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Contributions of co-chaperones and post-translational modifications towards Hsp90 drug sensitivity

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Abstract

Hsp90 is a molecular chaperone and important driver of stabilization and activation of several oncogenic proteins that are involved in the malignant transformation of tumor cells. Therefore, it is not surprising that Hsp90 has been reported to be a promising target for the treatment of several neoplasias, such as non-small-cell lung cancer and HER2-positive breast cancer. Hsp90 chaperone function depends on its ability to bind and hydrolyze ATP and Hsp90 inhibitors have been shown to compete with nucleotides for binding to Hsp90. Multiple factors, such as co-chaperones and post-translational modification, are involved in regulating Hsp90 ATPase activity. Here, the impact of post-translational modifications and co-chaperones on the efficacy of Hsp90 inhibitors are reviewed.

Hsp90 structure & function

Hsp90 is an evolutionarily conserved molecular chaperone and has two sides to its cellular function [1–3]. On one side, it is involved in the normal development and proliferation of cells [4–6], but conversely, it is an important driver of malignant transformation, growth, survival and possibly invasiveness of cancer cells (Figure 1) [7–9]. Therefore, it can be argued that cancer cells are ‘addicted’ to Hsp90 [10,11]. There are two isoforms of Hsp90 encoded by two separate genes in eukaryotes. These include the constitutively expressed human Hsp90β (yeast Hsc82), and the stress-induced human Hsp90α (hHsp90α) or yeast Hsp82 [12,13]. This **molecular chaperone** belongs to the ATPase/kinase GHKL (DNA Gyrase, Hsp90, Histidine Kinase, MutL) superfamily [14], sharing the unifying feature of an ATP-binding site. Each protomer of the Hsp90 dimer contains three domains: the N-domain

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that contains an ATP- and drug-binding site, and co-chaperone-interacting motifs; a middle domain that harbors sites for clients and cochaperones; and a carboxy-terminal domain that contains a dimerization motif, a second drug-binding region and interaction region for other co-chaperones (Figure 2) [15–21]. Driven by ATP, Hsp90 has the ability to undergo conformational changes, known as the chaperone cycle, allowing it to interact with other distinct co-chaperones (Figure 2). The cycle involves several conformational states that bind and release client proteins, ultimately altering their stability. An updated list of Hsp90 clients can be found online [18,19,22,23,201]. Hsp90 inhibitors interfere with this cycle by replacing ATP at the nucleotide-binding site and, consequently, leading to ubiquitination and proteasome degradation of the majority of client proteins (Figure 2) [24,25]. Several studies have assessed the effects of Hsp90 inhibitors on different tumor cells. A relatively recent and less developed area of investigation is the regulatory factors that affect drug sensitivity or resistance. This article will review the evidence assessing **post-translational modifications** and other regulatory mechanisms, such as co-chaperones, that affect and influence cells sensitivity and resistance to various Hsp90 inhibitors.

Hsp90 inhibitors

Hsp90 inhibitors and their clinical development are reviewed in depth elsewhere [2,26]. This section contains a brief summary of this area to provide background for the sections on sensitivity and resistance to Hsp90 inhibitors.

The first identified Hsp90 inhibitors were the natural products, radicicol (RD; macrocyclic antifungal antibiotic) and geldanamycin (GA; benzaquinoid ansamycin antibiotic) [2,27]. They work by mimicking the unusual structure ATP adopts when binding to the N-terminal nucleotide binding pocket, therefore blocking ATP binding and hydrolysis, and consequently interaction with Hsp90 client proteins, leading to their degradation. Both GA and RD are poorly soluble, unstable and highly toxic, minimizing their clinical value. However, they provided a chemical foundation to build clinically suitable, better tolerated drugs. An example is 17-allylamino-demothoxygeladanamycin (17-AAG: tanespimysin), a geldanamycin derivative with low toxicity and significant clinical response in HER2-positive breast cancer, used in combination with bortezomib in relapsed/refractory multiple myeloma (Figure 3) [28,29]. The water-soluble 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG, alvespimycin) and the soluble stabilized hydroquinone form of 17-AAG, IPI-504 (retaspimycin), have improved pharmokinetic properties, which circumvent the hepatotoxicity problems of 17-AAG in clinical trials [30,31]. RD has inhibitory effects *in vitro* but not *in vivo* [5,32,33]. Derivatives of resorcinals, such as ganetespib (formerly STA-9090 developed by Synta Pharmaeuticals, MA, USA), AUY922 [34–36], KW-2478 [37] and AT13387 [38], have been found to be more effective in multiple clinical trials (Figure 3).

The purine scaffold series were the first synthetic small molecules to inhibit Hsp90. They mimicked the shape adopted by ADP when bound to the nucleotide-binding site [27]. These inhibitors were based initially on the prototype PU3 [39,40], and led to the clinical candidates BIIB021 and BIIB028, as well as PU-H71, currently in Phase I clinical trials [41].

The resorcylic pyrazoles and isoxazoles (based on CCT018159 [42]) led to the development of NVP-AUY922 (Novartis)/VER52296 [43], the resorcylic dihydroxybenza-mide AT13387 [38], and the structurally related KW-2478 [44]. These purine derivatives proved to be more beneficial than 17-AAG because they were not affected by NAD(P)H:NQO1 or P-gp as discussed below [45].

Hsp90 also possess a second drug-binding site in the C-domain [46,47]. Coumarin antibiotics, such as novobiocin, target this site and recent works have made significant advances in improving the affinity of these compounds for Hsp90 (Figure 3). For example, KU135, a novel novobiocin derivative has the ability to induce apoptosis in cancer cells, in some cases with superior efficacy compared with tanespimycin (Figure 3) [48]. Another potential benefit that is associated with some of the Hsp90 C-domain inhibitors is their inability to activate *HSF1*. This is unlike the effect connected to the N-terminal inhibitors [49]. Existing data strongly support further medicinal chemistry optimization and preclinical evaluation of C-terminal Hsp90 inhibitors [48,50,51].

Impact of co-chaperones on Hsp90 drug binding

Co-chaperones are a group of proteins that regulate the function of chaperones and tend to interact with distinct conformations of Hsp90, therefore, forming a multi-protein complex also known as ‘the molecular chaperone machine’ [52]. Some co-chaperones such as Hop/Sti1, CHIP (E3 ubiquitin ligase), FKBP-51 and -52 (immunophilins-peptidyl-prolyl isomerases), PP5/Ppt1 (serine/threonine protein phosphatase 5) rely on their tetratricopeptide repeat (TPR) domains to interact with Hsp90. These co-chaperones have additional domains that catalyze reactions such as ubiquitin ligation, dephosphorylation and peptidylprolyl isomerization. Non-TPR co-chaperones include Aha1/Hch1, p23 (PGES3)/Sba1, Sgt1 and p50/Cdc37 [53–69]. A number of co-chaperones (e.g., p50/Cdc37, Hop/Sti1, p23 and Aha1) have the ability to modulate the rate of Hsp90 ATPase activity, thereby tailoring the chaperone machine for specific clients. Most Hsp90 co-chaperones are conserved in the single cell eukaryote budding yeast, *Saccharomyces cerevisiae.* Therefore, yeast has proven to be a valuable model organism to dissect the function of both Hsp90 and its co-chaperones in maintaining cellular homeostasis [70–75].

Cdc37

The co-chaperone Cdc37 recruits protein kinases to the Hsp90 chaperone complex [58,59,76]. This is supported by a 3D structure of Hsp90–Cdc37–Cdk4 obtained using single-particle electron microscopy [22]. Cdc37 also has an inhibitory effect on Hsp90 ATPase activity, thus, allowing kinase clients to be loaded on to the chaperone complex. [77]. Consequently, disruption of Hsp90–Cdc37 complex not only can affect the chaperoning of kinase clients but can also impact drug binding.

Gray *et al.* have demonstrated the role of Cdc37 in the proliferation of prostate cancer cells by using RNAi to knockdown *CDC37* expression. This leads to induced growth arrest both in androgen receptor-negative and -positive prostate carcinoma cells [78]. Furthermore, they reported that knockdown of Cdc37 was able to sensitize PC3 and DU145 cells to 17-AAG and proved to be highly effective in inhibiting cell growth in these cell lines. Of notice, they

describe that in spite of this effect, targeting Cdc37 does not significantly deplete intracellular levels of other Hsp90 clients [78]. Similarly, Smith *et al.* reported that silencing of Cdc37 in human colon carcinoma-derived HCT116 cells led to an increased sensitization to 17-AAG (threefold) and to VER49009 (twofold). Silencing of Cdc37 interfered with Hsp90 association with kinase clients and induced proteasome dependent degradation of clients such as ErbB2, c-Raf, CDK4 and CDK6. The levels of non-kinase clients were unaffected [79]. It has been reported that celastrol binds to the C-domain of Hsp90 and affects chaperone activity by disrupting the Hsp90–Cdc37 complex [80]. It would be of interest to know if celastrol or novobiocin-derived C-terminal inhibitors of Hsp90 (i.e., KU compounds) can provide synergistic effects when combined with N-domain inhibitors [50].

Post-translational modifications of co-chaperones add another layer of regulation in controlling Hsp90 chaperone function both in normal and cancer cells. Using yeast, Bandhakavi *et al.* have elegantly shown that CK2 targets two conserved serines, S14/17 in yeast Cdc37 (S13 in human Cdc37). This phosphorylation is necessary for Cdc37 interaction with Hsp90 and also for chaperoning of numerous kinase clients including CK2 itself, $Cdc28^{Cdc2}$, $Ste11^{RAF}$, Kin28 and Mps1 [81]. Lack of phosphorylation hypersensitizes cells to the Hsp90 inhibitor GA. Later studies confirmed that a similar process also exists for mammalian Cdc37 [82,83]. Finally, Vaughan *et al.* have reported that Cdc37 and PP5/Ppt1 form complexes with Hsp90 in yeast and in human tumor cells. The protein phosphatase PP5/Ppt1 dephosphorylates the S13 on Cdc37 *in vivo*, therefore, affecting its interaction with Hsp90 and negatively impacting the chaperoning of numerous kinase clients by the Hsp90–Cdc37 complex [60]. Recent work by Xu and colleagues demonstrated that the nonreceptor tyrosine kinase c-Yes can target Y4 and Y298 on Cdc37. It would be interesting to know if the phosphorylation status of these residues can contribute to Hsp90 inhibitor sensitivity [84].

Aha1

The co-chaperone Aha1 is an activator of Hsp90 ATPase activity [65,66]. Recent work by Retzlaff and co-workers demonstrated that both N- and C-domains of Aha1 interact in a cooperative manner with both the N-terminal and middle domains of Hsp90 via an asymmetric activation mechanism [85]. Aha1 is involved in various cellular processes, including the quality control of the cystic fibrosis transmembrane conductance regulator [86], as well as activation of glucocorticoid receptor and p60v-src [64,66,87]. Interestingly, Holmes *et al.* demonstrated that siRNA knockdown of *AHA1* in human colon carcinoma HCT116 cells and in the ovarian cancer cell line A2780 resulted in a three- to six-fold increase of sensitivity to 17-AAG after 48 h of treatment [87]. It would be of interest to know if downregulation of Aha1 also sensitizes cancer cells to the new generation of Hsp90 inhibitors.

p23/Sba1

p23 (Sba1 in yeast), is known to interact with ATP-bound Hsp90, slowing the chaperone cycle and stabilizing the conformation required for client–protein activation [61,88]. Hsp90 inhibitors such as GA and RD are known to interfere with the binding of p23/Sba1 by competing with ATP binding [89]. Forafonov *et al.* reported that when p23/Sba1p is absent,

both yeast and mammalian cells become hypersensitive to GA and RD. Furthermore, based on the fact that Hsp90 inhibitors induce apoptosis in several cell types, the group observed that low doses of GA inhibited cell growth in p23-null cells and induced cell death, as opposed to wild-type p23 cells, which continued to grow for 2 days and had greater survival. This suggests that p23 may have a protective role against Hsp90 inhibitors. In addition, p23 mutants A13S, R106A and K113A interfered with GA binding by at least twofold in yeast [74,90].

Hop/Sti1 & Cyp40/Cpr6–Cpr7

Hop is a TPR-containing co-chaperone with an important role in facilitating interactions between Hsp70 and Hsp90 [91–93]. Hop has the ability to bind to the MEEVD peptide in the C-domain of Hsp90, as well as to additional sites in the C-terminal and middle domain. Deletion of *Hop/Sti1* makes yeast cells hypersensitive to GA and RD [94]. The observed drug sensitivity was further enhanced in the absence of Hsc82 (constitutively expressed yeast Hsp90) compared with Hsp82 (stress-induced yeast Hsp90). These data highlight the functional differences between Hsp90 isoforms [95].

The immunophilin Cyp40 is another TPR-containing co-chaperone that binds solely to the C-domain of Hsp90 and is involved in the chaperoning of steroid hormone receptors [66,96,97]. Displacement of Hsp90–Hop/Sti1 complexes by Cpr6, (Cyp40 homolog in yeast), including formation of an Hsp90–Sti1–Cpr6 ternary complex has been proposed to be one of the key components of the Hsp90 cycle [7,97]. Work by Zuehlke *et al.* indicated that an Hsp90–Cpr6–Cpr7 ternary complex also exists [98]. The authors have proposed a model where Hsp90–Sti1–Cpr6 complex is followed by formation of an Hsp90–Cpr6–Cpr7 complex that is required for Cpr6 and Cpr7 dissociation. Li and colleagues recently reported that Cpr6 enhances the affinity between Aha1 and Hsp90 and further stimulates the Hsp90 ATPase activity [99]. Finally, single or double deletion of *CPR6, CPR7*in yeast hyposensitizes these mutants to GA [100]. Interestingly, Cyp40 interacts with Wee1 kinase [101]. This kinase directly targets a conserved tyrosine residue in the Hsp90 N-domain and regulates its chaperone function (see later discussion) [102]. Inhibition of Wee1 function hypersensitizes cells to Hsp90 inhibitors [103]. Whether deregulated or destabilized, Wee1 contributes to the hypersensitivity of *CPR7* knock out cells to Hsp90 inhibitors and requires further investigation.

Post-translational modifications that enhance sensitivity to Hsp90 inhibitors

Hsp90 is the target of various post-translational modifications that affect its chaperone function [8,104]. These include phosphorylation, acetylation, S-nitrosylation, oxidation and ubiquitination. Multiple studies have demonstrated the influence of post-translational modifications on Hsp90 function and their impact on drug sensitivity [102,105–107].

Phosphorylation

Hsp90 is a phosphoprotein and early work reported that treating NIH 3T3 cells with the serine/threonine phosphatase inhibitor okadaic acid led to hyper-phosphorylation of Hsp90

and compromised chaperoning of its client kinase p60v-src [108]. This was the first evidence linking Hsp90 phosphorylation to its ability to chaperone client proteins [108]. These data were supported by the fact that the co-chaperone PP5/Ppt1 has the ability to dephosphorylate Hsp90 *in vitro* and positively regulate its chaperone activity *in vivo* [109,110]. Recently, it was observed that phosphorylation of threonine and tyrosine sites on Hsp90 can influence its sensitivity to Hsp90 inhibitors [8].

Recent work has shown that CK2 phosphorylates a conserved threonine residue (T22) in the N-domain of yeast Hsp82 both *in vitro* and *in vivo* (Figure 4) [111]. Thr22, and adjacent amino acids, participate in a vital hydrophobic interaction with the catalytic loop in the middle domain of Hsp90 [15,112–115]. It was also reported that non-phosphorylatable T22A mutant did not compromise Hsp90 ATPase activity, while the phosphomimetic mutant (T22E) had only approximately 40% of wild-type ATPase activity. These mutants and their equivalent in hHsp90α (T36A and T36E), affected Hsp90-dependent chaperoning of kinase and non-kinase clients [111]. In addition, the phosphomimetic mutant (T22E) resulted in yeast sensitivity to Hsp90 inhibitors GA and ganetespib (formerly STA-9090, Synta Pharmaceuticals), and SNX-2112 (Serenex/Pfizer [NY, USA]) [116]. These findings identify T22 as an important determinant of Hsp90 drug sensitivity in yeast and suggest that hyperphosphorylation of T22 (hHsp90α-T36) may contribute to drug sensitivity *in vivo* [116]. Previous work has shown that members of the Src family kinases, c-Src and c-Yes, can specifically target hHsp90 [84,117]. Recent work has shown that Wee1 kinase, a key regulator of the cell cycle check-point and also an Hsp90 client, is responsible for phosphorylating a highly conserved tyrosine residue in the N-domain of yeast Hsp40 (Y24) and hHsp90 (Y38) (Figure 4) [102]. This phosphorylation occurs in the nucleus and eventually leads to ubiquitination and degradation of Hsp90 in the proteasome. Furthermore, regulation of Hsp90 chaperone function also depends on tyrosine phosphorylation. It was observed that lack of phosphorylation as a result of either *SWE1* deletion (*Saccharomyces cerevisiae WEE1)* in yeast or knocking down *WEE1* in prostate or cervical cancer cell lines led to hypersensitization of these cells to Hsp90 inhibitors (GA, RD, ganetespib and SNX-2112). This phenotype is explained by the increased binding affinity of non-phosphorylatable Y24 or Y38 mutants to Hsp90 inhibitors [107,118]. Our recent study has demonstrated that pharmacological inhibition of Wee1 made prostate cancer cells sensitive to Hsp90 inhibitors both *in vivo* and *in vitro*, and this led to the activation of the intrinsic apoptotic pathway [103]. This downstream effect also coincided with the transcriptional downregulation of survivin and Wee1. These findings provide a novel therapeutic strategy using combined *Wee1/Hsp90* inhibitors, and also support a mechanistic rationale for enhancing the pro-apoptotic activity of Hsp90 inhibitors.

Acetylation–deacetylation

Acetylation is another post-translational modification that impacts Hsp90 chaperone function [8,104,119,120]. Enzymes that catalyze the addition/removal of acetyl groups on lysine residues were initially discovered in histones, hence they were termed histone acetylases and HDAC. Initial work by Yu *et al.* reported that the HDACi depsipeptide (romidepsin) caused hyperacetylation of Hsp90 and decreased ATP binding. This post-translational modification also simultaneously abrogated Hsp90 interaction with several

client proteins, including ErbB2, Raf-1 and mutant p53 [121]. Subsequent work demonstrated that p300 promotes acetylation, and HDAC1, 6 and 10 cause deacetylation, of Hsp90, affecting chaperone function [122–127]. Rao *et al.* have specifically shown that silencing *HDAC6* by siRNA led to hyperacetylation of Hsp90 and also increased binding to the Hsp90 inhibitor 17-AAG [106]. Work by Yang *et al.* identified acetylation of seven lysine residues in Hsp90 when HEK293 cells were treated with the pan-HDACi panobinostat (LBH589) [105]. Acetylation of all seven lysines enhanced the binding of hHsp90α to 17-AAG [105]. Interestingly, recent work by Robbins *et al.* has shown that mutation of one of those seven lysines (K290) to arginine or glutamine conferred sensitivity to GA [105]. These observations suggest an evolutionarily conserved mechanism, where acetylation–deacetylation of Hsp90 impacts chaperone function and also influences its ability to bind to its inhibitors.

Molecular mechanisms of resistance to Hsp90 inhibitors

As more Hsp90 inhibitors enter clinical trials, the question of how to increase sensitivity to these drugs is of great interest. However, equally important, but a far less explored area, is the development of possible resistance to Hsp90 inhibitors and understanding the underlying mechanisms of this process.

Hsp90 inhibitors used in the clinic target the nucleotide-binding pocket in the N-domain of Hsp90. Therefore, mutations to this region that weaken drug binding can potentially give rise to drug resistance. Furthermore, most mutations within this pocket can potentially compromise the essential chaperone function of Hsp90. Recent work by Millson *et al.* demonstrated a partial resistance to GA or RD by engineering mutations to yeast Hsp90 [128,129]. Those mutations were based on unusual features of the N-domain ADP-/ATP-binding site of Hsp90 from drug producing organisms; *Streptomyces hygroscopicus* (GA) and *Humicola fuscoatra* (RD) [128,129]. Other work have shown that single amino acid substitution I123T in hHsp90β, and the corresponding hHsp90α-I128T, can make yeast and HEK293 cells resistant to Hsp90 inhibitors [130]. This mutation did not diminish drug binding *in vitro*, but it had strong Aha1 binding and increased ATPase activity, therefore, protecting Hsp90 from its inhibitors [130]. Although these data complement an earlier report that decreased *AHA1* expression increases cellular sensitivity to Hsp90 inhibitors [87], it does not explain that tumor cells have highly active Hsp90 with increased binding affinity to 17-AAG [131]. Holmes *et al.*, have also reported that over-expression of *AHA1* in human cancer cell lines did not significantly affect their sensitivity to 17-AAG [87]. Finally, recent work by Armstrong *et al.* has demonstrated that deletion of *HCH1*, related to co-chaperone *AHA1* in yeast, confers resistance to Hsp90 inhibitor NVP-AUT922 [132]. Although Hch1 does not exist in higher eukaryotes, it does, however, suggest a distinct role in the regulation of Hsp90 that may be independent of its ability to stimulate ATPase activity [132].

As mentioned in previous sections, NQO1, also known as DT-diaphorase, plays an important role in drug sensitivity or resistance. Cancer cells with high levels of NQO1 are particularly sensitive to 17-AAG, probably due to reduction of the quinone, which favors binding of more potent Hsp90 inhibitors [133]. Gaspar and colleagues reported an acquired resistance to 17-AAG in four glioblastoma cell lines *in vitro.* They proposed that decreased NQO1

expression and activity is the mechanism of resistance in these cells [134]. They obtained similar findings in melanoma cells (WM266.4). Benchekroun *et al.* observed that human breast cancer cells (MCF7/ADRR) are resistant to Hsp90 inhibitors such as GA and herbimycin A (a benzoquinonoid ansamycin antibiotic). They used photo-affinity labeling methods to show GA interaction with the P-gp efflux pump and that cells expressing this pump have lower concentrations of GA [135]. However, McCollum *et al.* demonstrated that even though P-gp plays a role in resistance to 17-AAG, Hsp70 and Hsp27 were the more important players in determining resistance to Hsp90 inhibitors [136]. A number of studies have linked the upregulation of these two chaperones to an increase in the cellular capacity for evasion of apoptosis [137,138]. It has been reported that downregulation of *HSP27* or treating the cells with buthionine sulfoximine, an inhibitor of GSH synthesis, sensitizes cells to 17-AAG [139]. Conversely, 17-AAG can upregulate Hsp27 and make cell lines resistant to 17-AAG. There are few molecular mechanisms that can contribute towards this drug resistance. First, 17-AAG can directly bind to GSH and, therefore, may reduce binding to Hsp90 *in vivo*, and could, therefore, explain protection of tumor cells by increased GSH levels. Second, ansamycins can produce reactive oxygen species in cells and the formation of free radicals. GSH can eliminate this by either direct binding to 17-AAG, binding to oxygen species formed during 17-AAG treatment, such as superoxide, or binding oxidative species from mitochondrial leakage observed during 17-AAG-mediated apoptosis. Third, GSH may play an important role in oxidative protein folding that is disrupted as a result of 17-AAG inhibiting Hsp90 chaperone functions. Increased levels of GSH in tumor cells could prevent cell damage and possibly death resulting from these oxidative species. In any of these events, GSH plays an important role in cellular homeostasis and inhibits cell death as a result of 17-AAG treatment. These data suggest an explanation for disparate sensitivity and also drug resistance to ansamycin derivatives of Hsp90 inhibitors. However, mechanisms that may lead to tumor resistance to other Hsp90 inhibitors, such as the synthetic purine analog PU-H71 (Samus Therapeutics, NY, USA), or the resorcinol analogs ganetespib and NVP-AUY922, remain to be examined. These drugs are currently being extensively evaluated in clinical trials.

Conclusion

Hsp90 is an essential and evolutionarily conserved molecular chaperone in eukaryotes. Cancer cells hijack Hsp90 chaperone machinery in order to protect an array of mutated and overexpressed oncoproteins from misfolding and degradation. Therefore, Hsp90 plays a crucial role as a facilitator of oncogenic addiction and cancer cell survival. Thus, it is not surprising that 17 Hsp90 inhibitors have entered into clinical trials during the past two decades. Hence, it is important to evaluate the factors that can influence cell susceptibility to Hsp90 inhibitors. Co-chaperones and post-translational modifications play a major role in fine-tuning Hsp90 chaperone function. Targeting and preventing the interaction of certain co-chaperones, including Aha1, Cdc37, p23, Hop/Sti1 and Cyp40/Cpr6–Cpr7 with Hsp90 has made cancer cells, or the model eukaryotic organism yeast, hypersensitive to Hsp90 inhibitors. Although Hsp90 is subject to numerous post-translational modifications, only a few studies have linked the contributions of phosphorylation or acetylation of certain amino acids to Hsp90 drug binding. Remarkably, the vast majority of these post-translational

modifications are catalyzed by Hsp90 clients and, therefore, targeting these enzymes may provide novel strategies to improve drug sensitivity and/or avoid drug resistance.

Future perspective

During the past two decades significant progress has been made in understanding the complex regulation of the Hsp90 machinery. Post-translational modifications of Hsp90 and co-chaperones have been recently the focus of intense scrutiny. A large number of studies have mapped various post-translational modifications on Hsp90 and their impact on chaperone activity (Figure 4). However, the contributions of these individual modifications to drug sensitivity or resistance remain to be explored. Other important areas of Hsp90 research that require further investigation are:

- Identification of the enzymes (e.g., kinases, phosphatases, histone acetylases and HDACs) that target Hsp90 and co-chaperones;
- Development of post-translational site-specific drugs to Hsp90 and co-chaperones to understand the impact of individual Hsp90 inhibitors or environmental stimuli on sub-cellular localization of the chaperone complex;
- Unraveling the cross-talk between several post-translational modifications of the Hsp90 chaperone machine (Figure 5).

These data will expand our knowledge of the multilayered control mechanisms used by both normal and cancer cells to fine-tune Hsp90 function. They will also provide information relevant to improving drug sensitivity and inhibiting drug resistance.

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Website

201. Updated list of Hsp90 clients www.picard.ch/downloads/downloads.html

Key Terms

Molecular chaperone: Group of proteins that assist in the folding or unfolding and the assembly or disassembly of other macromolecular structures.

Co-chaperones: Proteins that are involved in regulating chaperone function.

Post-translational modification: Covalent addition of functional groups or protein.

Excutive Summary

- Hsp90 is a promising target in cancer therapy and Hsp90 inhibitors have demonstrated efficacy in clinical trials in patients with non-small-cell lung cancer and HER2-positive breast cancer.
- Impact of co-chaperones on Hsp90 drug binding are discussed.
- Post-translational modifications regulate Hsp90 chaperone function.
- Targeting the regulators of Hsp90 function may provide a strategy to increase the potency of Hsp90 inhibitors and to prevent/minimize drug resistance.

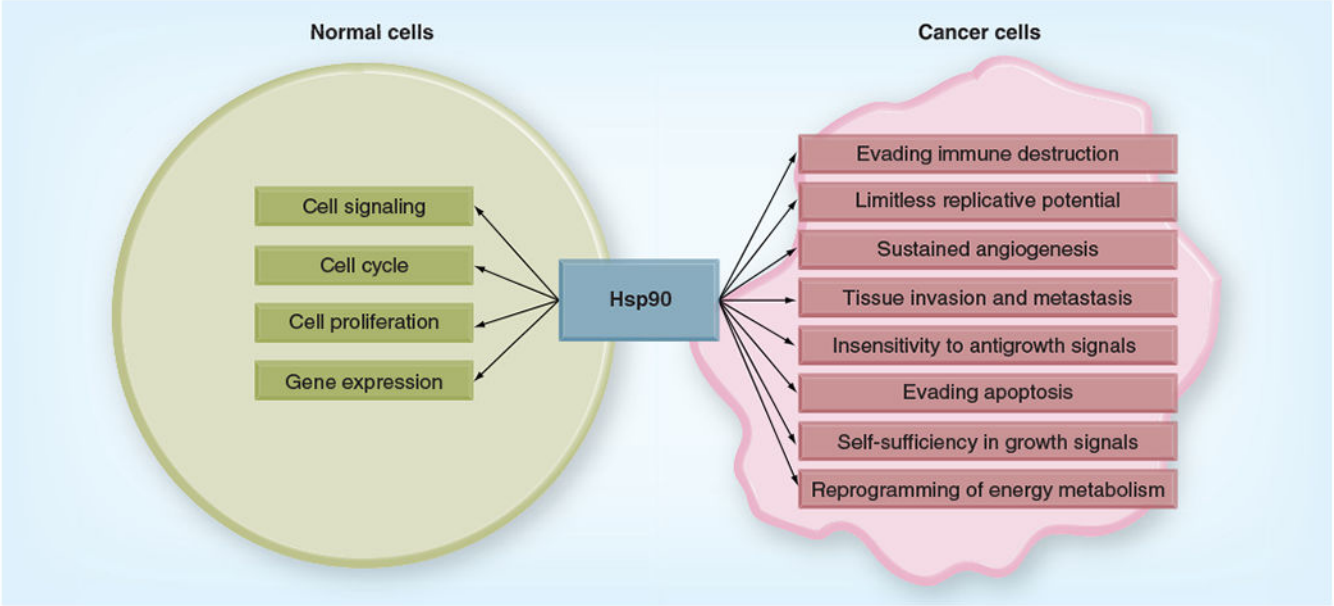

Figure 1. Two sides to Hsp90 function.

Hsp90 looks after proteins that are involved in normal cellular function. Hsp90 also chaperones clients that are crucial for the maintenance of each of the proposed hallmarks of cancer.

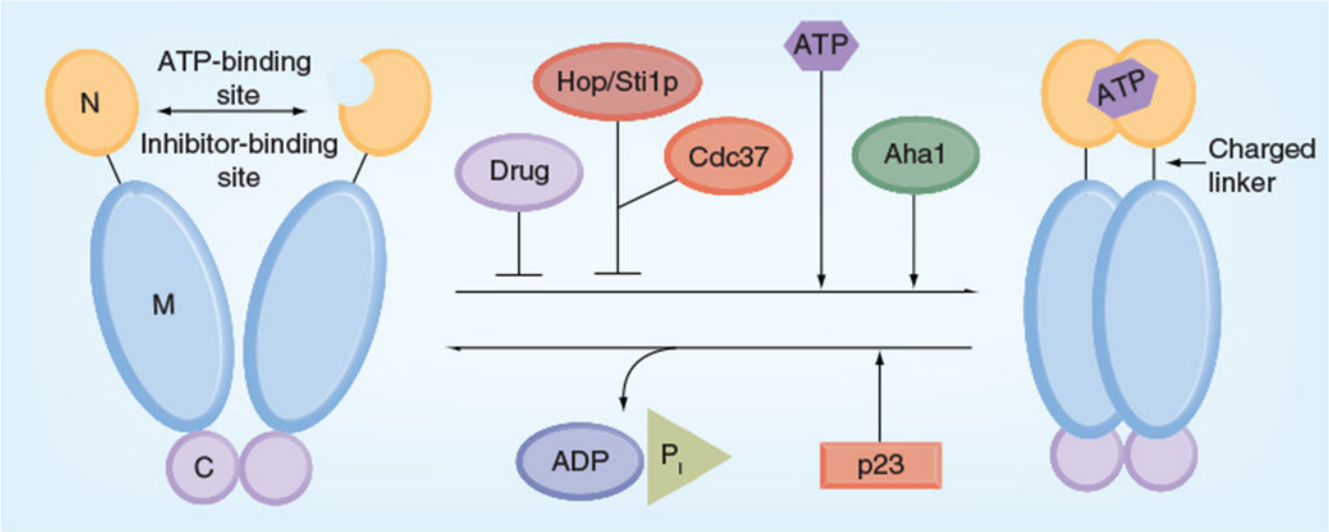

Figure 2. Hsp90 chaperone function.

ATP binding to the N-terminal domain of Hsp90 promotes transient dimerization of the N-domains. The co-chaperone Aha1 enhances Hsp90 ATPase activity by promoting various conformational changes, while Hop/Sti1 and Hsp90 inhibitors such as geldanamycin or radicicol exert the opposite effect by inhibiting N-domain dimerization. p23 slows ATP hydrolysis at a late stage of the chaperone cycle.

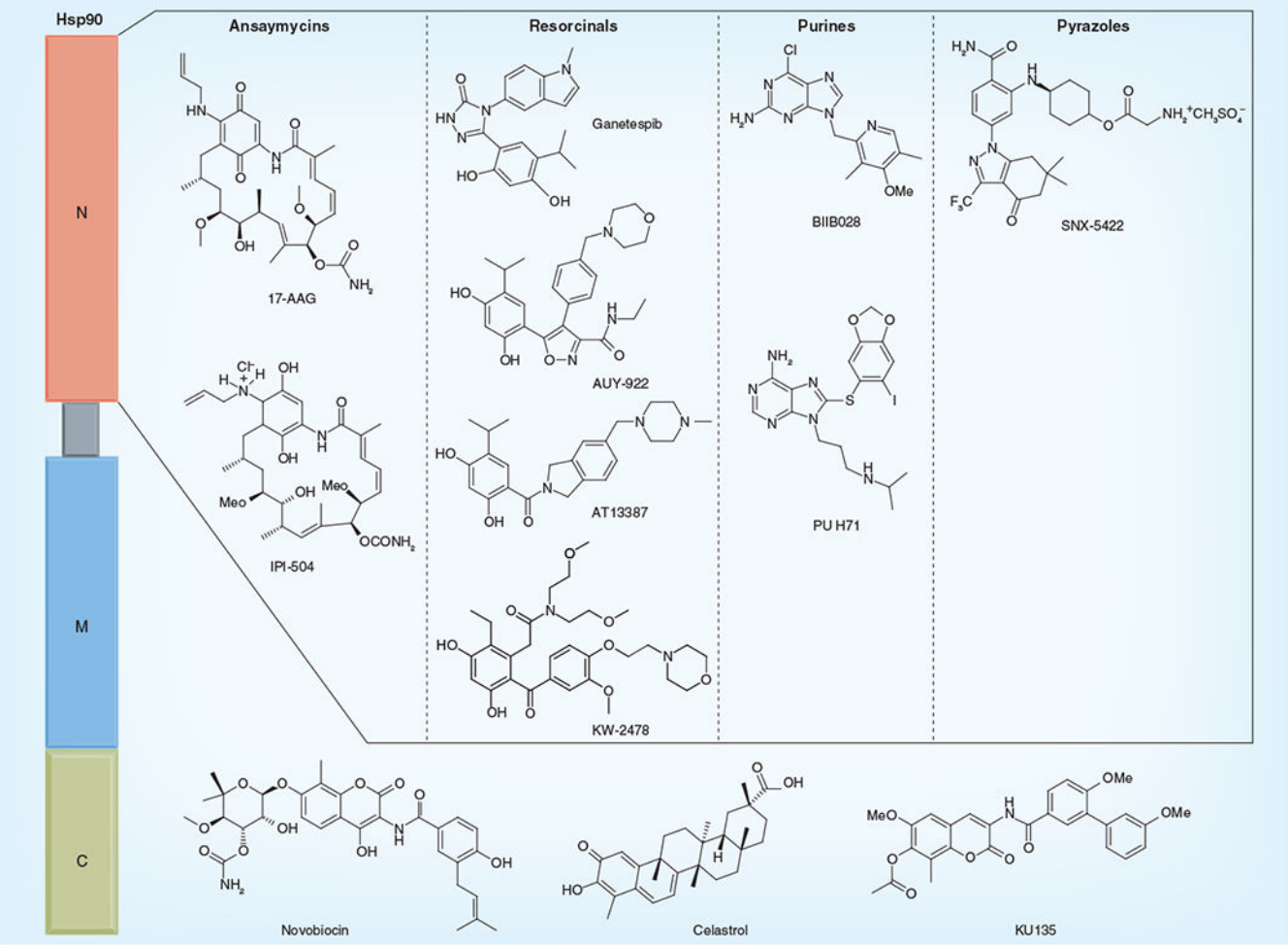

Figure 3.
Hsp90 N- and C-domain inhibitors.

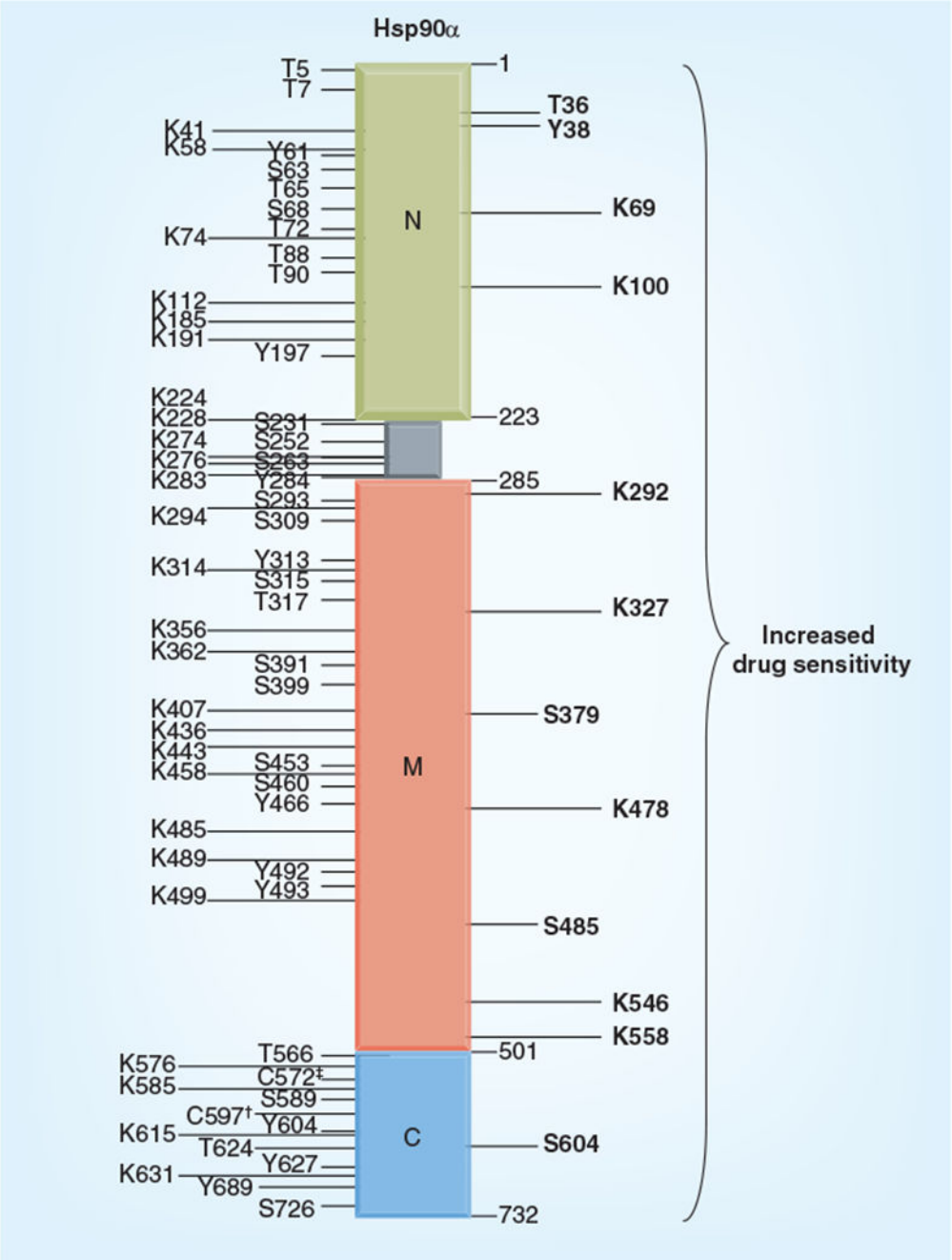

Figure 4. Post-translational modification residues on human Hsp90α.

Domain location of phosphorylated serine (S), theronine (T) and tyrosine (Y) sites for which kinases are known. Acetylated lysine (K) residues, S-nitrosylated cysteine (†) and cysteine oxidation sites (‡) on human Hsp90α. Phosphorylation and acetylation sites that enhance sensitivity to Hsp90 inhibitors are also marked in bold.

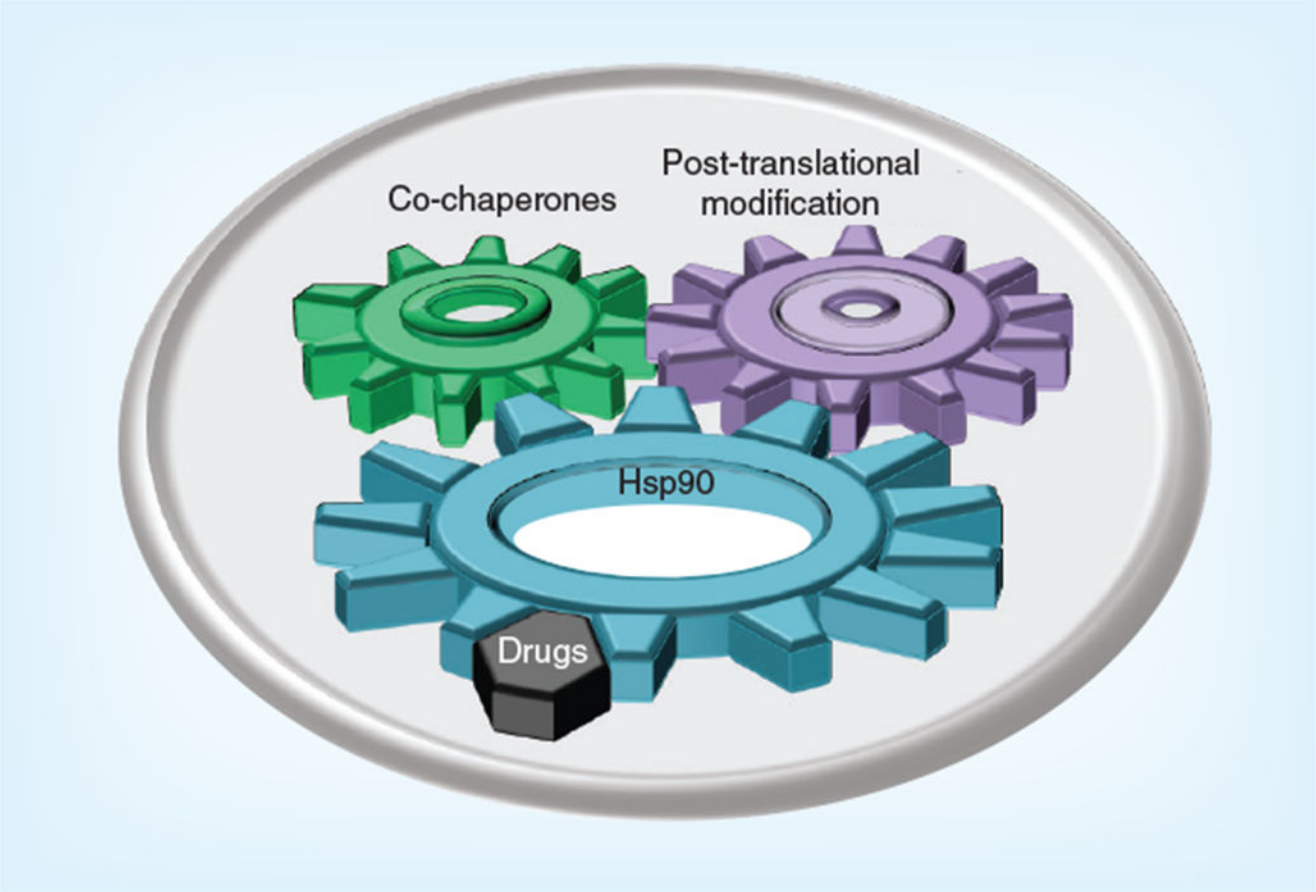

Figure 5. Post-translational modifications and co-chaperones fine-tune Hsp90 chaperone machinery.

The Hsp90 chaperone cycle is regulated by the interplay between ATP binding to Hsp90 and the regulated association/dissociation of various co-chaperones. The Hsp90 chaperone machine is also regulated by a number of diverse post-translational modifications of Hsp90 and co-chaperones, including phosphorylation, S-nitrosylation, oxidation, acetylation and ubiquitination. Hsp90 inhibitors effectively displace ATP from its binding pocket in the N-domain of Hsp90, disrupt this complex mechanism and prevent chaperone cycling.