquinoid ansamycins. Cancer Res 1994; 54:2724-30.
81. Liu SM, Carpenter G. Differential heat stress stability of epidermal growth factor receptor and erbB-2 receptor tyrosine kinase activities. J Cell Physiol 1993; 157:237-42.
82. Murakami Y, Fukazawa H, Mizuno S, Uehara Y. Conversion of epidermal growth factor (EGF) into a stimulatory ligand for A431-cell growth by herbimycin A by decreasing the level of expression of EGF receptor. Biochem J 1994; 301:57-62.
83. Sepp-Lorenzino L, Ma Z, Lebwohl DE, Vinitsky A, Rosen N. Herbimycin A induces the 20 S proteasome- and ubiquitin-dependent degradation of receptor tyrosine kinases. J Biol Chem 1995; 270:16580-7.
84. Isaacs JS, Xu W, Neckers L. Heat shock protein 90 as a molecular target for cancer therapeutics. Cancer Cell 2003; 3:213-7.
85. Tikhomirov O, Carpenter G. Geldanamycin induces ErbB-2 degradation by proteolytic fragmentation. J Biol Chem 2000; 275:26625-31.
86. Tikhomirov O, Carpenter G. Caspase-dependent cleavage of ErbB-2 by geldanamycin and staurosporin. J Biol Chem 2001; 276:33675-80.
87. Tikhomirov O, Carpenter G. Identification of ErbB-2 kinase domain motifs required for geldanamycin-induced degradation. Cancer Res 2003; 63:39-43.
88. Hartmann F, Horak EM, Cho C, Lupu R, Bolen JB, Stetler-Stevenson MA, Pfreundschuh M, Waldmann TA, Horak ID. Effects of the tyrosine-kinase inhibitor geldanamycin on ligand-induced Her-2/neu activation, receptor expression and proliferation of Her-2-positive malignant cell lines. Int J Cancer 1997; 70:221-9.
89. Cutforth T, Rubin GM. Mutations in Hsp83 and cdc37 impair signaling by the sevenless receptor tyrosine kinase in Drosophila. Cell 1994; 77:1027-36.
90. Stancato LF, Silverstein AM, Owens-Grillo JK, Chow YH, Jove R, Pratt WB. The hsp90-binding antibiotic geldanamycin decreases Raf levels and epidermal growth factor signaling without disrupting formation of signaling complexes or reducing the specific enzymatic activity of Raf kinase. J Biol Chem 1997; 272:4013-20.
91. Cadepond F, Schweizer-Groyer G, Segard-Maurel I, Jibard N, Hollenberg SM, Giguere V, e al. Heat shock protein 90 as a critical factor in maintaining glucocorticosteroid receptor in a nonfunctional state. J Biol Chem 1991; 266:5834-41.
92. Lin RZ, Hu ZW, Chin JH, Hoffman BB. Heat shock activates c-Src tyrosine kinases and phosphatidylinositol 3-kinase in NIH3T3 fibroblasts. J Biol Chem 1997; 272:31196-202.
93. Sachsenmaier C, Radler-Pohl A, Zinck R, Nordheim A, Herrlich P, Rahmsdorf HJ. Involvement of growth factor receptors in the mammalian UVC response. Cell 1994; 78:963-72.
94. Rao GN. Hydrogen peroxide induces complex formation of SHC-Grb2-SOS with receptor tyrosine kinase and activates Ras and extracellular signal-regulated protein kinases group of mitogen-activated protein kinases. Oncogene 1996; 13:713-9.
95. Webb CP, Hose CD, Koochekpour S, Jeffers M, Oskarsson M, Sausville E, et al. The geldanamycins are potent inhibitors of the hepatocyte growth factor/scatter factor-met-urokinase plasminogen activator-plasmin proteolytic network. Cancer Res 2000; 60:342-9.
96. An WG, Schulte TW, Neckers LM. 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 2000; 11:355-60.
97. Stamos J, Sliwkowski MX, Eigenbrot C. Structure of the epidermal growth factor receptor kinase domain alone and in complex with a 4-anilinoquinazoline inhibitor. J Biol Chem 2002; 277:46265-72.
98. Jeffrey PD, Russo AA, Polyak K, Gibbs E, Hurwitz J, Massague J, et al. Mechanism of CDK activation revealed by the structure of a cyclinA-CDK2 complex. Nature 1995; 376:313-20.
99. Helmbrecht K, Zeise E, Rensing L. Chaperones in cell cycle regulation and mitogenic signal transduction: A review. Cell Prolif 2000; 33:341-65.
100. Hostein I, Robertson D, DiStefano F, Workman P, Clarke PA. Inhibition of signal transduction by the Hsp90 inhibitor 17-allylamino-17-demethoxygeldanamycin results in cytostasis and apoptosis. Cancer Res 2001; 61:4003-9.
101. Stancato LF, Chow YH, Hutchison KA, Perdew GH, Jove R, Pratt WB. Raf exists in a native heterocomplex with hsp90 and p50 that can be reconstituted in a cell-free system. J Biol Chem 1993; 268:21711-6.
102. Fujita N, Sato S, Ishida A, Tsuruo T. Involvement of Hsp90 in signaling and stability of 3-phosphoinositide-dependent kinase-1. J Biol Chem 2002; 277:10346-53.
103. Basso AD, Solit DB, Chiosis G, Giri B, Tsichlis P, Rosen N. Akt forms an intracellular complex with heat shock protein 90 (Hsp90) and Cdc37 and is destabilized by inhibitors of Hsp90 function. J Biol Chem 2002; 277:39858-66.
104. Sato S, Fujita N, Tsuruo T. Modulation of Akt kinase activity by binding to Hsp90. Proc Natl Acad Sci USA 2000; 97:10832-7.
105. van der Straten A, Rommel C, Dickson B, Hafen E. The heat shock protein 83 (Hsp83) is required for Raf-mediated signalling in Drosophila. Embo J 1997; 16:1961-9.
106. Schulte TW, Blagosklonny MV, Ingui C, Neckers L. Disruption of the Raf-1-Hsp90 molecular complex results in destabilization of Raf-1 and loss of Raf-1-Ras association. J Biol Chem 1995; 270:24585-8.
107. Schulte TW, Blagosklonny MV, Romanova L, Mushinski JF, Monia BP, Johnston JF, et al. Destabilization of Raf-1 by geldanamycin leads to disruption of the Raf-1-MEK-mitogen-activated protein kinase signalling pathway. Mol Cell Biol 1996; 16:5839-45.
108. Xu W, Yuan X, Jung Y, Yang Y, Basso A, Rosen N, et al. The HSP90 inhibitor geldanamycin and the ErbB inhibitor ZD1839 promote rapid PP1 phosphatase-dependent inactivation of AKT in ErbB2 over-expressing breast cancer cells. Cancer Res 2003; In Press.
109. Smith V, Hobbs S, Court W, Eccles S, Workman P, Kelland LR. ErbB2 overexpression in an ovarian cancer cell line confers sensitivity to the HSP90 inhibitor geldanamycin. Anticancer Res 2002; 22:1993-9.
110. Munster PN, Marchion DC, Basso AD, Rosen N. Degradation of HER2 by ansamycins induces growth arrest and apoptosis in cells with HER2 overexpression via a HER3, phosphatidylinositol 3'-kinase-AKT-dependent pathway. Cancer Res 2002; 62:3132-7.
111. Slamon D. Herceptin((R)): Increasing survival in metastatic breast cancer. Eur J Oncol Nurs 2000; 4:24-9.
112. Dancey JE, Freidlin B. Targeting epidermal growth factor receptor—are we missing the mark? Lancet 2003; 362:62-4.
113. Goetz MP, Toft DO, Ames MM, Erlichman C. The Hsp90 chaperone complex as a novel target for cancer therapy. Ann Oncol 2003; 14:1169-76.
114. Omura S, Iwai Y, Takahashi Y, Sadakane N, Nakagawa A, Oiwa H, et al. Herbimycin, a new antibiotic produced by a strain of Streptomyces. J Antibiot (Tokyo) 1979; 32:255-61.
115. DeBoer C, Meulman PA, Wnuk RJ, Peterson DH. Geldanamycin, a new antibiotic. J Antibiot (Tokyo) 1970; 23:442-7.
116. Muroi M, Izawa M, Kosai Y, Asai M. Macbecins I and II, new antitumor antibiotics. II. Isolation and characterization. J Antibiot (Tokyo) 1980; 33:205-12.
117. Uehara Y, Hori M, Takeuchi T, Umezawa H. Phenotypic change from transformed to normal induced by benzoquinonoid ansamycins accompanies inactivation of p60src in rat kidney cells infected with Rous sarcoma virus. Mol Cell Biol 1986; 6:2198-206.
118. Okabe M, Uehara Y, Miyagishima T, Itaya T, Tanaka M, Kuni-Eda Y, et al. Effect of herbimycin A, an antagonist of tyrosine kinase, on bcr/abl oncoprotein-associated cell proliferations: Abrogative effect on the transformation of murine hematopoietic cells by transfection of a retroviral vector expressing oncoprotein P210bcr/abl and preferential inhibition on Ph1-positive leukemia cell growth. Blood 1992; 80:1330-8.
119. Miller P, Schnur RC, Barbacci E, Moyer MP, Moyer JD. Binding of benzoquinoid ansamycins to p100 correlates with their ability to deplete the erbB2 gene product p185. Biochem Biophys Res Commun 1994; 201:1313-9.
120. Prodromou C, Roe SM, O'Brien R, Ladbury JE, Piper PW, Pearl LH. Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. Cell 1997; 90:65-75.
121. 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.
122. Schulte TW, Neckers LM. The benzoquinone ansamycin 17-allylamino-17-demethoxygeldanamycin binds to HSP90 and shares important biologic activities with geldanamycin. Cancer Chemother Pharmacol 1998; 42:273-9.
123. 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 structureactivity relationships. J Med Chem 1995; 38:3813-20.
124. Neckers L. Heat shock protein 90 inhibition by 17-allylamino-17- demethoxygeldanamycin: A novel therapeutic approach for treating hormone-refractory prostate cancer. Clin Cancer Res 2002; 8:962-6.
125. Chiosis G, Rosen N, Sepp-Lorenzino L. LY294002-geldanamycin heterodimers as selective inhibitors of the PI3K and PI3K-related family. Bioorg Med Chem Lett 2001; 11:909-13.
126. Schulte TW, Akinaga S, Soga S, Sullivan W, Stensgard B, Toft D, et al. Antibiotic radicicol binds to the N-terminal domain of Hsp90 and shares important biologic activities with geldanamycin. Cell Stress Chaperones 1998; 3:100-8.
127. Marcu MG, Chadli A, Bouhouche I, Catelli M, Neckers LM. The heat shock protein 90 antagonist novobiocin interacts with a previously unrecognized ATP-binding domain in the carboxyl terminus of the chaperone. J Biol Chem 2000; 275:37181-6.
128. 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.
129. Drake JG, Becker JL. Aspirin-induced inhibition of ovarian tumor cell growth. Obstet Gynecol 2002; 100:677-82.
130. Korutla L, Kumar R. Inhibitory effect of curcumin on epidermal growth factor receptor kinase activity in A431 cells. Biochim Biophys Acta 1994; 1224:597-600.
131. Hong RL, Spohn WH, Hung MC. Curcumin inhibits tyrosine kinase activity of p185neu and also depletes p185neu. Clin Cancer Res 1999; 5:1884-91.

132. Omura S, Sasaki Y, Iwai Y, Takeshima H. Staurosporine, a potentially important gift from a microorganism. J Antibiot (Tokyo) 1995; 48:535-48.
133. Friedman B, Fujiki H, Rosner MR. Regulation of the epidermal growth factor receptor by growth-modulating agents: Effects of staurosporine, a protein kinase inhibitor. Cancer Res 1990; 50:533-8.
134. Huezo H, Vilenchik M, Rosen N, Chiosis G. Microtiter cell-based assay for detection of agents that alter cellular levels of Her2 and EGFR. Chem Biol 2003; 10:629-34.
135. Zheng FF, Kuduk SD, Chiosis G, Munster PN, Sepp-Lorenzino L, Danishefsky SJ, et al. Identification of a geldanamycin dimer that induces the selective degradation of HER-family tyrosine kinases. Cancer Res 2000; 60:2090-4.
136. Mandler R, Dadachova E, Brechbiel JK, Waldmann TA, Brechbiel MW. Synthesis and evaluation of antiproliferative activity of a geldanamycin-Herceptin immunoconjugate. Bioorg Med Chem Lett 2000; 10:1025-8.
137. 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.
138. 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.
139. Hargreaves R, David CL, Whitesell L, Skibo EB. Design of quinolinedione-Based geldanamycin analogues. Bioorg Med Chem Lett 2003; 13:3075-8.
140. Yu X, Guo ZS, Marcu MG, Neckers L, Nguyen DM, Chen GA, et al. Modulation of p53, ErbB1, ErbB2, and Raf-1 expression in lung cancer cells by depsipeptide FR901228. J Natl Cancer Inst 2002; 94:504-13.
141. Neckers L. Development of small molecule hsp90 inhibitors: Utilizing both forward and reverse chemical genomics for drug identification. Curr Med Chem 2003; 10:733-9.
142. de Bono JS, Tolcher AW, Rowinsky EK. The future of cytotoxic therapy: Selective cytotoxicity based on biology is the key. Breast Cancer Res 2003; 5:154-9.
143. Fry DW, Bridges AJ, Denny WA, Doherty A, Greis KD, Hicks JL, et al. Specific, irreversible inactivation of the epidermal growth factor receptor and erbB2, by a new class of tyrosine kinase inhibitor. Proc Natl Acad Sci USA 1998; 95:12022-7.
144. Gee JM. Chaperone-mediated destruction of erbB2: Relevance to tyrosine kinase inhibitors. Breast Cancer Res 2002; 4:205-6.
145. Lee AV, Schiff R, Cui X, Sachdev D, Yee D, Gilmore AP, et al. New mechanisms of signal transduction inhibitor action: Receptor tyrosine kinase down-regulation and blockade of signal transactivation. Clin Cancer Res 2003; 9:516S-23S.
146. Huynh H, Nguyen TT, Chan E, Tran E. Inhibition of ErbB-2 and ErbB-3 expression by quercetin prevents transforming growth factor alpha (TGF-alpha)- and epidermal growth factor (EGF)-induced human PC-3 prostate cancer cell proliferation. Int J Oncol 2003; 23:821-9.
147. Carniti C, Perego C, Mondellini P, Pierotti MA, Bongarzone I. PP1 inhibitor induces degradation of RETMEN2A and RETMEN2B oncoproteins through proteosomal targeting. Cancer Res 2003; 63:2234-43.
148. Osawa Y, Lowe ER, Everett AC, Dunbar AY, Billecke SS. Proteolytic degradation of nitric oxide synthase: Effect of inhibitors and role of hsp90-based chaperones. J Pharmacol Exp Ther 2003; 304:493-7.
149. Mohammadi M, McMahon G, Sun L, Tang C, Hirth P, Yeh BK, et al. Structures of the tyrosine kinase domain of fibroblast growth factor receptor in complex with inhibitors. Science 1997; 276:955-60.