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Heat-shock protein 90 (HSP90) in cell physiology
Buněčné funkce proteinu teplotního šoku 90 (HSP90)

Bachelor's thesis

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Poděkování
Slova nemohou vyjádřit mou vděčnost mému školiteli RNDr. Cyrilu Bařinkovi, Ph.D., za jeho trpělivost, podporu a neocenitelnou zpětnou vazbu, stejně jako RNDr. Zoře Novákové, Ph.D, za její moudré vedení a rady. Jsem rovněž vděčná svému manželovi, jehož optimismus a energie mi dodávaly sílu.

Prohlášení
Prohlašuji, že jsem závěrečnou práci vypracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 24.4.2023

Alina Karmazin

Abstract

Heat-shock protein 90 (HSP90) is a molecular chaperone that represents one of the most important proteins for cellular homeostasis in all life domains. Chaperones are proteins that assist other proteins in proper folding and refolding. First discovered as a protein of a heat-shock response, HSP90 eventually emerged as a hub connecting multiple cellular functions, such as transcription, translation, DNA repair, immune response, cell signaling, etc. Unsurprisingly, HSP90 also plays a role in the pathogenesis of human diseases: various cancers, and neurodegenerative and respiratory diseases. For that reason, it became a target of medical research. HSP90 is a homodimer consisting of two protomers, each of which is composed of three domains: N-terminal domain, middle domain, and C-terminal domain. To fulfill its functions, HSP90 goes through an ATP-dependent conformational cycle, tightly regulated by a large group of assisting proteins—co-chaperones, and several post-translational modifications, such as phosphorylation and acetylation. Acetylation is known to affect HSP90 binding to nucleotides, clients, and co-chaperones, and thus it is suggested as a control mechanism of HSP90 function. Potentially, HSP90 acetylation can be utilized in the treatment of hormone-dependent cancers. Therefore, regulators of HSP90 acetylation are currently under intensive investigation. Histone deacetylase 6 (HDAC6) is proposed to be the major deacetylase of HSP90, however, available data about HSP90—HDAC6 interaction are very limited up to date. This thesis summarizes current knowledge about the structure and function of HSP90 with a focus on the regulation of HSP90 acetylation status.

Keywords: proteostasis, chaperones, post-translational modifications, lysine acetylation, heat-shock protein 90, histone deacetylase 6.

Abstrakt

Heat-shock protein 90 (HSP90) je molekulární chaperon, který představuje jeden z nejdůležitějších proteinů pro buněčnou homeostázu ve všech doménách života. Chaperony plní funkci molekul, které pomáhají ostatním proteinům ve správném sbalování a uspořádání jejich trojrozměrné struktury. HSP90 byl poprvé objeven jako protein tepelného šoku, později byla prokázána jeho důležitá role uzlu propojujícího mnoho buněčných funkcí zahrnující transcripci, translaci, opravu DNA, imunitní odpověď, buněčnou signalizaci atd. Není tedy překvapením, že HSP90 hraje roli také v patogenezi lidských onemocnění, např. u různých druhů rakoviny a u neurodegenerativních a respiračních chorob. Z tohoto důvodu se HSP90 stal cílem lékařského výzkumu. HSP90 je homodimer skládající se ze dvou protomerů, z nichž každý se skládá ze tří domén: N-terminální domény, střední domény a C-terminální domény. Pro splnění svých funkcí prochází HSP90 ATP-dependentním konformačním cyklem, kde je striktně regulován velkou skupinou pomocných proteinů — ko-chaperonů, stejně jako několika posttranslačními modifikacemi typu fosforylace a acetylace. O acetylaci je známo, že ovlivňuje vazbu HSP90 na nukleotidy, klienty a ko-chaperony, a proto je považována za kontrolní mechanismus funkce HSP90. Potenciálně lze acetylaci HSP90 využít při léčbě hormonálně závislých nádorů. Proto jsou regulátory acetylace HSP90 v současné době intenzivně zkoumány. Histon deacetyláza 6 (HDAC6) je považována za hlavní deacetylázu HSP90, nicméně dostupné údaje o interakci mezi HSP90 a HDAC6 jsou až do dnešního dne velmi omezené. Tato práce shrnuje současné poznatky o struktuře a funkci HSP90 se zaměřením na regulaci acetylace HSP90.

Klíčová slova: proteostáza, chaperony, posttranslační modifikace, acetylace lysinu, heat-shock protein 90, histon deacetyláza 6.

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Introduction

The object of this thesis is one of the most important, abundant, and interconnected proteins that is known to science — HSP90, the so-called 90 kDa heat shock protein. Heat shock proteins were discovered serendipitously while studying eukaryotic chromosomes accidentally subjected to high heat, which led to cells developing a so-called heat shock response (Ritossa 1962). During the heat shock response, the expression of heat shock proteins in cells increases rapidly, allowing cells to quickly deal with damage caused to proteins by the high heat, such as denaturation and/or aggregation. Such helper proteins are called chaperones, because among many functions they protect proteins from "unwanted associations", compared to how female chaperones in Victorian England guarded young ladies. However, high temperature is not the only trigger of such a stress response. Other triggers include UV radiation, toxic chemicals, non-physiological pH, nutrient starvation, cancer, etc (Ellis and van der Vies 1991; Richter et al 2010).

It was soon discovered, however, that the HSP90 family is utilized by cells not only in case of emergency. For example, it was later found that HSP90 seemed to facilitate the folding of the hormone-binding domain (HBD) of several nuclear steroid hormone receptors (SHRs) (Pratt and Toft 1997). HSP90 was also found to be a part of many protein kinase complexes (Brugge and Erikson 1977; Brugge 1986). It is clear today that these two names, chaperones and heat-shock proteins, do not encompass the whole variety of functions that HSP90 fulfills: from development, transcription and translation, DNA repair, and intracellular transport, to immune response and cell signaling (Zhao et al 2005; McClellan et al 2007). HSP90 is central to many eukaryotic cellular processes, and in lower eukaryotes, namely *Saccharomyces cerevisiae*, it was shown to be essential for survival (Borkovich et al 1989). The same is true for HSP90 homologs in other eukaryotes, e.g. GRP94 (endoplasmic reticulum resident homolog) is vital for *Drosophila* so much that GRP94-deficient *Drosophila* does not survive the larval stage (Maynard et al 2010).

Substrate proteins that physically interact with HSP90 and whose activity is dependent on HSP90 are called clients. It is already known that HSP90 has hundreds of clients in various spheres of its functions (Echeverria et al 2011), with an estimated 10-20% of yeast and human proteins possibly interacting with HSP90 (Zhao and Houry 2007; Taipale et al 2010; Wu et al 2012). The most studied clients are protein kinases and steroid hormone receptors, and this extensive studying is justified: both of these groups of proteins are key to cell signaling, and cell signaling is one of the biggest spheres of influence of HSP90 (Echeverria and Picard 2010; Taipale et al 2012; Caplan et al 2007).

How does such a friendly protein, which likes to associate with many clients and perform in many fields, "know" what to bind and what function to execute? I would not be the first to ask this question, for HSP90 regulation has occupied many researchers' minds for years. And not in vain — it was discovered that HSP90 is regulated in at least four ways:

- expression — activated by stress, transcription factor heat shock factor 1 (HSF1) induces transcription of HSP90 genes (Voellmy and Boellmann 2007);

- nucleotides — binding of ATP regulates the conformational cycle of HSP90, allowing it to change client conformations (Panaretou et al. 1998);
- post-translational modifications (PTMs) — phosphorylation, methylation, and acetylation either modify HSP90 itself or modify its clients, influencing HSP90-client interactions (Backe et al. 2020);
- and co-chaperones — binding of special non-client proteins that form complexes with HSP90 and assist it in its function (Röhl, Rohrberg, and Buchner 2013).

In this work, I will focus primarily on post-translational modifications of HSP90, particularly on acetylation. Acetylation is a reversible modification, which is provided by opposite actions of acetyltransferases and deacetylases (Aoyagi and Archer 2005). HSP90 acetylation is known to modify its ATP- and inhibitors-binding ability, as well as its interactions with clients and co-chaperones (Scroggins et al. 2007). These modifications, in turn, affect the stability and function of protein kinases and steroid hormone receptors and alter cell migration (Murphy et al. 2005; Yang et al. 2008). It is suggested that knowledge of acetylation mechanisms and the ability to regulate them can provide a therapeutic target for hormone-dependent cancers, such as breast or prostate cancer, and increase stress resilience (Fiskus et al. 2007; Jochems et al. 2015).

To understand how post-translational modifications affect the function of HSP90, I must first explore the structure of the protein and how it goes through its conformational cycle. The next chapter is dedicated to this topic.

Structure of HSP90 and its chaperone cycle

HSP90 is an obligatory homodimer — dimerizing is essential for *in vivo* functioning of the protein (Wayne and Bolon 2007). Each monomer consists of three highly conserved domains: ATP-binding N-terminal domain (NTD), catalytic middle domain (MD), and dimerizing C-terminal domain (CTD) (Figure 1). Monomers are arranged in linear order along each other, resembling pincers, with NTDs on one end of a dimer, and CTDs on another (Ali et al. 2006).

The N-terminal domain is a two-layer sandwich with a β-sheet on one side and α-helices on another. NTD's main functions are ATP binding and hydrolysis ensured by a deep 15Å ATP-binding pocket, situated on the inside face of the β-sheet and surrounded by α-helices and loops. Just twenty years ago the ATP-binding and ATP-hydrolyzing nature of HSP90 was controversial since no catalytic residues were identified and the enzymatic activity of HSP90 was very low (Jakob et al. 1996). However, the catalytic residues were eventually found and ATPase activity was confirmed (Prodromou et al. 1997). Researchers noted that the ATPase activity of HSP90 resembles that of DNA gyrase B, and later, based on the similarity of the ATP-binding pocket and ATP hydrolysis, it was categorized as a member of GHKL superfamily of ATPases, consisting of prokaryotic DNA gyrase B,

HSP90, MutL DNA mismatch repair proteins, protein kinases, and eukaryotic topoisomerases type II (Dutta and Inouye 2000). This ATP-binding structure was named after one of its discoverers, Bergerat fold.

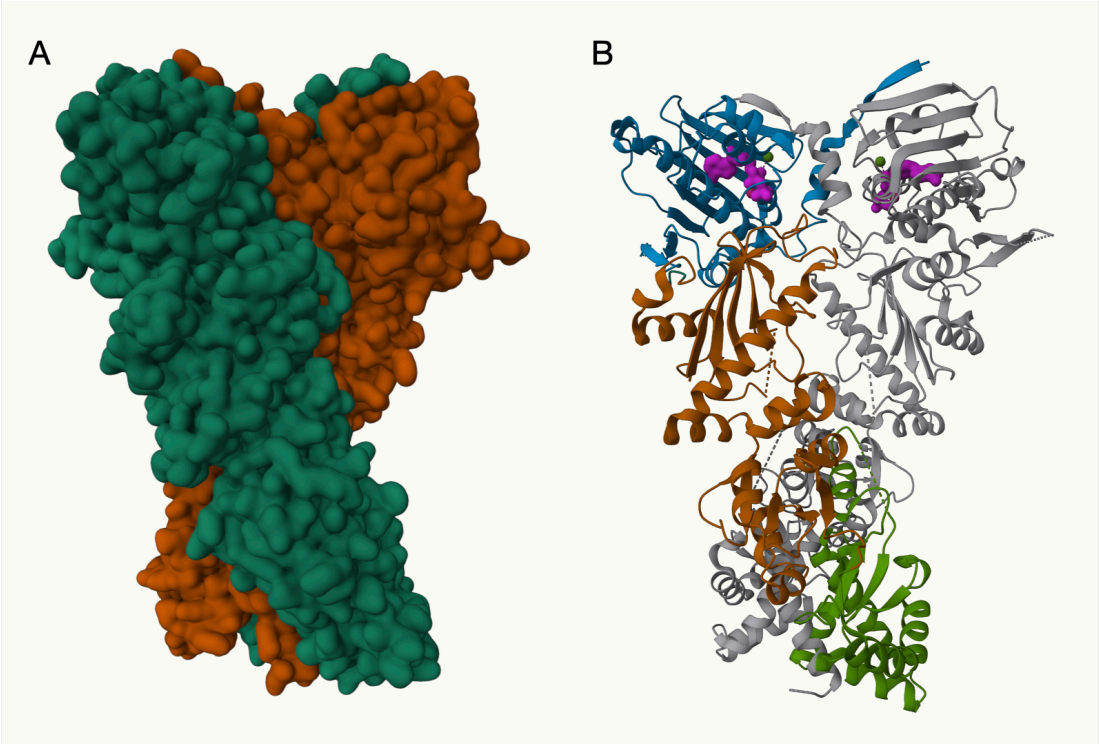

Figure 1: HSP90 3D structure. (**A**) Dimerized HSP90 protomers in Gaussian Surface representation. (**B**) Dimerized HSP90 protomers with ATP-analog molecules (*pink*) bound to both N-domains, where *blue* represents N-terminal domain, *orange* represents middle domain, *green* represents C-terminal domain, and *gray* represents another protomer; all in cartoon representation. Molecular structure was imported from PDB (PDB code 6XLE; Yanxin Liu et al., 2020) and edited in Mol* (Sehnal et al., 2021).

The middle domain plays an important role in ATP hydrolysis, for it contains amino acids essential for catalysis, but it also participates in the interactions with clients and several co-chaperones. The structure of MD consists of two subdomains: a larger N-terminal α/β/α domain, which is linked to a smaller α/β/α domain through a sequence of three short helices. In Hsp82 representing yeast homolog of HSP90, a hydrophobic patch with Trp300 exposed to solvent and an amphipathic sequence was found, suggesting a client or co-chaperone binding site (Meyer et al. 2003).

The C-terminal domain is formed by a mix of five short α-helices and a three-stranded antiparallel β-sheet. The two longest α-helices, H4 and H5, work as a dimerization interface: they interact with their equivalents from the partner CTD of a second HSP90 monomer, forming a four-helix bundle (Harris, Shiau, and Agard 2004). CTD fold has the important function of keeping the monomers together, which is essential for the ATPase activity, as NTDs' affinity for each other is rather low, and they are not capable of providing the proper inter-domain interaction and catalytic environment on their own (Ratzke et al. 2010). As a proof of that, deleting CTD cancels HSP90

ability to hydrolyze ATP (Prodromou et al. 2000). Another important function of CTD is providing a binding site — MEEVD (Met-Glu-Glu-Val-Asp) motif — for co-chaperones with tetratricopeptide repeat (TPR) domains (Scheufler et al. 2000). This motif, however, is only found in eukaryotic HSP90s, as they evolved in cooperation with a large pool of co-chaperones, in contrast to more "independent" prokaryotic HSP90 (Buchner 1999).

NTD and MD in eukaryotes are connected through a long flexible charged linker that facilitates intramolecular rearrangements necessary for the chaperone cycle progression and thus affects HSP90 function in a major way. Partial deletion of the charged linker was tested in yeast cells, and although the activity of ATPase was decreased, the cells were proven viable. However, when the linker was deleted completely, the HSP90 function was affected dramatically: it lost the ability to assist clients in maturation and be regulated by co-chaperones (Hainzl et al. 2009; Tsutsumi et al. 2012). The middle domain, in turn, is connected to CTD through an extended loop interacting with an α-helix at the start of the CTD (Ali et al. 2006).

The chaperone cycle of HptG, an *E.coli* ortholog of HSP90, is well-described. In an unbound state, the HptG dimer adopts an open "V" conformation. Fast ATP binding induces a sequence of conformational changes: a loop of highly conserved amino acid residues in the NTD spreads over the bound ATP like a "lid", that releases N-terminal segments of NTDs. These changes allow HptG to slowly reach intermediate state 1 (I1). After reaching I1, NTDs dimerize through their released N-terminal segments, arriving at the intermediate state 2 (I2) conformation. Now, as HptG is in a compact state, MDs and NTDs in each monomer relocate to closely interact with each other, and this signifies a fully closed conformation — active ATPase state. Finally, ATP hydrolysis takes place, then NTDs dissociate and ADP + Pi are released. HptG is back to an open conformation (Shiau et al. 2006; Mickler et al. 2009; Hessling, Richter, and Buchner 2009) (Figure 2). This simple model, however, may not apply fully to the eukaryotic HSP90. Prokaryotic HtpG and eukaryotic (yeast) HSP90 were found to differ substantially in conformational dynamics which came as a surprise at the time: HtpG fluctuates between the open and closed conformations randomly until it binds ATP — then it adopts a rigid closed conformation and proceeds to ATP hydrolysis; meanwhile, yeast HSP90 is considered to be a more flexible protein, adopting distinct conformations stochastically and maintaining a dynamic equilibrium, even after binding ATP. It seems that ATP binding only weakly influences the conformational dynamics of HSP90 and in the absence of co-chaperones and clients no directionality is observed in the conformational cycle (Ratzke et al., 2012).

HSP90 is sufficiently flexible and dynamic to accommodate the variety of its clients and functions, as mentioned in the introduction. To adjust to any of its clients and execute exactly the function that is required at the moment, HSP90 is regulated in a pretty complex manner.

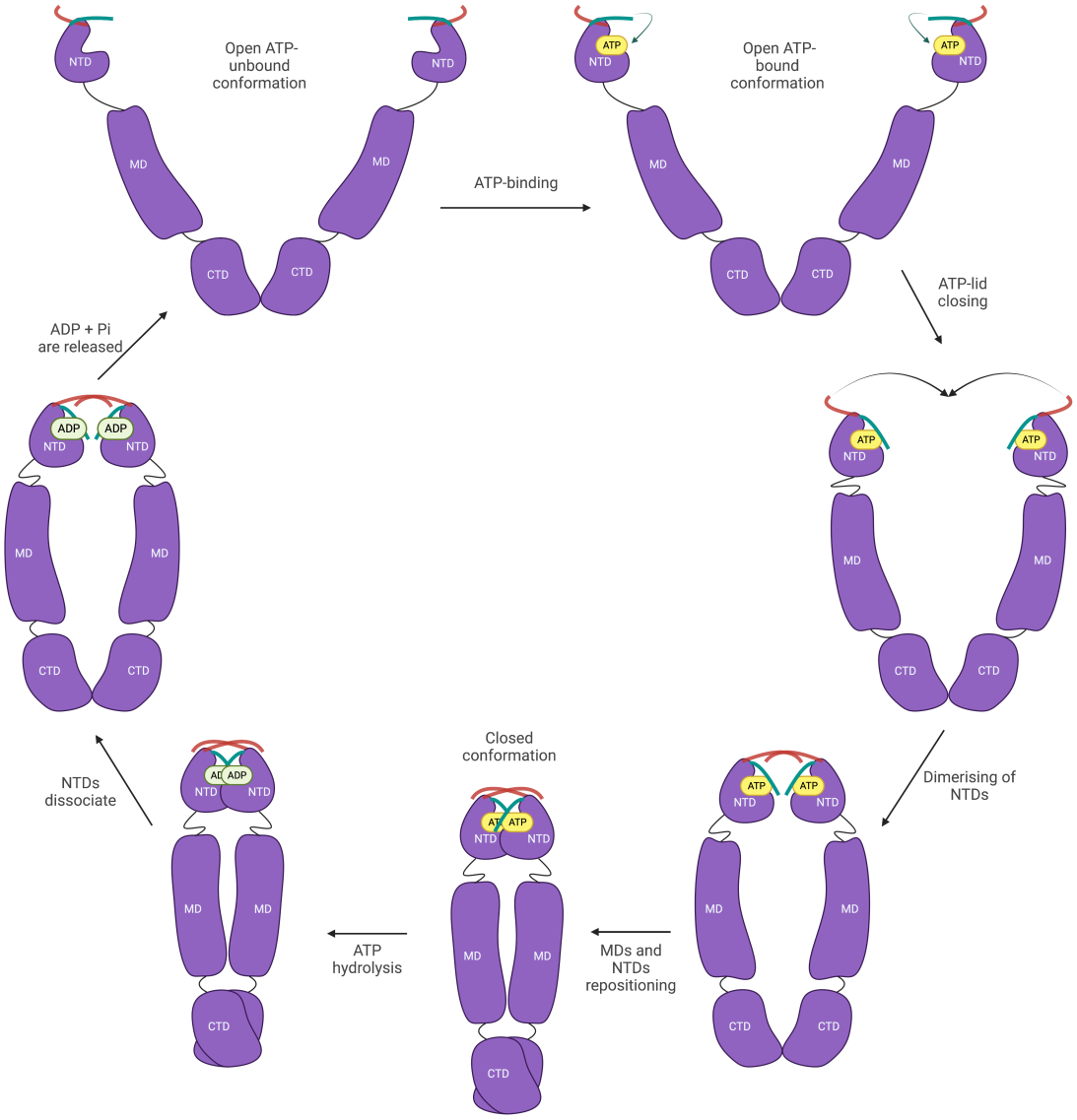

Figure 2: HSP90 conformational cycle in *E.coli* HptG. HSP90 protomers are connected through the C-terminal domain, and most of the time ATP-unbound HSP90 spends in an open, V-like conformation. Upon binding ATP, ATP "lids" (*green*) close, releasing the N-terminal conserved dimerizing sequences (*red*). NTD conserved sequences then dimerize, which causes a series of rearrangements leading to the closed conformation associated with ATP hydrolysis. After that, NTDs dissociate and ADP + Pi are released (Shiau et al. 2006; Mickler et al. 2009; Hessling, Richter, and Buchner 2009).

How is HSP90 regulated

As was noted in the introduction, HSP90 is regulated in a variety of ways, including transcription, nucleotide binding, co-chaperones, and post-translational modifications. Transcription regulation is the most "basic" one because it defines levels of HSP90 in the cell: the main heat-shock response (HSR) regulator, heat shock factor 1 (HSF1), is responsible for inducing expression of HSP90 as a reaction to cellular stress (Prodromou 2016). In yeast, HSF1 regulates HSP90 levels under

normal conditions as well (Solís et al. 2016). HSF1 is a highly conserved eukaryotic protein that consists of several domains: DNA-binding domain (DBD), trimerization domain of three Leucine Zippers (HR-A/B), HR-C region which negatively regulates HR-A/B, C-terminal trans-activation domain CTA, which is negatively regulated by central regulatory domain RD. Under non-stress conditions, HSP90 and HSP70 are both bound to HSF1, inhibiting it. When the cell experiences proteotoxic stress, however, chaperones and co-chaperones are needed in other areas, particularly to assist in the folding of accumulating denatured proteins. As a result of that, cytoplasmic HSF1 is released and forms a homotrimer. Homotrimers are transported into the nucleus, where they are free to bind heat-shock elements (HSE) in the upstream intron of the HSP gene with the help of their DNA-binding domain. HSF1 gets phosphorylated on binding DNA and becomes an active transcription factor. Quick expression of HSP90 and other chaperones then follows. Thus, HSF1 is a direct link between the stress response and the expression of HSPs (Prodromou 2016).

Another way of HSP90 regulation is through a range of different co-chaperones, which tend to bind to HSP90 in various stages of its cycle and influence its function through activation/inhibition of its ATPase activity or recruitment of specific clients. Some co-chaperones serve as adaptors for clients, others work as modulators of the cycle, and several of them can manage both functions. Interestingly, co-chaperones assemble in different complexes depending on specific clients and behave differently towards each other: they may compete for a binding site on HSP90, or work synergistically together (Smith 1993).

Co-chaperones can be arbitrarily divided into two major groups: TPR-domain-containing and TPR-less classes. The TPR (tetratricopeptide repeat) domain is a 34 amino acid repeats folded together in a bundle of helix-turn-helix motifs, serving as a binding site for the HSP90 C-terminal MEEVD motif (Scheufler et al. 2000). The most important co-chaperones with the TPR domain are HOP (Hsp70/Hsp90 organizing protein, so-called Sti1 in yeast) and PPIase (peptidyl-proline isomerase, PP5, so-called Ppt1 in yeast). Co-chaperones without a TPR domain seem to have a diverse range of specific binding sites on the surface of the HSP90 dimer. Such co-chaperones include AHA1 (“activator of Hsp90 ATPase''), CDC37 (a “cell division cycle control” protein, originally found in yeast), and PTGES3 (prostaglandin E synthase 3 cytosolic, so-called p23 protein, or Sba1 in yeast).

One of the most studied co-chaperone complexes is a steroid hormone receptor (SHR) maturation complex. This complex itself goes through several distinct intermediate complexes during the maturation cycle, forming from interacting co-chaperones: a TPR-containing co-chaperone HOP, which serves as an adaptor between HSP90 and HSP70; PPIase, which contains both a TPR-domain and a PPIase domain, and whose function in the complex is not completely understood yet; and TPR-less PTGES3, which deregulates ATPase and stabilizes the closed HSP90 conformation. The cycle begins with the binding of HOP to the TPR-binding site of the HSP90 dimer in an open conformation and thus stabilizing it, which inhibits the ATPase activity. At the same time, PPIase binds to another TPR-binding site, giving rise to an asymmetric intermediate complex. Next, HSP90 binds ATP at its

ATP-binding pocket in NTD, and PTGES3 binds HSP90, stabilizing a closed conformation, which in turn weakens the association of HSP90 with HOP and causes it to get released. The second PPIase or another TPR co-chaperone can bind the unoccupied TPR-binding site, and that induces the final complex capable of ATP hydrolysis. After ATP is hydrolyzed, co-chaperones and the folded client are released (Li, Richter, and Buchner 2011).

Another well-studied complex is the protein kinase activation complex, which resembles the SHR complex in some steps. For example, this complex also uses HOP for stabilization. As a first step, nascent or misfolded protein kinase binds chaperones Hsp70 and Hsp40, where Hsp70 hydrolyses ATP with the help of catalytic Hsp40, which stabilizes the ternary complex (Lee et al. 2004). CDC37 binds to a kinase through its N-terminal domain. Then, HOP, assisted by CDC37, facilitates the binding of the Hsp70/Hsp40/kinase complex to HSP90. CDC37 in this arrangement is thought to stabilize HSP90 in an open conformation and to inhibit its ATPase by preventing lid closure on NTD (Roe et al. 2004). Next, AHA1 binds HSP90 across NTD and MD (Meyer et al. 2004), displacing HOP and CDC37, and causing a quick transition from an open to closed conformation, drastically accelerating the cycle. In the presence of AHA1, ATP is hydrolyzed and the client kinase is activated (Retzlaff et al. 2010).

Although co-chaperones are very powerful regulators of HSP90 activity, they are not alone in their function. Another important level of regulation is represented by post-translational modifications, which have gained significant attention in recent years. PTMs, such as phosphorylation, acetylation, SUMOylation, S-nitrosylation, methylation, and ubiquitylation, are shown to serve in two roles: as local regulators of binding site accessibility, and as allosteric switch points regulating domain communication between dimers (Mollapour and Neckers 2012).

Phosphorylation is the most frequent post-translational modification of HSP90's Ser, Thr, and Tyr (Scroggins and Neckers 2007). Many phosphorylation sites were found all over the surface of the dimer. Phosphorylation seems to slow down the HSP90 conformational cycle and reduce client maturation, as well as affect the chaperone's general flexibility and interaction with co-chaperones, clients, and nucleotides (Soroka et al. 2012). For example, phosphorylation of Thr22 in yeast Hsp90 (Thr31 in human) NTD impairs ATPase function and seriously weakens interaction with both Aha1 and Cdc37 (Mollapour et al. 2011a), while phosphorylation of CTD Thr725 affects the binding of two co-chaperones in opposite ways: increases binding of HOP and decreases binding of CHIP (C terminus of Hsp70-interacting protein; Muller et al. 2013). Hyperphosphorylation in the absence of protein phosphatase PP5/Ppt1 leads to impaired HSP90 activity and decreased client activation in yeast (Wandinger et al. 2006). Apart from regulating co-chaperone and client binding, phosphorylation is also linked to apoptosis inhibition (Kurokawa et al. 2008), cell cycle regulation (Mollapour, Tsutsumi, and Neckers 2010), and sensitivity to inhibitors (Mollapour et al. 2011b). In summary, phosphorylation sites must be precisely and specifically regulated to support and control HSP90 function.

Acetylation and deacetylation of HSP90

Acetylation represents an acetyl group (CH3CO) bound to a molecule — a protein in our case. Approximately 85% of eukaryotic proteins are co-translationally acetylated at their Nα-termini (Nt-acetylation) in an irreversible manner (Polevoda and Sherman 2000). Nt-acetylation plays a role in protein half-life regulation, protein folding, membrane targeting and sub-cellular localization, and other cellular activities.

Another type of protein acetylation is N-ε-Lysine acetylation, which, on the contrary, is post-translational and reversible. It is highly regulated and allows the cell to turn on and off various cellular pathways. ε-Lysine acetylation proceeds as a transfer of an acetyl group onto a Nε-group of the lysine side chain, which increases the size of the side chain and neutralizes its positive charge (charge change from +1 to 0 when pH < pKa of lysine), leading to impaired ability to form hydrogen bonds. Lysine residues, being positively charged at physiological pH, are often used by proteins to bind negatively charged substrates. By acetylating lysines on proteins, the cell decreases the binding of these proteins to negatively charged substrates and increases the steric hindrance, altering protein-DNA and protein-protein interactions, protein stability, and enzymatic activity (Yang and Seto 2008; Xiong and Guan 2012). Studies show that lysine acetylation is often found in highly structured regions, like α-helices and β-sheets, and acetylated lysines are more numerous there than their non-acetylated counterparts (Choudhary et al. 2009). Phosphorylation, in contrast, mostly occurs in flexible and unstructured regions, like hinges and loops (Gnad et al. 2007; Malik et al. 2008).

Lysines on proteins are acetylated by so-called KATs (lysine acetyltransferases), previously known as HATs (histone acetyltransferases) because lysine acetylation was first found on histones in 1964 (Allfrey et al. 1964). It was later discovered that HATs acetylate many other proteins different from histones, thus they were renamed to KATs. Today 22 different human and mouse KATs are known to science, which can be divided into three groups: GNAT (GCN5 (general control non-derepressible 5)-related acetyltransferase) family, MYST (MOZ, Ybf2/Sas3, Sas2, TIP60) family and p300/CBP (CREB-binding protein) family (Allis et al. 2007; Berndsen and Denu 2008).

Deacetylation is carried out by KDACs (lysine deacetylases), also called HDACs (histone deacetylases) — similarly to KATs, HDACs were at first discovered in relation to histone modifications. There are several classes of KDACs: classes I, II, and IV, consisting of 11 members, correspond to “classical” HDACs; and class III, consisting of 7 members, corresponds to sirtuins. The difference between classical HDACs and sirtuins is based on their catalytic mechanisms: the former use Zn^{2+} ion as a cofactor; the latter use NAD+. Classical HDACs are under investigation as potential cancer targets, and most of the HDAC inhibitors studied are specific for classical HDACs (Witt et. al. 2009; Drazic et. al. 2016).

HSP90 acetyltransferase

Available data uncovering HSP90 acetylation are very limited. Yang et. al. investigated whether p300 is a lysine acetyltransferase efficient towards HSP90: when HSP90 was incubated with p300 and acetyl-CoA, immediate acetylation of HSP90 was detected by Western blot using anti-acetyl-lysine (AcK) antibodies. It was also demonstrated that p300 is required for HSP90 acetylation, and higher concentrations of p300 correspond to higher levels of HSP90 acetylation. However, p300 might not be the only KAT of HSP90, because when it was down-regulated, HSP90 was still acetylated, just less so (Yang et. al. 2008).

p300/CBP family is known to interact with at least 411 proteins, from them, approximately 100 proteins represent acetylation substrates. As there is no data on how p300 acetylates HSP90, we can look at the mechanism of acetylation of its more common substrates — histones. Contrary to the KAT usual ordered ternary complex mechanism of catalysis, p300 was found to have a less frequent Theorell-Chance, or hit-and-run, mechanism. Here, no stable ternary complex is formed, the substrate binds to the enzyme only shortly and leaves immediately after the reaction is complete. Dancy and Cole in their excellent review provide a detailed description of the acetylation catalysis steps by p300/CBP (Dancy and Cole 2015).

Where is HSP90 acetylated and how it affects its function

Scroggins et al. were the first researchers who identified a specific acetylation site on HSP90. They treated cells with an HDAC inhibitor trichostatin A (TSA), isolated HSP90, and analyzed it by mass spectrometry. As a result, K294 in HSP90α (corresponds to K286 in HSP90β) was identified. K294 is located at the beginning of the middle domain in close proximity to a charged linker. This location suggests that K294 acetylation plays an important role in the chaperone function, as it is in a very flexible region and on the surface of the protein, thus the change in its charge can affect binding to clients and co-chaperones. To test this hypothesis, HSP90 was produced with mutations mimicking acetylated lysine, where glutamine (Q) or alanine (A) was incorporated in place of lysine (neutral side chains), and mutations mimicking non-acetylatable lysine, where arginine (R) was incorporated in place of lysine (the positively charged side chain). As a result, K294Q and K294A mutants showed much weaker interaction with client tyrosine kinases ErbB2 and $p60^{v\text{-}Src}$, serine/threonine kinase Chk1, tumor suppressor p53, transcriptional regulator HIF1A (hypoxia-inducible factor 1-α), and androgen receptor, than K294R mutants and wild-type HSP90. The same effects were observed for co-chaperone binding: K294Q and K294A mutants showed no interaction or almost no interaction with co-chaperones p23, $p50^{Cdc37}$ (or HSP90 co-chaperone Cdc37), FKBP52 (or FK506 binding protein 4), Aha1, Hsp70, $p60^{Hop}$, and CHIP; while K294R and wild-type (WT) interacted with co-chaperones without any change. Interestingly, the non-acetylatable lysine-mimicking K294R

mutant interacted with FKBP52 and Aha1 much stronger than WT HSP90. These results can be explained by the fact that many clients and co-chaperones bind to HSP90 in the area of MD, where K294 is located, and several residues in MD regulate the ATPase activity of HSP90 (Scroggins et. al. 2007).

The next big finding was made by Yang and others, who identified seven new acetylated lysines with the help of an HDAC inhibitor LBH589. After treatment with LBH589, acetylated HSP90-α proteins were affinity purified using anti-acetyl-lysine antibodies. Mass spectrometry then detected seven acetylated lysines, K69, K100, K292, K327, K478, K546, and K558, which were all found to be on the surface of the protein, easily accessible for modification. Strikingly, K294 was not detected as acetylated, possibly due to different protocols and inhibitors used. The authors of this study also used acetylation-mimicking and non-acetylatable lysine mutations to see how acetylation affects the function of HSP90. Their findings were consistent with previous data. Most acetylation-mimicking mutants (K -> Q) in the area of MD (K292, K327, K478, K546, K558) and NTD (K69, K100) showed reduced binding to co-chaperones p23, Hsp40, Hsp70, and CHIP, as well as to the client serine/threonine kinase c-Raf. The only exception was K292Q, which bound CHIP in the same way as WT. Another interesting finding of the group was the effect of acetylation on ATP binding: all acetylation-mimicking (K -> Q) mutants also showed reduced binding to ATP; again, except for K292Q, which bound ATP with even higher affinity. Incidentally, all these K -> Q mutants bound more strongly to HSP90 inhibitor biotinylated-geldanamycin (B-GA). It was previously demonstrated that HSP90, where all seven lysines are acetylated (hyperacetylated state), binds more strongly to another HSP90 inhibitor 17-AAG (geldanamycin derivative used in cancer treatment). Authors suggested that HDAC inhibitors inducing hyperacetylation of HSP90, and HSP90 inhibitors such as 17-AAG can be used together for more effective cancer treatment (Yang et. al. 2008) (Figure 3).

Another high-resolution mass spectrometry study found several more HSP90α (K275, K284, K346, K354, K399, K402, K405, K414, K484, K529, K565, K580, K589, K611 and K698) and HSP90β (K435, K481, K568, K573) acetylation sites in all domains of HSP90 (Choudhary et al. 2009).

Eight more lysine acetylation sites were discovered after treatment with selective HDAC6 inhibitor tubastatin A, with the help of anti-AcK antibody beads and tandem mass spectrometry and chromatography: K185, K293, K437, K490, K540, K560, K568, and K633. The authors showed that acetylation levels of K490, K560, and K568 increased upon HDAC6 inhibition, while K633 acetylation levels decreased. On the other hand, when HDAC6 was overexpressed in cells, acetylation levels of K490 decreased, levels of acetylated K560 and K633 increased, and acetylation of K568 did not change. This data suggests that HDAC6 directly regulates only the acetylation status of K490, partially regulates the acetylation status of K560 and K568, and does not affect directly the acetylation of K633 (Du et al. 2021).

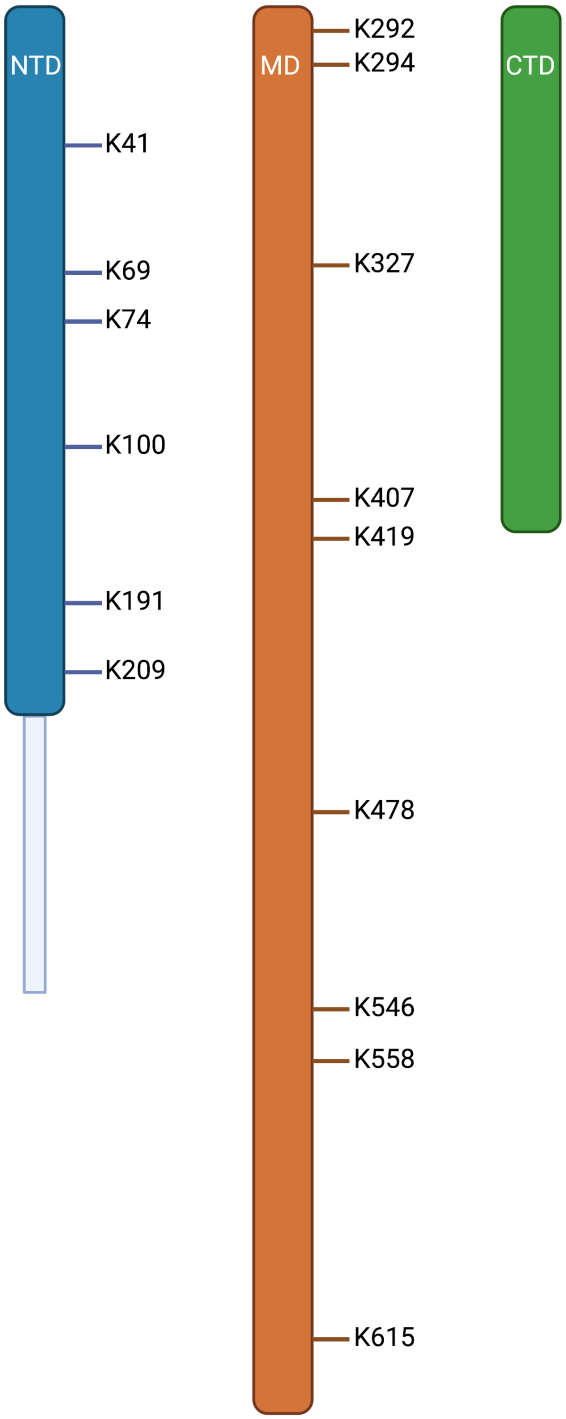

Figure 3: HSP90α acetylation sites. This figure represents a schematic diagram of three HSP90 domains: N-terminal domain (*blue*), middle domain (*orange*), and C-terminal domain (*green*). Only lysines that were shown to be acetylated and their contribution to HSP90 function was experimentally confirmed are marked on this diagram. No functional importance was confirmed for lysines on CTD yet. Diagram is adapted from Backe et al., 2020; created with BioRender.com

HDAC6 is an HSP90 deacetylase

The first hint to the question of HSP90 deacetylation was offered when a group of researchers studied HDAC inhibitor depsipeptide FR901228 (FK228) to use it for cancer treatment. They knew that HDAC inhibition leads to cancer cell growth arrest and apoptosis, but suspected it is not only due to dysfunctional histone deacetylation machinery, so they tried to uncover other effects of FK228 on cancer cells. FK228 was found to decrease levels of mutant (cancerous) p53, ErbB1, ErbB2, and Raf-1 (proteins usually stabilized by HSP90) as a result of decreased binding of HSP90 to its clients p53 and Raf-1 due to HSP90 hyperacetylation. HSP90 client degradation contributes to cell cycle disruption and may ultimately lead to apoptosis (Yu et al. 2002).

Similar results were shown when cancer cells were treated with another HDAC inhibitor LAQ824. It was shown that treatment with LAQ824 increased HSP90 acetylation levels, which led to decreased HSP90-ATP binding resulting in reduced association of HSP90 with its clients Bcr-Abl and c-Raf-1. Such a change in the interaction led to improper chaperoning of clients, a decrease of their levels in cells, and eventually proteasomal degradation (Nimmanapalli et al. 2003).

Murphy and colleagues went one step further and knocked down specifically HDAC6, assuming that HDAC6 may be directly targeting HSP90, and studied the effects on cells. They found that HDAC6 knockdown leads to HSP90 hyperacetylation, consistent with previous research, and causes reduced HSP90 binding to ATP, as well as to co-chaperone p23. The authors of the study suggested simultaneous binding of co-chaperone p23 to HSP90 when it assumes its ATP-bound conformation. If HSP90 is not able to bind ATP, then the HSP90-p23 complex cannot get stabilized. An unstable HSP90-p23 complex is unable to form a stable complex with the client glucocorticoid receptor (GR), which leads to GR malfunctioning ligand binding, nuclear translocation, and gene activation (Murphy et al. 2005).

Another study confirmed HDAC6 to be an HSP90 deacetylase. The study proceeded from the previous finding that treatment with hydroxamic acid analog HDAC inhibitors (HA-HDIs), such as LAQ824 and LBH589, lead to HSP90 hyperacetylation, decreased HSP90-ATP binding and impaired HSP90 chaperone function, which altogether causes polyubiquitination and degradation of pro-growth and pro-survival HSP90 client proteins in cancer cells: Bcr-Abl, FLT-3, c-Raf, and AKT (Nimmanapalli et al., 2003; Fuino et al., 2003; Bali et al., 2004). In the current study, HDAC6 was co-immunoprecipitated via HSP90 which proved that a specific interaction between HDAC6 and HSP90 exists. The consequences of HDAC6 knockdown were studied further: the data confirmed that HSP90 revealed significantly reduced ATP binding and impaired association with clients, which led to their degradation. On the other hand, when HDAC6 was overexpressed in cells, previously impaired HSP90 returned to normal function, and client (Bcr-Abl, AKT, and c-Raf) depletion ceased. When other HDACs were inhibited except HDAC6, no hyperacetylation of HSP90 occurred. Described relationships presented good evidence in support of HDAC6 being the HSP90 deacetylase (Bali et al. 2005). The findings were confirmed by other studies that proved HDAC6 to regulate HSP90 through

deacetylation. It was shown that HDAC6 inactivation or knockdown causes HSP90 hyperacetylation, its inability to bind p23, and loss of chaperone activity, leading to defective clients, such as GR. Furthermore, the HSP90 functionality was rescued by adding purified HSP90 from cells with functional HDAC6 (Kovacs et al. 2005).

Fiskus and others treated cells with HA-HDIs and discovered that HSP90 hyperacetylation (following the HDAC6 inhibition) further affects its binding to another client, estrogen receptor α (ERα), which causes ERα increased polyubiquitination and eventual depletion. Levels of other HSP90 clients, such as p-Akt and phospho-extracellular signal-regulated kinase 1/2 (ERK), were also found to be decreased (Fiskus et al. 2007). HDAC6 function *in vivo* was investigated in mice, and the results were similar to the results from human cells: depletion of HDAC6 caused hyperacetylation of HSP90, resulting in its malfunction, which led to defective GR activation and malfunctioning translocation of GR to the nucleus (Zhang et al. 2008). One of the recent studies confirmed anew that HDACi-induced HSP90 hyperacetylation results in disrupted HSP90-GR interaction, which leads to ubiquitin-proteasome-mediated GR degradation after prolonged exposure to the inhibitor (Zhu et al. 2020).

Another nuclear receptor affected by HDAC6-HSP90 interaction is the androgen receptor (AR), which was already mentioned concerning acetylation sites. HSP90 binds AR and maintains it in a high-affinity ligand-binding conformation, while also influencing its nuclear localization. It seems that HDAC6 regulates AR sensitivity to androgens via the deacetylation of HSP90 in the same manner as it was described for GR. Following HDAC6 inhibition or knockdown, the ability of AR to bind its ligand is compromised and its localization to the nucleus is significantly reduced (Ai et al, 2009). Mineralocorticoid receptor (MR) is another steroid nuclear receptor with a high affinity for aldosterone and cortisol. Similar to GR and AR, HSP90 interacts with MR to keep it in the ligand-binding form and regulate its nuclear localization. However, in this case, HDAC6 inhibition does not affect MR expression levels, furthermore, it enhances ligand-dependent nuclear localization, contrary to reducing the effect in the case of AR. In particular, these changes are induced by increased acetylation of a particular HSP90 residue, K295, and reversed by co-expression of non-acetylatable lysine-mimicking mutant K295R, suggesting that K295 acetylation directly regulates MR localization (Jimenez-Canino et al, 2016).

The list of HSP90 clients depleted due to malfunctional HDAC6 extends beyond nuclear receptors. For example, vascular endothelial growth factor receptors (VEGFRs) are important for tumor growth and the formation of new blood vessels. The group of Park treated cancer cells with HDIs (SAHA and LAQ824), resulting in reduced expression and decreased binding of VEGFR-1 and VEGFR-2 to HSP90. At the same time, increased binding of VEGFR-1/2 to HSP70 led to their proteasomal degradation. Furthermore, the complete knockdown of HDAC6 led to the depletion of VEGFR-1 and VEGFR-2. Interestingly, the deletion of another deacetylase, HDAC10, revealed the same result. Authors suggested that HDAC6 and HDAC10 may both regulate HSP90 (Park et al, 2008).

Another example of a client is represented by hypoxia-inducible factor-1α (HIF-1α) which is an oxygen-dependent major transcription factor playing an important regulatory role in many cellular processes. HIF-1α is stabilized by HSP90/HSP70 complex. Overexpression of HSP90/HSP70 was found to increase HIF-1α stability, while expression of defective HDAC6 (with impaired deacetylation function) or its complete absence led to HIF-1α degradation (Zhang et al, 2010).

Other putative deacetylases

Interesting evidence suggests that HDAC6 may not be the only deacetylase capable of deacetylating HSP90. The study describing the effects of HDACi or HDAC knockdown on DNA methyltransferase 1 (DNMT1) in the nucleus of human breast cancer cells showed that knockdown (KD) of HDAC6 has no effect on DNMT1 in the nucleus, while HDAC1 KD causes a decrease in DNMT1 expression. Furthermore, HDAC1 KD was shown to lead to HSP90 hyperacetylation in the nucleus. Inhibition of HDAC1 also caused HSP90 hyperacetylation in the nucleus, which in turn caused DNMT1 proteasomal degradation. Interestingly, HDAC6 inhibition did not reveal an effect on HSP90 acetylation in the nucleus in contrast to the cytoplasm (Zhou et al, 2008). Another study determined the regulatory relationship between the HDACs and a TAp73 protein, one of the forms produced by the p73 gene (a member of the p53 tumor suppressor family). Deletion of HDAC1 was followed by a significant decrease in TAp73 expression in both normal and DNA damage-induced states. Moreover, the half-life of TAp73 was reduced by 24% and 37% in normal and DNA-damage conditions, respectively. Interestingly, HDAC1 KD also led to HSP90 hyperacetylation. In addition to that, when HSP90 was inhibited by 17-AAG, it also caused a decrease in TAp73 expression, and concomitant KD of HDAC1 did not have an additive effect on the down-regulation of TAp73. HSP90 was suggested to chaperone and stabilize TAp73. Furthermore, HSP90 was found to associate with TAp73 *in vitro* and this association was weakened by HDAC1 KD. In summary, HDAC1 probably regulates TAp73 through HSP90 acetylation, which leads to TAp73 proteasomal degradation (Zhang et al, 2013).

The interplay between nuclear HSP90 and another deacetylase HDAC3 was examined in human breast cancer cells. HDAC3 was found to interact with HSP90 in the nucleus, moreover, the inhibition of HDAC3 was followed by the rise of nuclear HSP90 acetylation levels. Interestingly, HSP90 inhibition by 17-AAG caused a drop in nuclear HDAC3 levels, suggesting that HDAC3 could be a client of HSP90 (Kotwal and Subbarao, 2020).

Another candidate for HSP90 deacetylase is NAD+-dependent deacetylase SIRT2, which operates in the cytoplasm. SIRT2 was found to decrease HSP90 acetylation levels, even in cells specifically treated with Trichostatin A (TSA), a potent HDAC6 inhibitor. Meanwhile, the knockdown of SIRT2 caused a substantial increase in HSP90 acetylation; SIRT2 inhibition increased HSP90

acetylation as well. Expression of the SIRT2 mutant, which was unable to deacetylate, at least did not decrease HSP90 acetylation levels (Min et al, 2018). SIRT2 was also shown to regulate HSP90-GR interaction. When SIRT2 was overexpressed, the HSP90-GR association weakened, and GR was translocated into the nucleus. Conversely, when SIRT2 was knocked down, HSP90 acetylation levels increased, the HSP90-GR association strengthened, and GR was retained in the cytoplasm (Sun et at, 2020).

HSP90 Inhibitors and their use in disease treatment

Considering the importance of HSP90 for a large number of cellular processes, and its role in chaperoning many clients involved in signal transduction, transcriptional regulation, cell cycle regulation, and generally in cell survival, proliferation, and apoptosis, it is not surprising that HSP90 has attracted the attention of biomedical researchers. A lot of the clients are known oncogenes, for example, kinases ErbB2/Her2, v-Src, Bcr-Abl, Raf-1, Cdk4, etc. Another big portion of clients involves steroid hormone receptors, e.g. estrogen, androgen, glucocorticoid, and progesterone receptors. Dysregulation of them may easily lead to the development of cancerous phenotype. Interestingly, HSP90 expression is significantly enhanced in cancer cells in comparison to normal cells (Ferrarini et al, 1992), and HSP90 inhibitors tend to concentrate in cancer cells (Kamal et al, 2003). These facts make HSP90 a convenient target for anti-cancer treatment. Moreover, HSP90 binds ATP in a rather atypical way: the base and the sugar are buried in the pocket, while phosphates are exposed to solvent, and γ-phosphate submerges in the pocket only after the conformational changes caused by the interaction of MD and NTD. In addition, the ATP itself is bound in a compact conformation, as opposed to the usual extended conformation, that many other ATPases bind. This special binding was employed by a bacterium *Streptomyces hygroscopius*, that survives via the production of an antibiotic Geldanamycin (GA). GA competes with ATP for the ATP-binding site in the NTD of HSP90, and it has a 100-fold higher affinity than ATP. By binding to the ATP-binding pocket, Geldanamycin stabilizes HSP90 closed conformation and halts the progression of the conformational cycle, bringing on the degradation of HSP90 clients (Prodromou et al, 1997; Stebbins et al, 1997; Obermann et al, 1998). Unfortunately, Geldanamycin in its original form could not be used as a drug in cancer treatment, because it is poorly water-soluble and highly hepatotoxic (Neckers, 2006). Advanced derivatives, e.g. 17-AAG and 17-DMAG, are more potent, while being well-soluble in water and less toxic (Schulte and Neckers, 1998; Jez et al, 2003).

Radicicol (RDC) is another natural inhibitor isolated from *Monosporium bonorden* (Ki et al, 2000). Its mechanism of inhibition is similar to that of Geldanamycin. It competitively binds to the ATP-binding pocket in NTD with even higher affinity than GA and prevents HSP90 from forming complexes with clients (Roe et al, 1999; Sharma et al, 1998; Schulte et al, 1998). Radicicol was not effective as an anticancer agent, since *in vivo* it is metabolized into a compound with almost no

affinity for HSP90, but its oxime derivatives show significantly higher efficiency (Soga et al, 1999; Shiotsu et al, 2000; Agatsuma et al, 2002).

Interesting results were achieved when a chimeric HSP90 inhibitor Radamide was created based on GA's quinone component and RDC's resorcinol ring: the HSP90 inhibition and client depletion was more potent as a result of Radamide treatment than that of GA *in vivo* and RDC *in vitro*, individually (Clevenger and Blagg, 2004; Wang et at, 2006; Hadden and Blagg, 2009).

The function of all these three inhibitors and their derivatives is based on the inhibition of ATP binding to HSP90 NTD, which stops the ATPase cycle, and thus the clients cannot get chaperoned and activated. There are other ways to inhibit HSP90. One of them is the disruption of co-chaperone binding to HSP90 by blocking CTD. Novobiocin, an aminocoumarin antibiotic isolated from a bacterium *Streptomyces niveus* (Heide, 2009), binds to CTD and causes the dimers to split up, which forces the client to be released, as well as blocks HSP70 and p23 co-chaperones binding, and subsequently leads to the degradation of clients (Marcu et al, 2000a; Garnier et al, 2002; Allan et al, 2006). Novobiocin demonstrated a weak binding to HSP90 and a very poor inhibitory activity compared to NTD-binding inhibitors, but derivatives developed later showed a 1000-fold higher potency (Yu et al, 2005; Burlison and Blagg, 2006; Burlison et al, 2008; Radanyi et al, 2008).

Epigallocatechin-3-gallate (EGCG) is a well-known inhibitor that binds to CTD and prevents CTD dimerization, disrupts HSP90 interaction with co-chaperones HSP70 and p23, and induces degradation of clients Akt, Cdk4, Raf-1, Her2, pERK and aryl hydrocarbon receptor (AhR) (Palermo et al, 2005; Ann Beltz et al, 2006; Yin et al, 2009; Li et al, 2009). It demonstrates anti-proliferative effects in pancreatic cancer cells and otherwise shows great anticancer activity (Khan et al, 2006; Tran et al, 2010).

It was mentioned previously that HSP90 emerges as a convenient target for antitumor therapies, but it is also a very desirable target because its clients participate in the manifestation of and are important for all six hallmarks of cancer, according to Hanahan and Weinberg: "Self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis" (Hanahan and Weinberg, 2000). Therefore, disabling HSP90 could render useless many proteins mediating the hallmarks of cancer, particularly signaling pathways, at once (Zhang and Burrows, 2004; Neckers, 2007; Eustace et al, 2004).

HSP90 inhibition can be utilized also in neurodegenerative diseases. It is hypothesized that misfolded proteins, accumulating in plaques, result in the development of several diseases: such as Alzheimer's (AD), Parkinson's (PD), Huntington's, and prion diseases (Luheshi and Dobson, 2009). HSP90 along with other chaperones is playing a key role in refolding and solubilization of aggregating proteins. Experiments show that increased levels of HSP90 result in higher solubilization of tau protein (microtubule-associated protein in the CNS, its hyperphosphorylation leads to aggregation of neurofibrillary tangles, and AD or PD pathogenesis) and its binding to microtubules, as

well as its lower phosphorylation (Alonso et al, 2001; Dou et al, 2003; Dickey et al, 2005). Now, inhibition of HSP90 significantly decreased levels of tau via proteasomal degradation, possibly because the HSP90 client, tau kinase p35, was unstable (Luo et al, 2007). The same effect was found in the case of other clients, for example, tau kinases GSK3β (Glycogen synthase kinase-3 β) or Akt (Dou et al, 2007; Dickey et al, 2008).

In summary, HSP90 is an attractive target for drug development because it plays an important role in the maintenance of proteins essential to the six hallmarks of cancer, and its inhibition can lead to the destabilization and subsequent degradation of those proteins. It also allows for significant selectivity for cancers due to its enhanced expression in cancerous tissues. HSP90 inhibitors accumulate in cancer tissues, allowing for lower toxicity for the patient's healthy tissue. Geldanamycin, radicicol, and Novobiocin are among the natural inhibitors of HSP90; derivatives of those molecules, such as 17-AAG, 17-DMAG, and Radamide, have also been developed. Although HSP90 as a drug target gives high hope for helping to battle cancer and neurodegenerative diseases, it cannot stand as a sole target. To date, only two HSP90 inhibitors were approved due to the frequent significant adverse effects of many studied drugs (Garnock-Jones, 2015; Seo, 2015). HSP90 is capable, however, of lowering the drug resistance of cancers and infectious diseases, and that allows cooperation with other drugs resulting in efficient treatment.

Conclusions

HSP90, originally discovered as a stress-response protein, plays a crucial role in various cellular functions, including immune response, protein folding, DNA repair, and signal transduction. Its clients include steroid hormone receptors, tyrosine and serine/threonine kinases, HSF1, p53, cytoskeleton proteins, and telomerase. HSP90 consists of three main domains and is tightly regulated through transcriptional control, ATP binding, post-translational modifications (PTMs), and co-chaperone binding. Acetylation is a significant PTM, with p300 identified as a putative acetyltransferase for HSP90 and HDAC6 as the primary deacetylase.

Given its numerous clients involved in the pathogenesis of cancer, neurodegenerative diseases, and other disorders, HSP90 is a promising target for drug discovery and development. Natural HSP90 inhibitors, such as geldanamycin and radicicol, have led to the development of more potent derivatives like 17-AAG and 17-DMAG. HSP90 inhibitors can be employed not only as antitumor agents but also to reduce drug resistance in cancers when combined with established drugs. The proper functioning of HSP90 and its tight regulation are essential for the maintenance of cellular physiology.

References

- Agatsuma, T., Ogawa, H., Akasaka, K., Asai, A., Yamashita, Y., Mizukami, T., Akinaga, S., Saitoh, Y., 2002. Halohydrin and Oxime Derivatives of Radicicol: Synthesis and Antitumor Activities. Bioorganic & Medicinal Chemistry 10, 3445–3454. https://doi.org/10.1016/S0968-0896(02)00260-2
- Ai, J., Wang, Y., Dar, J.A., Liu, J., Liu, L., Nelson, J.B., Wang, Z., Wang, Z., 2009. HDAC6 regulates androgen receptor hypersensitivity and nuclear localization via modulating Hsp90 acetylation in castration-resistant prostate cancer. Molecular Endocrinology 23, 1963–1972. https://doi.org/10.1210/me.2009-0188
- Ali, M.M.U., Roe, S.M., Vaughan, C.K., Meyer, P., Panaretou, B., Piper, P.W., Prodromou, C., Pearl, L.H., 2006. Crystal structure of an Hsp90–nucleotide–p23/Sba1 closed chaperone complex. Nature 440, 1013–1017. https://doi.org/10.1038/nature04716
- Allan, R.K., Mok, D., Ward, B.K., Ratajczak, T., 2006. Modulation of Chaperone Function and Cochaperone Interaction by Novobiocin in the C-terminal Domain of Hsp90. Journal of Biological Chemistry 281, 7161–7171. https://doi.org/10.1074/jbc.M512406200
- Allfrey, V.G., Faulkner, R., Mirsky, A.E., 1964. Acetylation and methylation of histones and their possible role in the regulation of rna synthesis*. Proceedings of the National Academy of Sciences 51, 786–794. https://doi.org/10.1073/pnas.51.5.786
- Allis, C.D., Berger, S.L., Cote, J., Dent, S., Jenuwien, T., Kouzarides, T., Pillus, L., Reinberg, D., Shi, Y., Shiekhattar, R., Shilatifard, A., Workman, J., Zhang, Y., 2007. New Nomenclature for Chromatin-Modifying Enzymes. Cell 131, 633–636. https://doi.org/10.1016/j.cell.2007.10.039
- Alonso, A., Zaidi, T., Novak, M., Grundke-Iqbal, I., Iqbal, K., 2001. Hyperphosphorylation induces self-assembly of tau into tangles of paired helical filaments/straight filaments. Proc Natl Acad Sci U S A 98, 6923–6928. https://doi.org/10.1073/pnas.121119298
- Ann Beltz, L., Kay Bayer, D., Lynn Moss, A., Mitchell Simet, I., 2006. Mechanisms of Cancer Prevention by Green and Black Tea Polyphenols. Anti-Cancer Agents in Medicinal Chemistry-Anti-Cancer Agents) 6, 389–406. https://doi.org/10.2174/187152006778226468
- Aoyagi, S., Archer, T.K., 2005. Modulating molecular chaperone Hsp90 functions through reversible acetylation. Trends in Cell Biology 15, 565–567. https://doi.org/10.1016/j.tcb.2005.09.003
- Backe, S.J., Sager, R.A., Woodford, M.R., Makedon, A.M., Mollapour, M., 2020. Post-translational modifications of Hsp90 and translating the chaperone code. Journal of Biological Chemistry 295, 11099–11117. https://doi.org/10.1074/jbc.rev120.011833
- Bali, P., George, P., Cohen, P., Tao, J., Guo, F., Sigua, C., Vishvanath, A., Scuto, A., Annavarapu, S., Fiskus, W., Moscinski, L., Atadja, P., Bhalla, K., 2004. Superior Activity of the Combination of Histone Deacetylase Inhibitor LAQ824 and the FLT-3 Kinase Inhibitor PKC412 against Human Acute Myelogenous Leukemia Cells with Mutant FLT-3. Clinical Cancer Research 10, 4991–4997. https://doi.org/10.1158/1078-0432.CCR-04-0210
- Bali, P., Pranpat, M., Bradner, J.E., Balasis, M.E., Fiskus, W., Fiskus, W., Guo, F., Rocha, K., Kumaraswamy, S., Boyapalle, S., Atadja, P., Seto, E., Kapil Bhalla, Bhalla, K.N., 2005. Inhibition of Histone Deacetylase 6 Acetylates and Disrupts the Chaperone Function of Heat Shock Protein 90 A NOVEL BASIS FOR ANTILEUKEMIA ACTIVITY OF HISTONE DEACETYLASE INHIBITORS. Journal of Biological Chemistry 280, 26729–26734. https://doi.org/10.1074/jbc.c500186200
- Berndsen, C.E., Denu, J.M., 2008. Catalysis and substrate selection by histone/protein lysine acetyltransferases. Current Opinion in Structural Biology, Catalysis and regulation / Proteins 18, 682–689. https://doi.org/10.1016/j.sbi.2008.11.004
- Borkovich, K.A., Farrelly, F.W., Finkelstein, D.B., Taulien, J., Lindquist, S., 1989. hsp82 is an essential protein that is required in higher concentrations for growth of cells at higher temperatures. Molecular and Cellular Biology 9, 3919–3930. https://doi.org/10.1128/mcb.9.9.3919-3930.1989
- Brugge, J.S., 1986. Interaction of the Rous Sarcoma Virus Protein pp60srcwith the Cellular Proteins pp50 and pp90, in: Vogt, P.K., Koprowski, H. (Eds.), Retroviruses 4, Current Topics

in Microbiology and Immunology. Springer, Berlin, Heidelberg, pp. 1–22. https://doi.org/10.1007/978-3-642-70810-7_1
- Brugge, J.S., Erikson, R.L., 1977. Identification of a transformation-specific antigen induced by an avian sarcoma virus. Nature 269, 346–348. https://doi.org/10.1038/269346a0
- Buchner, J., 1999. Hsp90 & Co. – a holding for folding. Trends in Biochemical Sciences 24, 136–141. https://doi.org/10.1016/S0968-0004(99)01373-0
- Burlison, J.A., Avila, C., Vielhauer, G., Lubbers, D.J., Holzbeierlein, J., Blagg, B.S.J., 2008. Development of Novobiocin Analogues That Manifest Anti-proliferative Activity against Several Cancer Cell Lines. J. Org. Chem. 73, 2130–2137. https://doi.org/10.1021/jo702191a
- Burlison, J.A., Blagg, B.S.J., 2006. Synthesis and Evaluation of Coumermycin A1 Analogues that Inhibit the Hsp90 Protein Folding Machinery. Org. Lett. 8, 4855–4858. https://doi.org/10.1021/ol061918j
- Caplan, A.J., Mandal, A.K., Theodoraki, M.A., 2007. Molecular chaperones and protein kinase quality control. Trends in Cell Biology 17, 87–92. https://doi.org/10.1016/j.tcb.2006.12.002
- Choudhary, C., Kumar, C., Gnad, F., Nielsen, M.L., Rehman, M., Walther, T.C., Olsen, J.V., Mann, M., 2009. Lysine Acetylation Targets Protein Complexes and Co-Regulates Major Cellular Functions. Science 325, 834–840. https://doi.org/10.1126/science.1175371
- Clevenger, R.C., Blagg, B.S.J., 2004. Design, Synthesis, and Evaluation of a Radicicol and Geldanamycin Chimera, Radamide. Org. Lett. 6, 4459–4462. https://doi.org/10.1021/ol048266o
- Dancy, B.M., Cole, P.A., 2015. Protein Lysine Acetylation by p300/CBP. Chem. Rev. 115, 2419–2452. https://doi.org/10.1021/cr500452k
- Dickey, C.A., Eriksen, J., Kamal, A., Burrows, F., Kasibhatla, S., Eckman, C.B., Hutton, M., Petrucelli, L., 2005. Development of a High Throughput Drug Screening Assay for the Detection of Changes in Tau Levels - Proof of Concept with HSP90 inhibitors. Current Alzheimer Research 2, 231–238. https://doi.org/10.2174/1567205053585927
- Dickey, C.A., Koren, J., Zhang, Y.-J., Xu, Y., Jinwal, U.K., Birnbaum, M.J., Monks, B., Sun, M., Cheng, J.Q., Patterson, C., Bailey, R.M., Dunmore, J., Soresh, S., Leon, C., Morgan, D., Petrucelli, L., 2008. Akt and CHIP coregulate tau degradation through coordinated interactions. Proceedings of the National Academy of Sciences 105, 3622–3627. https://doi.org/10.1073/pnas.0709180105
- Dou, F., Chang, X., Ma, D., 2007. Hsp90 Maintains the Stability and Function of the Tau Phosphorylating Kinase GSK3β. International Journal of Molecular Sciences 8, 51–60. https://doi.org/10.3390/i8010060
- Dou, F., Netzer, W.J., Tanemura, K., Li, F., Hartl, F.U., Takashima, A., Gouras, G.K., Greengard, P., Xu, H., 2003. Chaperones increase association of tau protein with microtubules. Proceedings of the National Academy of Sciences 100, 721–726. https://doi.org/10.1073/pnas.242720499
- Drazic, A., Myklebust, L.M., Ree, R., Arnesen, T., 2016. The world of protein acetylation. Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics 1864, 1372–1401. https://doi.org/10.1016/j.bbapap.2016.06.007
- Du, Y., Yang, X., Li, Z., Le, W., Hao, Y., Song, Y., Wang, F., Guan, Y., 2021. HDAC6-mediated Hsp90 deacetylation reduces aggregation and toxicity of the protein α-synuclein by regulating chaperone-mediated autophagy. Neurochemistry International 149, 105141. https://doi.org/10.1016/j.neuint.2021.105141
- Dutta, R., Inouye, M., 2000. GHKL, an emergent ATPase/kinase superfamily. Trends in Biochemical Sciences 25, 24–28. https://doi.org/10.1016/S0968-0004(99)01503-0
- Echeverría, P.C., Bernthaler, A., Dupuis, P., Mayer, B., Picard, D., 2011. An Interaction Network Predicted from Public Data as a Discovery Tool: Application to the Hsp90 Molecular Chaperone Machine. PLOS ONE 6, e26044. https://doi.org/10.1371/journal.pone.0026044
- Echeverria, P.C., Picard, D., 2010. Molecular chaperones, essential partners of steroid hormone receptors for activity and mobility. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, Molecular Chaperones and Intracellular Protein Transport 1803, 641–649. https://doi.org/10.1016/j.bbamcr.2009.11.012

- Ellis, R.J., van der Vies, S.M., 1991. Molecular Chaperones. Annual Review of Biochemistry 60, 321–347. https://doi.org/10.1146/annurev.bi.60.070191.001541
- Eustace, B.K., Sakurai, T., Stewart, J.K., Yimlamai, D., Unger, C., Zehetmeier, C., Lain, B., Torella, C., Henning, S.W., Beste, G., Scroggins, B.T., Neckers, L., Ilag, L.L., Jay, D.G., 2004. Functional proteomic screens reveal an essential extracellular role for hsp90α in cancer cell invasiveness. Nat Cell Biol 6, 507–514. https://doi.org/10.1038/ncb1131
- Ferrarini, M., Heltai, S., Zocchi, M.R., Rugarli, C., 1992. Unusual expression and localization of heat-shock proteins in human tumor cells. International Journal of Cancer 51, 613–619. https://doi.org/10.1002/ijc.2910510418
- Finka, A., Mattoo, R.U.H., Goloubinoff, P., 2016. Experimental Milestones in the Discovery of Molecular Chaperones as Polypeptide Unfolding Enzymes. Annu Rev Biochem 85, 715–742. https://doi.org/10.1146/annurev-biochem-060815-014124
- Fiskus, W., Ren, Y., Mohapatra, A., Bali, P., Mandawat, A., Rao, R., Rao, R., Herger, B., Yang, Y., Atadja, P., Wu, J., Kapil N. Bhalla, Bhalla, K.N., 2007. Hydroxamic Acid Analogue Histone Deacetylase Inhibitors Attenuate Estrogen Receptor-α Levels and Transcriptional Activity: A Result of Hyperacetylation and Inhibition of Chaperone Function of Heat Shock Protein 90. Clinical Cancer Research 13, 4882–4890. https://doi.org/10.1158/1078-0432.ccr-06-3093
- Fuino, L., Bali, P., Wittmann, S., Donapaty, S., Guo, F., Yamaguchi, H., Wang, H.-G., Atadja, P., Bhalla, K., 2003. Histone deacetylase inhibitor LAQ824 down-regulates Her-2 and sensitizes human breast cancer cells to trastuzumab, taxotere, gemcitabine, and epothilone B. Molecular Cancer Therapeutics 2, 971–984.
- Garnier, C., Lafitte, D., Tsvetkov, P.O., Barbier, P., Leclerc-Devin, J., Millot, J.-M., Briand, C., Makarov, A.A., Catelli, M.G., Peyrot, V., 2002. Binding of ATP to Heat Shock Protein 90: EVIDENCE FOR AN ATP-BINDING SITE IN THE C-TERMINAL DOMAIN *. Journal of Biological Chemistry 277, 12208–12214. https://doi.org/10.1074/jbc.M111874200
- Garnock-Jones, K.P., 2015. Panobinostat: First Global Approval. Drugs 75, 695–704. https://doi.org/10.1007/s40265-015-0388-8
- Gnad, F., Ren, S., Cox, J., Olsen, J.V., Macek, B., Oroshi, M., Mann, M., 2007. PHOSIDA (phosphorylation site database): management, structural and evolutionary investigation, and prediction of phosphosites. Genome Biol 8, R250. https://doi.org/10.1186/gb-2007-8-11-r250
- Hadden, M.K., Blagg, B.S.J., 2009. Synthesis and Evaluation of Radamide Analogues, A Chimera of Radicicol and Geldanamycin. J. Org. Chem. 74, 4697–4704. https://doi.org/10.1021/jo900278g
- Hainzl, O., Lapina, M.C., Buchner, J., Richter, K., 2009. The Charged Linker Region Is an Important Regulator of Hsp90 Function *. Journal of Biological Chemistry 284, 22559–22567. https://doi.org/10.1074/jbc.M109.031658
- Hanahan, D., Weinberg, R.A., 2000. The Hallmarks of Cancer. Cell 100, 57–70. https://doi.org/10.1016/S0092-8674(00)81683-9
- Harris, S.F., Shiau, A.K., Agard, D.A., 2004. The Crystal Structure of the Carboxy-Terminal Dimerization Domain of htpG, the Escherichia coli Hsp90, Reveals a Potential Substrate Binding Site. Structure 12, 1087–1097. https://doi.org/10.1016/j.str.2004.03.020
- Heide, L., 2009. The aminocoumarins: biosynthesis and biology. Nat. Prod. Rep. 26, 1241–1250. https://doi.org/10.1039/B808333A
- Hessling, M., Richter, K., Buchner, J., 2009. Dissection of the ATP-induced conformational cycle of the molecular chaperone Hsp90. Nat Struct Mol Biol 16, 287–293. https://doi.org/10.1038/nsmb.1565
- Jakob, U., Scheibel, T., Bose, S., Reinstein, J., Buchner, J., 1996. Assessment of the ATP Binding Properties of Hsp90 (∗). Journal of Biological Chemistry 271, 10035–10041. https://doi.org/10.1074/jbc.271.17.10035
- Jez, J.M., Chen, J.C.-H., Rastelli, G., Stroud, R.M., Santi, D.V., 2003. Crystal Structure and Molecular Modeling of 17-DMAG in Complex with Human Hsp90. Chemistry & Biology 10, 361–368. https://doi.org/10.1016/S1074-5521(03)00075-9
- Jimenez-Canino, R., Lorenzo-Díaz, F., Jaisser, F., Farman, N., Giraldez, T., de la Rosa, D.A., 2016. Histone Deacetylase 6–Controlled Hsp90 Acetylation Significantly Alters

Mineralocorticoid Receptor Subcellular Dynamics But Not its Transcriptional Activity. Endocrinology 157, 2515–2532. https://doi.org/10.1210/en.2015-2055

- Jochems, J., Teegarden, S.L., Chen, Y., Yong Chen, Boulden, J., Challis, C., Ben-Dor, G.A., Kim, S.F., Olivier Berton, Berton, O., Olivier Berton, 2015. Enhancement of stress resilience through histone deacetylase 6-mediated regulation of glucocorticoid receptor chaperone dynamics. Biological Psychiatry 77, 345–355. https://doi.org/10.1016/j.biopsych.2014.07.036
- Kamal, A., Thao, L., Sensintaffar, J., Zhang, L., Boehm, M.F., Fritz, L.C., Burrows, F.J., 2003. A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors. Nature 425, 407–410. https://doi.org/10.1038/nature01913
- Khan, N., Afaq, F., Saleem, M., Ahmad, N., Mukhtar, H., 2006. Targeting Multiple Signaling Pathways by Green Tea Polyphenol (−)-Epigallocatechin-3-Gallate. Cancer Research 66, 2500–2505. https://doi.org/10.1158/0008-5472.CAN-05-3636
- Ki, S.W., Ishigami, K., Kitahara, T., Kasahara, K., Yoshida, M., Horinouchi, S., 2000. Radicicol Binds and Inhibits Mammalian ATP Citrate Lyase *. Journal of Biological Chemistry 275, 39231–39236. https://doi.org/10.1074/jbc.M006192200
- Kotwal, A., Subbarao, S.A., 2020. Hsp90 regulates HDAC3-dependent gene transcription while HDAC3 regulates the functions of Hsp90. Cellular Signalling 76, 109801–109801. https://doi.org/10.1016/j.cellsig.2020.109801
- Kovacs, J.J., Murphy, P.J., Gaillard, S., Zhao, X., Wu, J.-T., Nicchitta, C.V., Yoshida, M., Toft, D.O., Pratt, W.B., Yao, T.-P., 2005. HDAC6 Regulates Hsp90 Acetylation and Chaperone-Dependent Activation of Glucocorticoid Receptor. Molecular Cell 18, 601–607. https://doi.org/10.1016/j.molcel.2005.04.021
- Kurokawa, M., Zhao, C., Reya, T., Kornbluth, S., 2008. Inhibition of Apoptosome Formation by Suppression of Hsp90β Phosphorylation in Tyrosine Kinase-Induced Leukemias. Molecular and Cellular Biology 28, 5494–5506. https://doi.org/10.1128/MCB.00265-08
- Lee, P., Shabbir, A., Cardozo, C., Caplan, A.J., 2004. Sti1 and Cdc37 Can Stabilize Hsp90 in Chaperone Complexes with a Protein Kinase. MBoC 15, 1785–1792. https://doi.org/10.1091/mbc.e03-07-0480
- Li, J., Richter, K., Buchner, J., 2011. Mixed Hsp90–cochaperone complexes are important for the progression of the reaction cycle. Nat Struct Mol Biol 18, 61–66. https://doi.org/10.1038/nsmb.1965
- Li, Y., Zhang, T., Jiang, Y., Lee, H.-F., Schwartz, S.J., Sun, D., 2009. (−)-Epigallocatechin-3-gallate Inhibits Hsp90 Function by Impairing Hsp90 Association with Cochaperones in Pancreatic Cancer Cell Line Mia Paca-2. Mol. Pharmaceutics 6, 1152–1159. https://doi.org/10.1021/mp900037p
- Luheshi, L.M., Dobson, C.M., 2009. Bridging the gap: From protein misfolding to protein misfolding diseases. FEBS Letters, Protein Folding, Misfolding and Disease 583, 2581–2586. https://doi.org/10.1016/j.febslet.2009.06.030
- Luo, W., Dou, F., Rodina, A., Chip, S., Kim, J., Zhao, Q., Moulick, K., Aguirre, J., Wu, N., Greengard, P., Chiosis, G., 2007. Roles of heat-shock protein 90 in maintaining and facilitating the neurodegenerative phenotype in tauopathies. Proceedings of the National Academy of Sciences 104, 9511–9516. https://doi.org/10.1073/pnas.0701055104
- Malik, R., Nigg, E.A., Körner, R., 2008. Comparative conservation analysis of the human mitotic phosphoproteome. Bioinformatics 24, 1426–1432. https://doi.org/10.1093/bioinformatics/btn197
- Marcu, M.G., Chadli, A., Bouhouche, I., Catelli, M., Neckers, L.M., 2000a. The Heat Shock Protein 90 Antagonist Novobiocin Interacts with a Previously Unrecognized ATP-binding Domain in the Carboxyl Terminus of the Chaperone *. Journal of Biological Chemistry 275, 37181–37186. https://doi.org/10.1074/jbc.M003701200
- Maynard, J.C., Pham, T., Zheng, T., Jockheck-Clark, A., Rankin, H.B., Newgard, C.B., Spana, E.P., Nicchitta, C.V., 2010. Gp93, the Drosophila GRP94 ortholog, is required for gut epithelial homeostasis and nutrient assimilation-coupled growth control. Developmental Biology, Special Section: Gene Expression and Development 339, 295–306. https://doi.org/10.1016/j.ydbio.2009.12.023

- McClellan, A.J., Xia, Y., Deutschbauer, A.M., Davis, R.W., Gerstein, M., Frydman, J., 2007. Diverse Cellular Functions of the Hsp90 Molecular Chaperone Uncovered Using Systems Approaches. Cell 131, 121–135. https://doi.org/10.1016/j.cell.2007.07.036
- Meyer, P., Prodromou, C., Hu, B., Vaughan, C., Roe, S.M., Panaretou, B., Piper, P.W., Pearl, L.H., 2003. Structural and Functional Analysis of the Middle Segment of Hsp90: Implications for ATP Hydrolysis and Client Protein and Cochaperone Interactions. Molecular Cell 11, 647–658. https://doi.org/10.1016/S1097-2765(03)00065-0
- Meyer, P., Prodromou, C., Liao, C., Hu, B., Mark Roe, S., Vaughan, C.K., Vlasic, I., Panaretou, B., Piper, P.W., Pearl, L.H., 2004. Structural basis for recruitment of the ATPase activator Aha1 to the Hsp90 chaperone machinery. The EMBO Journal 23, 511–519. https://doi.org/10.1038/sj.emboj.7600060
- Mickler, M., Hessling, M., Ratzke, C., Buchner, J., Hugel, T., 2009. The large conformational changes of Hsp90 are only weakly coupled to ATP hydrolysis. Nature Structural & Molecular Biology 16, 281–286. https://doi.org/10.1038/nsmb.1557
- Min, J.S., Kim, J.C., Kim, J.A., Kang, I., Ahn, J.K., 2018. SIRT2 reduces actin polymerization and cell migration through deacetylation and degradation of HSP90. Biochimica et Biophysica Acta 1865, 1230–1238. https://doi.org/10.1016/j.bbamcr.2018.06.005
- Mollapour, M., Neckers, L., 2012. Post-translational modifications of Hsp90 and their contributions to chaperone regulation. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, Heat Shock Protein 90 (Hsp90) 1823, 648–655. https://doi.org/10.1016/j.bbamcr.2011.07.018
- Mollapour, M., Tsutsumi, S., Kim, Y.S., Trepel, J., Neckers, L., 2011b. Casein kinase 2 phosphorylation of Hsp90 threonine 22 modulates chaperone function and drug sensitivity. Oncotarget 2, 407–417.
- Mollapour, M., Tsutsumi, S., Neckers, L., 2010. Hsp90 phosphorylation, Wee1 and the cell cycle. Cell Cycle 9, 2310–2316. https://doi.org/10.4161/cc.9.12.12054
- Mollapour, M., Tsutsumi, S., Truman, A.W., Xu, W., Vaughan, C.K., Beebe, K., Konstantinova, A., Vourganti, S., Panaretou, B., Piper, P.W., Trepel, J.B., Prodromou, C., Pearl, L.H., Neckers, L., 2011a. Threonine 22 Phosphorylation Attenuates Hsp90 Interaction with Cochaperones and Affects Its Chaperone Activity. Molecular Cell 41, 672–681. https://doi.org/10.1016/j.molcel.2011.02.011
- Muller, P., Ruckova, E., Halada, P., Coates, P.J., Hrstka, R., Lane, D.P., Vojtesek, B., 2013. C-terminal phosphorylation of Hsp70 and Hsp90 regulates alternate binding to co-chaperones CHIP and HOP to determine cellular protein folding/degradation balances. Oncogene 32, 3101–3110. https://doi.org/10.1038/onc.2012.314
- Murphy, P.J., Morishima, Y., Kovacs, J.J., Yao, T.-P., Pratt, W.B., 2005. Regulation of the dynamics of hsp90 action on the glucocorticoid receptor by acetylation/deacetylation of the chaperone. Journal of Biological Chemistry 280, 33792–33799. https://doi.org/10.1074/jbc.m506997200
- Neckers, L., 2006. Chaperoning Oncogenes: Hsp90 as a Target of Geldanamycin, in: Starke, K., Gaestel, M. (Eds.), Molecular Chaperones in Health and Disease, Handbook of Experimental Pharmacology. Springer, Berlin, Heidelberg, pp. 259–277. https://doi.org/10.1007/3-540-29717-0_11
- Neckers, L., 2007. Heat shock protein 90: the cancer chaperone. J Biosci 32, 517–530. https://doi.org/10.1007/s12038-007-0051-y
- Nimmanapalli, R., Fuino, L., Bali, P., Gasparetto, M., Glozak, M., Tao, J., Moscinski, L., Smith, C., Wu, J., Jove, R., Atadja, P., Bhalla, K., 2003. Histone Deacetylase Inhibitor LAQ824 Both Lowers Expression and Promotes Proteasomal Degradation of Bcr-Abl and Induces Apoptosis of Imatinib Mesylate-sensitive or -refractory Chronic Myelogenous Leukemia-Blast Crisis Cells. Cancer Research 63, 5126–5135.
- Obermann, W.M.J., Sondermann, H., Russo, A.A., Pavletich, N.P., Hartl, F.U., 1998. In Vivo Function of Hsp90 Is Dependent on ATP Binding and ATP Hydrolysis. Journal of Cell Biology 143, 901–910. https://doi.org/10.1083/jcb.143.4.901
- Palermo, C.M., Westlake, C.A., Gasiewicz, T.A., 2005. Epigallocatechin Gallate Inhibits Aryl Hydrocarbon Receptor Gene Transcription through an Indirect Mechanism Involving Binding

to a 90 kDa Heat Shock Protein. Biochemistry 44, 5041–5052. https://doi.org/10.1021/bi047433p

- Panaretou, B., Prodromou, C., Roe, S.M., O'Brien, R., Ladbury, J.E., Piper, P.W., Pearl, L.H., 1998. ATP binding and hydrolysis are essential to the function of the Hsp90 molecular chaperone in vivo. The EMBO Journal 17, 4829–4836. https://doi.org/10.1093/emboj/17.16.4829
- Park, J., Kim, S.H., Choi, M.C., Lee, J.-H., Oh, D.-Y., Oh, D.-Y., Im, S.-A., Bang, Y.-J., Tae-You Kim, Kim, T.-Y., 2008. Class II histone deacetylases play pivotal roles in heat shock protein 90-mediated proteasomal degradation of vascular endothelial growth factor receptors. Biochemical and Biophysical Research Communications 368, 318–322. https://doi.org/10.1016/j.bbrc.2008.01.056
- Polevoda, B., Sherman, F., 2000. Nα-terminal Acetylation of Eukaryotic Proteins*. Journal of Biological Chemistry 275, 36479–36482. https://doi.org/10.1074/jbc.R000023200
- Pratt, W.B., Toft, D.O., 1997. Steroid Receptor Interactions with Heat Shock Protein and Immunophilin Chaperones[[sup.*]]. Endocrine Reviews 18, 306–361.
- Prodromou, C., 2016. Mechanisms of Hsp90 regulation. Biochemical Journal 473, 2439–2452. https://doi.org/10.1042/bcj20160005
- Prodromou, C., Panaretou, B., Chohan, S., Siligardi, G., O'Brien, R., Ladbury, J.E., Roe, S.M., Piper, P.W., Pearl, L.H., 2000. The ATPase cycle of Hsp90 drives a molecular 'clamp' via transient dimerization of the N-terminal domains. The EMBO Journal 19, 4383–4392. https://doi.org/10.1093/emboj/19.16.4383
- Prodromou, C., Roe, S.M., O'Brien, R., Ladbury, J.E., Piper, P.W., Pearl, L.H., 1997. Identification and Structural Characterization of the ATP/ADP-Binding Site in the Hsp90 Molecular Chaperone. Cell 90, 65–75. https://doi.org/10.1016/S0092-8674(00)80314-1
- Radanyi, C., Le Bras, G., Messaoudi, S., Bouclier, C., Peyrat, J.-F., Brion, J.-D., Marsaud, V., Renoir, J.-M., Alami, M., 2008. Synthesis and biological activity of simplified denoviose-coumarins related to novobiocin as potent inhibitors of heat-shock protein 90 (hsp90). Bioorganic & Medicinal Chemistry Letters 18, 2495–2498. https://doi.org/10.1016/j.bmcl.2008.01.128
- Ratzke, C., Mickler, M., Hellenkamp, B., Buchner, J., Hugel, T., 2010. Dynamics of heat shock protein 90 C-terminal dimerization is an important part of its conformational cycle. Proceedings of the National Academy of Sciences 107, 16101–16106. https://doi.org/10.1073/pnas.1000916107
- Retzlaff, M., Hagn, F., Mitschke, L., Hessling, M., Gugel, F., Kessler, H., Richter, K., Buchner, J., 2010. Asymmetric Activation of the Hsp90 Dimer by Its Cochaperone Aha1. Molecular Cell 37, 344–354. https://doi.org/10.1016/j.molcel.2010.01.006
- Richter, K., Haslbeck, M., Buchner, J., 2010. The Heat Shock Response: Life on the Verge of Death. Molecular Cell 40, 253–266. https://doi.org/10.1016/j.molcel.2010.10.006
- Ritossa, F., 1962. A new puffing pattern induced by temperature shock and DNP in drosophila. Experientia 18, 571–573. https://doi.org/10.1007/BF02172188
- Roe, S.M., Ali, M.M.U., Meyer, P., Vaughan, C.K., Panaretou, B., Piper, P.W., Prodromou, C., Pearl, L.H., 2004. The Mechanism of Hsp90 Regulation by the Protein Kinase-Specific Cochaperone p50cdc37. Cell 116, 87–98. https://doi.org/10.1016/S0092-8674(03)01027-4
- Roe, S.M., Prodromou, C., O'Brien, R., Ladbury, J.E., Piper, P.W., Pearl, L.H., 1999. Structural Basis for Inhibition of the Hsp90 Molecular Chaperone by the Antitumor Antibiotics Radicicol and Geldanamycin. J. Med. Chem. 42, 260–266. https://doi.org/10.1021/jm980403y
- Röhl, A., Rohrberg, J., Buchner, J., 2013. The chaperone Hsp90: changing partners for demanding clients. Trends in Biochemical Sciences 38, 253–262. https://doi.org/10.1016/j.tibs.2013.02.003
- Scheufler, C., Brinker, A., Bourenkov, G., Pegoraro, S., Moroder, L., Bartunik, H., Hartl, F.U., Moarefi, I., 2000. Structure of TPR Domain–Peptide Complexes: Critical Elements in the Assembly of the Hsp70–Hsp90 Multichaperone Machine. Cell 101, 199–210. https://doi.org/10.1016/S0092-8674(00)80830-2

- Schulte, T.W., Akinaga, S., Soga, S., Sullivan, W., Stensgard, B., Toft, D., Neckers, L.M., 1998. Antibiotic radicicol binds to the N-terminal domain of Hsp90 and shares important biologic activities with geldanamycin. Cell Stress Chaperones 3, 100–108.
- Schulte, T.W., Neckers, L.M., 1998. The benzoquinone ansamycin 17-allylamino-17-demethoxygeldanamycin binds to HSP90 and shares important biologic activities with geldanamycin. Cancer Chemother Pharmacol 42, 273–279. https://doi.org/10.1007/s002800050817
- Scroggins, B.T., Neckers, L., 2007. Post-translational modification of heat-shock protein 90: impact on chaperone function. Expert Opinion on Drug Discovery 2, 1403–1414. https://doi.org/10.1517/17460441.2.10.1403
- Scroggins, B.T., Robzyk, K., Wang, D., Marcu, M.G., Monica G. Marcu, Shinji Tsutsumi, Shinji Tsutsumi, Tsutsumi, S., Beebe, K., Cotter, R.J., Felts, S.J., Toft, D.O., Karnitz, L.M., Rosen, N., Neckers, L., 2007. An acetylation site in the middle domain of Hsp90 regulates chaperone function. Molecular Cell 25, 151–159. https://doi.org/10.1016/j.molcel.2006.12.008
- Sehnal, D., Bittrich, S., Deshpande, M., Svobodová, R., Berka, K., Bazgier, V., Velankar, S., Burley, S.K., Koča, J., Rose, A.S., 2021. Mol* Viewer: modern web app for 3D visualization and analysis of large biomolecular structures. Nucleic Acids Research 49, W431–W437. https://doi.org/10.1093/nar/gkab314
- Seo, Y.H., 2015. Repositioning Irsogladine to Hsp90 Inhibitor. Bulletin of the Korean Chemical Society 36, 1495–1499. https://doi.org/10.1002/bkcs.10291
- Sharma, S.V., Agatsuma, T., Nakano, H., 1998. Targeting of the protein chaperone, HSP90, by the transformation suppressing agent, radicicol. Oncogene 16, 2639–2645. https://doi.org/10.1038/sj.onc.1201790
- Shiau, A.K., Harris, S.F., Southworth, D.R., Agard, D.A., 2006. Structural Analysis of E. coli hsp90 Reveals Dramatic Nucleotide-Dependent Conformational Rearrangements. Cell 127, 329–340. https://doi.org/10.1016/j.cell.2006.09.027
- Shiotsu, Y., Neckers, L.M., Wortman, I., An, W.G., Schulte, T.W., Soga, S., Murakata, C., Tamaoki, T., Akinaga, S., 2000. Novel oxime derivatives of radicicol induce erythroid differentiation associated with preferential G1 phase accumulation against chronic myelogenous leukemia cells through destabilization of Bcr-Abl with Hsp90 complex. Blood 96, 2284–2291. https://doi.org/10.1182/blood.V96.6.2284
- Smith, D.F., 1993. Dynamics of heat shock protein 90-progesterone receptor binding and the disactivation loop model for steroid receptor complexes. Molecular Endocrinology 7, 1418–1429. https://doi.org/10.1210/mend.7.11.7906860
- Soga, S., Neckers, L.M., Schulte, T.W., Shiotsu, Y., Akasaka, K., Narumi, H., Agatsuma, T., Ikuina, Y., Murakata, C., Tamaoki, T., Akinaga, S., 1999. KF25706, a Novel Oxime Derivative of Radicicol, Exhibits in Vivo Antitumor Activity via Selective Depletion of Hsp90 Binding Signaling Molecules. Cancer Research 59, 2931–2938.
- Solís, E.J., Pandey, J.P., Zheng, X., Jin, D.X., Gupta, P.B., Airoldi, E.M., Pincus, D., Denic, V., 2016. Defining the Essential Function of Yeast Hsf1 Reveals a Compact Transcriptional Program for Maintaining Eukaryotic Proteostasis. Molecular Cell 63, 60–71. https://doi.org/10.1016/j.molcel.2016.05.014
- Soroka, J., Wandinger, S.K., Mäusbacher, N., Schreiber, T., Richter, K., Daub, H., Buchner, J., 2012. Conformational Switching of the Molecular Chaperone Hsp90 via Regulated Phosphorylation. Molecular Cell 45, 517–528. https://doi.org/10.1016/j.molcel.2011.12.031
- Stebbins, C.E., Russo, A.A., Schneider, C., Rosen, N., Hartl, F.U., Pavletich, N.P., 1997. Crystal Structure of an Hsp90–Geldanamycin Complex: Targeting of a Protein Chaperone by an Antitumor Agent. Cell 89, 239–250. https://doi.org/10.1016/S0092-8674(00)80203-2
- Sun, K., Wang, X., Fang, N., Xu, A., lin, Y., Zhao, X., Nazarali, A.J., Ji, S., 2020. SIRT2 suppresses expression of inflammatory factors via Hsp90-glucocorticoid receptor signalling. Journal of cellular and molecular medicine 24, 7439–7450. https://doi.org/10.1111/jcmm.15365
- Taipale, M., Jarosz, D.F., Lindquist, S., 2010. HSP90 at the hub of protein homeostasis: emerging mechanistic insights. Nat Rev Mol Cell Biol 11, 515–528. https://doi.org/10.1038/nrm2918

- Taipale, M., Krykbaeva, I., Koeva, M., Kayatekin, C., Westover, K.D., Karras, G.I., Lindquist, S., 2012. Quantitative Analysis of Hsp90-Client Interactions Reveals Principles of Substrate Recognition. Cell 150, 987–1001. https://doi.org/10.1016/j.cell.2012.06.047
- Tran, P.L., Kim, S.-A., Choi, H.S., Yoon, J.-H., Ahn, S.-G., 2010. Epigallocatechin-3-gallate suppresses the expression of HSP70 and HSP90 and exhibits anti-tumor activity in vitro and in vivo. BMC Cancer 10, 276. https://doi.org/10.1186/1471-2407-10-276
- Tsutsumi, S., Mollapour, M., Prodromou, C., Lee, C.-T., Panaretou, B., Yoshida, S., Mayer, M.P., Neckers, L.M., 2012. Charged linker sequence modulates eukaryotic heat shock protein 90 (Hsp90) chaperone activity. Proceedings of the National Academy of Sciences 109, 2937–2942. https://doi.org/10.1073/pnas.1114414109
- Voellmy, R., Boellmann, F., 2007. Chaperone Regulation of the Heat Shock Protein Response, in: Csermely, P., Vígh, L. (Eds.), Molecular Aspects of the Stress Response: Chaperones, Membranes and Networks, Advances in Experimental Medicine and Biology. Springer, New York, NY, pp. 89–99. https://doi.org/10.1007/978-0-387-39975-1_9
- Wandinger, S.K., Suhre, M.H., Wegele, H., Buchner, J., 2006. The phosphatase Ppt1 is a dedicated regulator of the molecular chaperone Hsp90. The EMBO Journal 25, 367–376. https://doi.org/10.1038/sj.emboj.7600930
- Wang, M., Shen, G., Blagg, B.S.J., 2006. Radanamycin, a macrocyclic chimera of radicicol and geldanamycin. Bioorganic & Medicinal Chemistry Letters, David W. Robertson Memorial Issue 16, 2459–2462. https://doi.org/10.1016/j.bmcl.2006.01.086
- Wayne, N., Bolon, D.N., 2007. Dimerization of Hsp90 Is Required for in Vivo Function. Journal of Biological Chemistry 282, 35386–35395. https://doi.org/10.1074/jbc.M703844200
- Witt, O., Deubzer, H.E., Milde, T., Oehme, I., 2009. HDAC family: What are the cancer relevant targets? Cancer Letters 277, 8–21. https://doi.org/10.1016/j.canlet.2008.08.016
- Wu, Z., Gholami, A.M., Kuster, B., 2012. Systematic Identification of the HSP90 Regulated Proteome. Molecular & Cellular Proteomics 11. https://doi.org/10.1074/mcp.M111.016675
- Xiong, Y., Guan, K.-L., 2012. Mechanistic insights into the regulation of metabolic enzymes by acetylation. Journal of Cell Biology 198, 155–164. https://doi.org/10.1083/jcb.201202056
- Yang, X.-J., Seto, E., 2008. Lysine Acetylation: Codified Crosstalk with Other Posttranslational Modifications. Molecular Cell 31, 449–461. https://doi.org/10.1016/j.molcel.2008.07.002
- Yang, Y., Rao, R., Jie Shen, Jie Shen, Jie Shen, Shen, J., Tang, Y., Fiskus, W., Fiskus, W., Nechtman, J., Atadja, P., Kapil Bhalla, Bhalla, K.M., Bhalla, K.N., 2008. Role of Acetylation and Extracellular Location of Heat Shock Protein 90α in Tumor Cell Invasion. Cancer Research 68, 4833–4842. https://doi.org/10.1158/0008-5472.can-08-0644
- Yanxin Liu, Ming Sun, Alexander G. Myasnikov, Daniel Elnatan, Nicolas Delaeter, Michael Nguyenquang, David A. Agard, 2020. Cryo-EM structures reveal a multistep mechanism of Hsp90 activation by co-chaperone Aha1. bioRxiv 2020.06.30.180695. https://doi.org/10.1101/2020.06.30.180695
- Yin, Z., Henry, E.C., Gasiewicz, T.A., 2009. (−)-Epigallocatechin-3-gallate Is a Novel Hsp90 Inhibitor. Biochemistry 48, 336–345. https://doi.org/10.1021/bi801637q
- Yu, X., Guo, Z.S., Marcu, M.G., Neckers, L., Nguyen, D.M., Chen, G.A., Schrump, D.S., 2002. Modulation of p53, ErbB1, ErbB2, and Raf-1 expression in lung cancer cells by depsipeptide FR901228. Journal of the National Cancer Institute 94, 504–513. https://doi.org/10.1093/jnci/94.7.504
- Yu, X.M., Shen, G., Neckers, L., Blake, H., Holzbeierlein, J., Cronk, B., Blagg, B.S.J., 2005. Hsp90 Inhibitors Identified from a Library of Novobiocin Analogues. J. Am. Chem. Soc. 127, 12778–12779. https://doi.org/10.1021/ja0535864
- Zhang, D., Li, J., Costa, M., Costa, M.H.M., Gao, J., Huang, C., 2010. JNK1 Mediates Degradation HIF-1α by a VHL-Independent Mechanism that Involves the Chaperones Hsp90/Hsp70. Cancer Research 70, 813–823. https://doi.org/10.1158/0008-5472.can-09-0448
- Zhang, H., Burrows, F., 2004. Targeting multiple signal transduction pathways through inhibition of Hsp90. J Mol Med 82, 488–499. https://doi.org/10.1007/s00109-004-0549-9

- Zhang, J., Xu, E., Chen, X., 2013. TAp73 Protein Stability Is Controlled by Histone Deacetylase 1 via Regulation of Hsp90 Chaperone Function. Journal of Biological Chemistry 288, 7727–7737. https://doi.org/10.1074/jbc.m112.429522
- Zhang, Y., Kwon, S., Yamaguchi, T., Cubizolles, F., Rousseaux, S., Kneissel, M., Cao, C., Li, N., Cheng, H.-L., Chua, K., Lombard, D., Mizeracki, A., Matthias, G., Alt, F.W., Khochbin, S., Matthias, P., 2008. Mice Lacking Histone Deacetylase 6 Have Hyperacetylated Tubulin but Are Viable and Develop Normally. Molecular and Cellular Biology 28, 1688–1701. https://doi.org/10.1128/MCB.01154-06
- Zhao, R., Davey, M., Hsu, Y.-C., Kaplanek, P., Tong, A., Parsons, A.B., Krogan, N., Cagney, G., Mai, D., Greenblatt, J., Boone, C., Emili, A., Houry, W.A., 2005. Navigating the Chaperone Network: An Integrative Map of Physical and Genetic Interactions Mediated by the Hsp90 Chaperone. Cell 120, 715–727. https://doi.org/10.1016/j.cell.2004.12.024
- Zhao, R., Houry, W.A., 2007. Molecular Interaction Network of the Hsp90 Chaperone System, in: Csermely, P., Vígh, L. (Eds.), Molecular Aspects of the Stress Response: Chaperones, Membranes and Networks, Advances in Experimental Medicine and Biology. Springer, New York, NY, pp. 27–36. https://doi.org/10.1007/978-0-387-39975-1_3
- Zhou, Q., Agoston, A.T., Atadja, P., Nelson, W.G., Davidson, N.E., 2008. Inhibition of Histone Deacetylases Promotes Ubiquitin-Dependent Proteasomal Degradation of DNA Methyltransferase 1 in Human Breast Cancer Cells. Molecular Cancer Research 6, 873–883. https://doi.org/10.1158/1541-7786.MCR-07-0330
- Zhu, K., Zhang, Y., Zhang, J., Zhou, F., Zhang, L., Wang, S., Zhu, Q., Liu, Q., Wang, X., Zhou, L., 2020. Acetylation of Hsp90 reverses dexamethasone-mediated inhibition of insulin secretion. Toxicology Letters 320, 19–27. https://doi.org/10.1016/j.toxlet.2019.11.022