Chapter 11
Impact of Co-chaperones and Posttranslational Modifications Toward Hsp90 Drug Sensitivity

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Abstract Posttranslational modifications (PTMs) regulate myriad cellular processes by modulating protein function and protein-protein interaction. Heat shock protein 90 (Hsp90) is an ATP-dependent molecular chaperone whose activity is responsible for the stabilization and maturation of more than 300 client proteins. Hsp90 is a substrate for numerous PTMs, which have diverse effects on Hsp90 function. Interestingly, many Hsp90 clients are enzymes that catalyze PTM, demonstrating one of the several modes of regulation of Hsp90 activity. Approximately 25 co-chaperone regulatory proteins of Hsp90 impact structural rearrangements, ATP hydrolysis, and client interaction, representing a second layer of influence on Hsp90 activity. A growing body of literature has also established that PTM of these co-chaperones fine-tune their activity toward Hsp90; however, many of the identified PTMs remain uncharacterized. Given the critical role of Hsp90 in supporting signaling in cancer, clinical evaluation of Hsp90 inhibitors is an area of great interest. Interestingly, differential PTM and co-chaperone interaction have been shown to impact Hsp90 binding to its inhibitors. Therefore, understanding these layers of Hsp90 regulation will provide a more complete understanding of the chaperone code, facilitating the development of new biomarkers and combination therapies.

Keywords Hsp90 · Chaperone · Co-chaperone · PTM · Phosphorylation · Chaperone code · Hsp90 inhibitors

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Introduction

Heat shock protein 90 (Hsp90) is a ubiquitous and essential molecular chaperone. Approximately 300 client proteins depend on the 90-kDa chaperone for proper folding, stability, and activation (Schopf et al. 2017). Hsp90 clients include protein kinases, transcription factors, oncoproteins, and tumor suppressors (https://www.picard.ch/downloads/Hsp90interactors.pdf). Through a highly dynamic process known as the chaperone cycle, Hsp90 ATP hydrolysis is coupled to large conformational changes and consequent client chaperoning (Panaretou et al. 1998; Obermann et al. 1998; Schopf et al. 2017). This chaperone cycle is tightly regulated by a class of regulators known as co-chaperones. These proteins bind to distinct conformations of Hsp90 and regulate its progression through the chaperone cycle, client loading and release, and posttranslational modification (PTM) (Cox and Johnson 2018; Sahasrabudhe et al. 2017; Zierer et al. 2016; Rohl et al. 2013; Hohrman et al. 2021).

Cancer cells often rely on the Hsp90 chaperone machinery to support dysregulated proliferation and metastasis, making Hsp90 an attractive therapeutic target (Neckers and Workman 2012; Barrott and Haystead 2013; Rodina et al. 2016; Wang et al. 2016). Due to the breadth of the Hsp90 clientome, Hsp90 inhibitors can simultaneously disrupt numerous oncogenic pathways, making Hsp90 inhibitor development an area of intense focus. Despite promising preclinical results, Hsp90 inhibitors have yet to be approved for the treatment of human cancers. Notably, Hsp90 PTMs and co-chaperone dynamics modulate cellular sensitivity to Hsp90 inhibitors, suggesting that a comprehensive understanding of Hsp90 regulation is paramount to the clinical success of Hsp90 inhibitors (Walton-Diaz et al. 2013; Woodford et al. 2016a; Backe et al. 2020; Cloutier and Coulombe 2013).

The Chaperone Cycle

The Hsp90 chaperone cycle comprises an ordered series of conformational changes coupled to its ATPase activity (Graf et al. 2009; Mickler et al. 2009). Hsp90 consists of three structural domains (Ali et al. 2006; Verba et al. 2016). The amino-terminal domain (NTD) contains the nucleotide-binding pocket (Prodromou et al. 1997a, b), which is connected to the middle domain (MD) by a highly charged, flexible linker region (Tsutsumi et al. 2012; Hainzl et al. 2009; Jahn et al. 2014). Many Hsp90-interacting proteins bind to the Hsp90-MD, which also contains the catalytic loop that is required for ATP hydrolysis (Meyer et al. 2003; Biebl and Buchner 2019; Schopf et al. 2017). The carboxyl-terminal domain (CTD) contains the highly conserved, extreme C-terminal MEEVD sequence which is the docking site for tetratricopeptide repeat (TPR) domain-containing co-chaperones (Young et al. 1998; Carrello et al. 1999; Russell et al. 1999; Ramsey et al. 2000). Notably, the functional unit of Hsp90 is a dimer, and the CTD is the site of constitutive dimerization of the Hsp90 protomers (Harris et al. 2004; Prodromou and Pearl 2003; Wayne and Bolon 2007).

Apo-Hsp90 that is dimerized at the CTD adopts an open V-shaped conformation. Upon ATP binding to the nucleotide pocket of the NTD, Hsp90 undergoes large conformational rearrangements resulting in transient dimerization of the Hsp90 NTDs to form the "closed" conformation. Subsequent ATP hydrolysis causes a return to the open V shape, resetting the chaperone for the next cycle (Zierer et al. 2016; Mickler et al. 2009; Prodromou and Pearl 2003; Hessling et al. 2009; Neckers et al. 2009; Shiau et al. 2006). Interestingly, ATP binding in the NTD leads to conformational changes throughout the length of the entire protein (Cunningham et al. 2008). This ability has also been attributed to several PTMs of Hsp90, demonstrating the complex interdomain connectivity and communication throughout the Hsp90 protein (Rehn et al. 2020; Stetz et al. 2018; Xu et al. 2019).

To meet the differing needs of ~300 client proteins, the Hsp90 chaperone cycle is regulated by co-chaperones and PTMs. These regulators provide directionality to the cycle by altering conformational dwell time, coordinating assembly of chaperone-client complexes, and modulating Hsp90 affinity for ATP and ATP hydrolysis rate. The general progression of the cycle is well established and is outlined below and in Fig. 11.1.

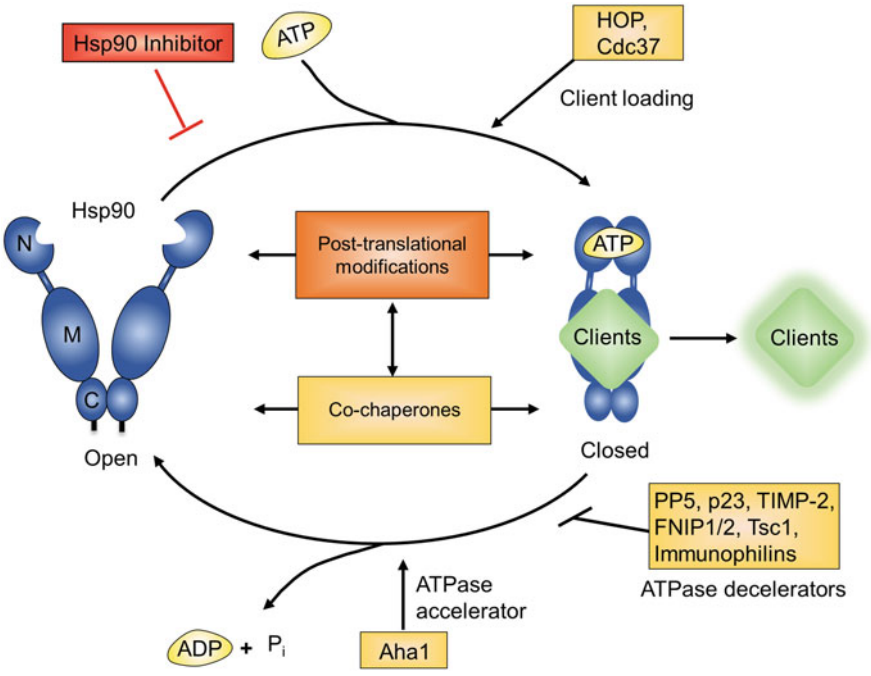

Fig. 11.1 The Hsp90 chaperone cycle. Open Hsp90 is dimerized only through contacts in the CTD. ATP binding and an ordered series of conformational changes allow Hsp90 to adopt a closed conformation, which is N-terminally dimerized. ATP hydrolysis leads Hsp90 to return to the open conformation and is ready to begin another chaperone cycle. This cycle allows for the activation of client proteins. Throughout the cycle, co-chaperones bind to Hsp90 and regulate its function. PTM of Hsp90 and PTM of co-chaperones provide further regulation of the chaperone cycle

An early event in the Hsp90 chaperone cycle is binding of the co-chaperone Hsp70-Hsp90-organizing protein (HOP) to the open conformation of Hsp90 via its TPR2A domain. HOP slows Hsp90 ATPase activity and helps transfer client proteins from the "early" chaperone Hsp70 to Hsp90 (Wegele et al. 2006; Li et al. 2011; Prodromou et al. 1999). HOP is then displaced by the co-chaperone activator of Hsp90 ATPase (Aha1) (Harst et al. 2005), which aids Hsp90 N-domain dimerization, thereby increasing the rate of ATP hydrolysis (Panaretou et al. 2002; Meyer et al. 2004; Retzlaff et al. 2010; Mercier et al. 2019). The co-chaperone cell division cycle 37 (Cdc37) assists in loading of protein kinase clients to Hsp90 (Keramisanou et al. 2016; Siligardi et al. 2002). Cdc37 co-chaperone function requires systematic phosphorylation and subsequent dephosphorylation by another Hsp90 co-chaperone protein phosphatase 5 (PP5) (Miyata and Nishida 2005; Vaughan et al. 2008; Bandhakavi et al. 2003). Late in the client maturation process, the co-chaperone prostaglandin E synthase 3 (p23) slows ATP hydrolysis by binding to and stabilizing Hsp90 in the closed conformation (Ali et al. 2006; Richter et al. 2004). Many of the co-chaperone/Hsp90 complex dynamics are heavily regulated by PTM. Regulation of co-chaperones' functions by PTM and the subsequent impact on Hsp90 function and downstream cellular processes will be discussed in detail below (Table 11.1).

Canonical Co-chaperones

Hsp70/Hsp90-Organizing Protein (HOP)

The co-chaperone HOP (stress-inducible protein 1, STI1, STIP1) catalyzes client protein transfer from ADP-bound Hsp70 to ADP-bound Hsp90 by binding each chaperone with a unique TPR domain (Chen and Smith 1998; Johnson et al. 1998; Odunuga et al. 2004; Brinker et al. 2002; Carrigan et al. 2004). Interestingly, HOP binds to Hsp90 with greater affinity than other TPR-containing co-chaperones (Hildenbrand et al. 2011). A high-affinity Hsp90/HOP complex may explain the observation that Hsp90 binding to HOP is mutually exclusive from some other co-chaperones including Aha1 and CHIP (Harst et al. 2005; Kundrat and Regan 2010a; Xu et al. 2019). Recent work has also demonstrated a critical role for HOP in assembly of the 26S proteasome, via its activity as an Hsp90 co-chaperone (Bhattacharya et al. 2020).

PTMs have been previously shown to regulate the formation of the Hsp70-HOP-Hsp90 chaperone heterocomplex, a critical initiation event in the Hsp90 chaperone cycle. HOP was shown to be phosphorylated by both Cdc2 (Cdk1) and casein kinase 2 (CK2), affecting HOP chaperone interactions and subcellular localization. Phosphorylation of HOP-T198 by Cdk1 resulted in cytoplasmic localization, whereas CK2 phosphorylation of HOP at S189 induced translocation to the nucleus (Fig. 11.2a) (Longshaw et al. 2004; Daniel et al. 2008). Interestingly, CK2 phosphorylation of the Hsp90 C-terminus (T725 and S726) enhanced binding to HOP and prevented the binding of CHIP (Muller et al. 2012), while the phosphomimetic

Table 11.1 Co-chaperone posttranslational modifications. Identified modification sites of co-chaperones are listed. When known, the modifying enzyme and impact on Hsp90 binding are shown. Increased binding to Hsp90 are identified by ↑, decreased ↓, and no change nc; no entry is provided in the table if not determined or examined

Co-chaperone	Modification	Residue	Enzyme	Hsp90 binding	References
HOP	Phosphorylation	S16		nc	Rohl et al. (2015)
HOP	Phosphorylation	S189	CK2	nc	Rohl et al. (2015), Longshaw et al. (2004)
HOP	Phosphorylation	T198	Cdk1	↓	Longshaw et al. (2004), Daniel et al. (2008), Rohl et al. (2015)
HOP	Phosphorylation	Y354		nc	Rohl et al. (2015)
HOP	Phosphorylation	S481		nc	Rohl et al. (2015)
Cdc37	Phosphorylation	Y4	Yes	↓	Xu et al. (2012).
Cdc37	Phosphorylation	S13	CK2, PP5	↑, ↓	Shao et al. (2003b), Bandhakavi et al. (2003), Miyata and Nishida (2005, 2007, 2008), Verba et al. (2016), Vaughan et al. (2008), Oberoi et al. (2016)
Cdc37	Phosphorylation	S97	PKA	↑	Pan et al. (2018)
Cdc37	Phosphorylation	Y298	Yes	↓	Xu et al. (2012), Bachman et al. (2018)
Cdc37	Phosphorylation	S339	Ulk1	nc	Li et al. (2017)
PP5	Ubiquitination	K185	VHL		Dushukyan et al. (2017)
PP5	Ubiquitination	K199	VHL		Dushukyan et al. (2017)
PP5	Phosphorylation	T362	CK1δ	nc	Dushukyan et al. (2017)
Aha1	Phosphorylation	Y223	c-Abl	↑	Dunn et al. (2015), Woodford et al. (2017)
p23	Phosphorylation	S113	CK2	↑	Kobayashi et al. (2004), Nakanishi et al. (2007)
p23	Phosphorylation	S118	CK2	↑	Kobayashi et al. (2004)
FKBP51	Acetylation	K28			Yu et al. (2017)
FKBP51	Acetylation	K155			Yu et al. (2017)
FKBP51	SUMOylation	K422	PIAS4	↑	Antunica-Noguerol et al. (2016)
FKBP52	Phosphorylation	T143	CK2	↓	Miyata et al. (1997), Cox et al. (2007)
SGTA	Phosphorylation	S305	Akt2		Moritz et al. (2010)
Sgt1	Phosphorylation	S249	CK2	↑	Prus et al. (2011)
Sgt1	Phosphorylation	S299	CK2	↑	Prus et al. (2011)
Sgt1	Phosphorylation	S331	Plk1	nc	Bansal et al. (2009), Liu et al. (2012a, b)
CHIP	Phosphorylation	S20	Cdk5, protein kinase G		Kim et al. (2016), Kim et al. (2018), Ranek et al. (2020)

(continued)

Table 11.1 (continued)

Co-chaperone	Modification	Residue	Enzyme	Hsp90 binding	References
FNIP1	Oxidation	C580			Manford et al. (2020)
FNIP1	Oxidation	C582			Manford et al. (2020)
FNIP1	Oxidation	C585			Manford et al. (2020)
FNIP1	Phosphorylation, O-GlcNAcylation	S938	CK2, PP5, OGT	↑, ↓	Sager et al. (2019)
FNIP1	Phosphorylation	S939	CK2, PP5	↑, ↓	Sager et al. (2019)
FNIP1	Phosphorylation	S941	CK2, PP5	↑, ↓	Sager et al. (2019)
FNIP1	Phosphorylation	S946	CK2, PP5	↑, ↓	Sager et al. (2019)
FNIP1	Phosphorylation	S948	CK2, PP5	↑, ↓	Sager et al. (2019)
FNIP1	Ubiquitination	K1119			Sager et al. (2019)
Tsc1	Phosphorylation	T310	Cdk1		Astrinidis et al. (2003, 2006)
Tsc1	Phosphorylation	S332	Cdk1		Astrinidis et al. (2003)
Tsc1	Phosphorylation	T417			Inoue et al. (2010)
Tsc1	Phosphorylation	S467	Plk1		Li et al. (2018b)
Tsc1	Phosphorylation	S487	IKKβ		Lee et al. (2007)
Tsc1	Phosphorylation	S511	IKKβ		Lee et al. (2007)
Tsc1	Phosphorylation	T578	Plk1		Li et al. (2018b)
Tsc1	Phosphorylation	T1047	Cdk1		Astrinidis et al. (2003)
TIMP-2	Phosphorylation	Y90	c-Src		Sanchez-Pozo et al. (2018)

HOP-T198E decreased HOP binding to Hsp90 (Daniel et al. 2008). HOP phosphorylation at residues S16, S189, Y354, and S481 was shown to regulate its interaction with Hsp70, but not Hsp90 (Daniel et al. 2008; Rohl et al. 2015). Interestingly, phosphorylation of HOP-S16, Y354, and S481 was also shown to reduce Hsp90-dependent GR activity (Rohl et al. 2015); however, the kinases regulating these phosphorylation events have yet to be reported (Fig. 11.2b). Hsp90α-T36 is also subject to phosphorylation by CK2, though its impact on HOP interaction has not been explored. Taken together, these data position CK2 as a master regulator of Hsp70-HOP-Hsp90 interactions (Lees-Miller and Anderson 1989; Mollapour et al. 2011a, b).

Cell Division Cycle 37 (Cdc37)

The kinase-specific co-chaperone Cdc37 facilitates recruitment and loading of kinase clients to the Hsp90 chaperone machinery (Siligardi et al. 2002; Roe et al.

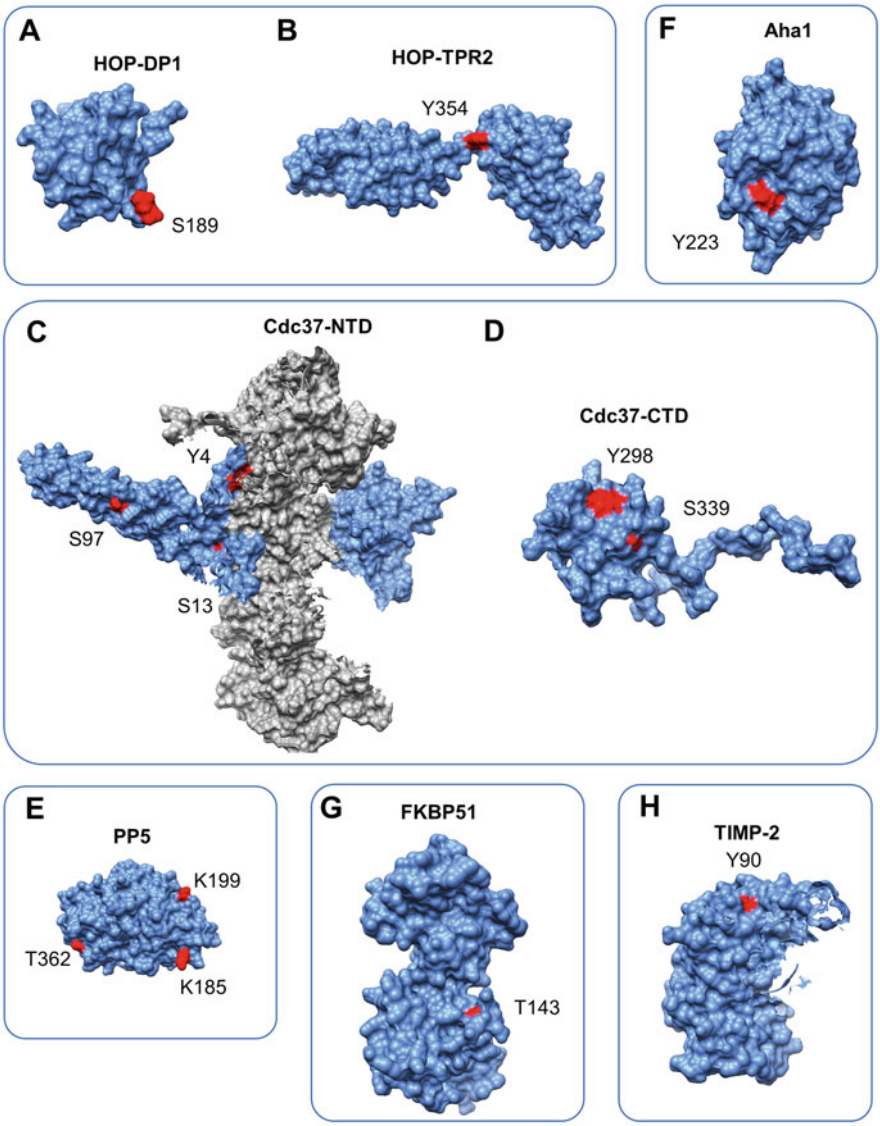

Fig. 11.2 PTM sites of Hsp90 co-chaperones. Surface structure of co-chaperones with confirmed sites of PTM highlighted in red. (**a**) HOP rich in aspartate and proline domain-1 (DP1), PDB ID, 2llv; (**b**) HOP-tetratricopeptide repeat domain-2 (TPR2), PBB ID, 3uq3; (**c**) Aha1, PDB ID, 1x53; (**d**) Cdc37 amino-terminal domain (NTD), PDB ID, 5fwp; (**e**) Cdc37-carboxy terminal domain (CTD), PDB ID, 2n5x; (**f**) PP5, PDB ID, 5hpe; (**g**) FKBP51, PDB ID, 5njx; (**h**) TIMP-2, PDB ID, 4ilw

2004; Verba et al. 2016). Cdc37 binds to the open conformation of the Hsp90 dimer which impedes Hsp90 conformational rearrangement, allowing time for client loading to the Hsp90 chaperone complex (Eckl et al. 2013; Vaughan et al. 2006; Shao et al. 2003a). Phosphorylation of the Cdc37-S13 by CK2 is required for Cdc37 co-chaperone activity (Fig. 11.2c) (Shao et al. 2003b; Bandhakavi et al. 2003); specifically, S13 phosphorylation is required for client recruitment and Hsp90 binding (Miyata and Nishida 2005, 2007, 2008). Mechanistically, S13 phosphorylation causes Cdc37 to adopt a more compact conformation with enhanced secondary structure. This is necessary for Cdc37 recognition of the unfolded kinase clients prior to binding Hsp90. Additionally, phosphorylation of Cdc37-S13 forms a salt bridge with Hsp90-K406, contributing to Cdc37-Hsp90 interaction (Verba et al. 2016). Subsequent dephosphorylation of S13 by protein phosphatase 5 (PP5) is required for client release from the chaperone complex, resetting Hsp90 to begin another chaperone cycle (Vaughan et al. 2008; Oberoi et al. 2016).

Cdc37 is also phosphorylated by protein kinase A (PKA) and the non-receptor tyrosine kinase Yes (Xu et al. 2012). Yes phosphorylates Cdc37-Y4 and Cdc37-Y298. Phosphorylation of Cdc37-Y298, and to a lesser extent Y4, causes dissociation of Cdc37 from the Hsp90/client complex (Fig. 11.2c, d) (Xu et al. 2012). Prior to dissociation, Cdc37-Y298 phosphorylation induces partial unfolding of the Cdc37-CTD (Bachman et al. 2018). Partial unfolding of Cdc37-CTD unmasks the polypeptide region of Hsp90 containing Y197, which is subsequently phosphorylated by Yes causing Cdc37 to dissociate from the complex and allow for progression through the chaperone cycle. PKA-mediated phosphorylation of Cdc37-S97 was subsequently found to be important for Cdc37/Hsp90 interaction and chaperoning of the kinase AXL. Interestingly, nonsteroidal anti-inflammatory drugs (NSAIDs) inhibited Cdc37-S97 phosphorylation leading to incomplete folding and eventual degradation of AXL by the co-chaperone CHIP (Fig. 11.2c) (Pan et al. 2018).

Posttranslational modification of Hsp90 also alters Hsp90/Cdc37 complex formation and chaperone function. Protein kinase A (PKA) phosphorylates Hsp90-T90 which perturbs the interaction between Cdc37 and Hsp90, leading to degradation of the kinase clients Src, Akt, and PKCγ (Wang et al. 2012). Interestingly, the Hsp90 client PKCγ phosphorylates three residues of Hsp90 and causes profound impacts on the chaperone cycle (Lu et al. 2014). Mutation of one of these residues, Hsp90-T115, to the phosphomimetic T115E, decreased interaction between Hsp90 and Cdc37. Further, Hsp90-T115E, Hsp90-T425E, and Hsp90-T603E all showed decreased binding to ATP and decreased ATPase activity. The authors propose a model where Hsp90:Cdc37 binds to newly synthesized and inactive PKCγ and progresses through the chaperone cycle leading to activation of mature PKCγ. Active PKCγ then phosphorylates Hsp90 triggering the release of the client from the chaperone complex. Notably, Hsp90-T115 was also shown to be phosphorylated by the mitotic checkpoint kinase, Mps1, which negatively impacts Cdc37 binding (Woodford et al. 2016c), and previous work has shown Cdc37 interaction with Mps1 is important for spindle pole body duplication (Schutz et al. 1997). Although the consequence of Hsp90-pT115 on Cdc37 function remains unknown, these works demonstrate the

importance of phosphorylation of both Cdc37 and Hsp90 to ensure efficient client chaperoning.

The co-chaperone activity of Cdc37 is critical to the activation of oncogenic kinase clients such as PKCγ, c-Src, and c-Abl (Gould et al. 2008; Dey et al. 1996; Keramisanou et al. 2016). Interestingly, Cdc37 expression is increased in some tumors, suggesting a direct role for Cdc37 in tumorigenesis (Stepanova et al. 2000; Zhu et al. 2018; McDowell et al. 2009). In contrast, Hsp90-Cdc37 also plays a role in starvation-induced autophagy through stabilization and activation of Unc-51 like autophagy-activating kinase (Ulk1) (Joo et al. 2011). Ulk1 was shown to phosphorylate Atg13 in an Hsp90-Cdc37-dependent manner, which led to Atg13 activation and release from the Hsp90-Cdc37-Ulk1 complex (Joo et al. 2011). Interestingly, Li et al. showed that phosphorylation of Cdc37 by Ulk1 enhances sensitivity of several colon cancer cell lines to Hsp90 inhibitors. Presumably after its activation by Hsp90-Cdc37, Ulk1 phosphorylates Cdc37-S339 leading to Hsp90 kinase client degradation (Fig. 11.2d). Ulk1 knockout (KO) DLD1 and HCT116 cells treated with an Hsp90 inhibitor (17-AAG or AUY922) were less sensitive to Hsp90 inhibition compared to Ulk1 WT cells (Li et al. 2017). Their finding supports a model by which Ulk1 phosphorylation of Cdc37 plays a role in proteasomal degradation of Hsp90 clients upon Hsp90 inhibition.

Protein Phosphatase 5 (PP5)

Protein phosphatase 5 (PP5) is a unique serine/threonine phosphatase and a member of the phosphoprotein phosphatase (PPP) family, which has recently been reviewed in great detail (Sager et al. 2020). PP5 regulates the chaperoning of numerous kinases and steroid hormone receptors and has been shown to work with the co-chaperones FNIP1 (Sager et al. 2019), FKBP51/52 (Gallo et al. 2007; Banerjee et al. 2008; Hamilton et al. 2018) and most notably Cdc37 (Vaughan et al. 2008; Oberoi et al. 2016; Prodromou 2017). Interaction of the amino-terminal TPR domain of PP5 with its C-terminal αJ helix locks PP5 in an auto-inhibited state, and association of the TPR domain with the Hsp90-MEEVD releases the auto-inhibition, enabling PP5 activation (Russell et al. 1999; Connarn et al. 2014; Haslbeck et al. 2015).

PP5-mediated dephosphorylation of Cdc37-S13 is a well-established mechanism to regulate the chaperoning of Hsp90 kinase clients (Vaughan et al. 2008). Mechanistically, it was shown that PP5 only dephosphorylates Cdc37 when both co-chaperones are bound to the same Hsp90 dimer (Vaughan et al. 2008). Interestingly, recent work has shown that an activating phosphorylation of PP5 allows for Hsp90-independent Cdc37 dephosphorylation (Dushukyan et al. 2017). Casein kinase 1δ (CK1δ) phosphorylates PP5-T362 in the absence of Hsp90, promoting its phosphatase activity and subsequent dephosphorylation of its substrates Cdc37 and GR (Oberoi et al. 2016; Dushukyan et al. 2017). Modulating PP5 activity also impacts Hsp90-binding affinity to its inhibitor ganetespib, likely via Cdc37

dephosphorylation, as expression of nonphosphorylatable Cdc37-S13A hypersensitized cells to the Hsp90 inhibitor geldanamycin (GA) (Oberoi et al. 2016; Vaughan et al. 2008). These works demonstrate a critical role for understanding the context-dependent modification of Hsp90 co-chaperones.

PP5 exhibits elevated expression in tumor cells and has an important role in tumorigenesis (Golden et al. 2008; Wang et al. 2015, 2018; Sager et al. 2020). Specifically, PP5 is overexpressed and hyperphosphorylated on T362 in clear cell renal cell carcinoma cells lacking the tumor suppressor ubiquitin ligase VHL (Dushukyan et al. 2017). Mechanistically, VHL ubiquitinates PP5-K185 and K199, leading to PP5 degradation and inactivation (Fig. 11.2e) (Dushukyan et al. 2017). Silencing PP5 using siRNA induces apoptosis, suggesting a pro-survival role for PP5 in cancer (Dushukyan et al. 2017). Accordingly, downregulation of PP5 has been also shown to facilitate apoptosis via G0/G1 phase cell cycle arrest (Wang et al. 2015; Zhi et al. 2015). PP5 depletion has the added effect of inducing Hsp90 hyperphosphorylation, which negatively regulates its chaperone function and the processing of client proteins (Wandinger et al. 2006). Taken together, these studies suggest that combined inhibition of Hsp90 and PP5 may show selectivity toward cancers addicted to Hsp90 client kinases.

Activator of Hsp90 ATPase 1 (Aha1)

Aha1 is a highly conserved 39-kDa protein that accelerates Hsp90 ATP hydrolysis by facilitating Hsp90 dimer N-terminal domain closure (Richter et al. 2008; Li et al. 2013; Retzlaff et al. 2010). Two-step binding of one Aha1 molecule to sites on both protomers of the Hsp90 dimer is sufficient to maximally stimulate Hsp90 ATPase activity (Retzlaff et al. 2010; Panaretou et al. 2002). Aha1 binding to Hsp90 impacts Hsp90-NTD and Hsp90-MD conformation, priming the catalytic Hsp90-R380 (yeast) for interaction with ATP, ultimately impacting client dwell time (Koulov et al. 2010; Meyer et al. 2003). Aha1 is the only co-chaperone known to accelerate Hsp90 ATPase activity in higher eukaryotes. Recently, Ids2 was identified as a co-chaperone for the yeast Hsp90 ortholog, Hsc82, which stimulates Hsc82 ATPase activity. Coordinated phosphorylation and dephosphorylation of Ids2-S148 by PKA and PP2C, respectively, was found to be imperative for Ids2 co-chaperone function (Chen et al. 2018). Notably, Ids2 does not have a known ortholog in higher eukaryotes.

Aha1 is recruited to Hsp90 by SUMOylation of Hsp90α-K191 on a single Hsp90 protomer (Mollapour et al. 2014). In addition to SUMOylation, phosphorylation, methylation, and acetylation of residues in Hsp90-NTD and Hsp90-MD have been shown to modulate the impact of Aha1 on Hsp90 activity (Mollapour et al. 2010, 2011b; Scroggins et al. 2007; Soroka et al. 2012; Xu et al. 2012, 2019; Rehn et al. 2020). These Hsp90 modifications have recently been reviewed in greater detail (Backe et al. 2020).

The tyrosine kinase c-Abl phosphorylates Aha1-Y223 promoting Aha1 association with Hsp90 and precedes Aha1 ubiquitination (Fig. 11.2f) (Dunn et al. 2015). The nonphosphorylatable Aha1-Y223F mutant did not stimulate the ATPase activity of Hsp90 nor form complexes with Hsp90, co-chaperones, or clients (Dunn et al. 2015). Notably, c-Abl-mediated phosphorylation of Aha1 displaces the co-chaperone Tsc1 from Hsp90 (Woodford et al. 2017). Mass spectrometric analysis revealed that phosphomimetic Aha1-Y223E preferentially interacted with proteins involved in metabolism, ribosomal components, and transcription and translation. Further, pharmacologic or genetic c-Abl ablation, and subsequent hypo-phosphorylation of Aha1-Y223, sensitized prostate cancer and renal cancer cells to Hsp90 inhibition (Dunn et al. 2015).

Aha1 expression is elevated in some cancers (Holmes et al. 2008). Moreover, knockdown of Aha1 increased cellular sensitivity to 17-AAG suggesting Aha1 levels could play a role in cellular response to Hsp90 inhibitors. Abrogating the function of the Aha1-Hsp90 complex might be beneficial in the context of the depletion of client proteins and kinase clients that are involved in tumor cell proliferation and survival. Several studies have examined the relationship between Aha1 expression and function and the efficacy of Hsp90 inhibitors. Aha1 overexpression in *L. donovani* increased radicicol (RD)-mediated inhibition of Hsp90, but not by geldanamycin (GA) (Bartsch et al. 2017). Conversely, Aha1 knockdown was shown to sensitize human colon cancer cells to the Hsp90 inhibitor 17-AAG (Holmes et al. 2008). The c-Abl inhibitor GNF-5, treatment of which led to hypo-phosphorylation of Aha1-Y223, hypersensitized tumors to Hsp90 inhibitors and caused increased apoptosis in prostate cancer and renal cancer cells (Dunn et al. 2015). Interestingly, the flavonoid TL-2-8 promotes breast cancer cell death, immature mitophagy, and downregulation of Hsp90 client proteins by disrupting the Aha1-Hsp90 complex (Liu et al. 2017). Aha1 has also been implicated in the increased production of pathogenic tau aggregates in Alzheimer's disease (AD). The Aha1 inhibitor KU-177 reduced the in vitro accumulation of toxic tau oligomers that correlated with (AD) progression (Shelton et al. 2017). These studies establish the importance of understanding the function and regulation of Aha1, as it shows promise as a therapeutic target in cancer and neurodegenerative disease.

Prostaglandin E Synthase 3 (p23)

There is a well-established role for the co-chaperone p23 in the maturation of Hsp90-dependent steroid hormone receptors (SHRs) such as glucocorticoid receptor (GR), estrogen receptor (ER), and progesterone receptor (PR) (Johnson and Toft 1995; McLaughlin et al. 2006; Graf et al. 2014; Xiao and Liu 2020). Upon ATP binding and Hsp90 N-terminal dimerization, p23 binds and stabilizes the closed, ATP-bound chaperone complex, providing the necessary dwell time for SHR maturation (Richter et al. 2004; Ali et al. 2006; McLaughlin et al. 2006; Martinez-Yamout et al. 2006; Woo et al. 2009; Blacklock and Verkhivker 2013; Cano et al. 2015; Borges et al.

2016; Gano and Simon 2010). Other co-chaperones including Aha1 and the p23-like Aarsd1L compete with p23 for binding to Hsp90 (Harst et al. 2005; Martinez-Yamout et al. 2006; Echeverria et al. 2016), while Hsp70/Hsp90-organizing protein (HOP) prevents the conversion of the ADP-Hsp90 to a state that can bind p23 (Johnson et al. 1998). Notably, recent work by Buchner's lab has provided evidence for Hsp90-independent chaperone functions of p23 (Biebl et al. 2021). The authors showed that a helical region in the unstructured tail of p23 is involved in direct interactions with the GR ligand-binding domain (LBD), which may stabilize the GR-LBD even in the absence of Hsp90. This is consistent with previous studies related to Hsp90-independent chaperone functions of p23 (Echtenkamp et al. 2011, 2016).

While posttranslational modification of Hsp90 regulates co-chaperone interaction (Backe et al. 2020), modification of the co-chaperones themselves provides an additional layer of regulation of the Hsp90 chaperone machinery. CK2-mediated phosphorylation of p23-S113 and p23-S118 is essential for p23 activation and association with Hsp90 (Kobayashi et al. 2004; Nakanishi et al. 2007). Interestingly, CK2 also phosphorylates the co-chaperones HOP (Lassle et al. 1997; Longshaw et al. 2000), Sgt1 (Bansal et al. 2009), Cdc37 (Shao et al. 2003b; Bandhakavi et al. 2003), FKBP52 (Miyata et al. 1997), and FNIP1 (Sager et al. 2019), as well as Hsp90 itself (Mollapour et al. 2011b, Lees-Miller and Anderson 1989), again highlighting the importance of CK2 activity to Hsp90 regulation (Miyata 2009).

Upregulation of p23 contributes to the progression of several cancers including prostate, breast, and lung as well as acute lymphoblastic leukemia (Mollerup et al. 2003; Oxelmark et al. 2006; Elmore et al. 2008; Reebye et al. 2012; Liu et al. 2012a; Cano et al. 2015). As p23 interaction with Hsp90 is nucleotide-dependent, ATP-competitive inhibitors such as geldanamycin (GA), macbecin, and radicicol (RD) could interfere with complex formation (Johnson and Toft 1995; Rehn and Buchner 2015). Concordantly, depletion of p23 hypersensitizes cells to Hsp90 inhibitors GA and RD (Forafonov et al. 2008; Bartsch et al. 2017). Interestingly, cancer cells treated with the C-terminal Hsp90 inhibitor gedunin induced caspase-dependent cleavage of p23 and cell death by apoptosis (Patwardhan et al. 2013).

Previous work has shown that treatment with the Hsp90 inhibitor 17-AAG shifts the binding of HDAC6 from Hsp90 to Hsp70, suppressing HDAC6 activity and promoting Hsp90 hyperacetylation (Kovacs et al. 2005). Hyperacetylation of Hsp90 decreases its affinity for ATP and subsequent p23 binding, resulting in the depletion of several Hsp90 client proteins (Kovacs et al. 2005, Scroggins et al. 2007, Rao et al. 2008, Yang et al. 2008). Taken together, targeting the Hsp90-p23 interaction can influence chaperone complex formation and drug sensitivity.

Immunophilins

Immunophilins are a class of peptidyl-prolyl cis/trans isomerases (PPIase) that include Cyp40, FKBP51, and FKBP52 and bind to Hsp90 through their TPR domain

(Pirkl et al. 2001). These co-chaperones can be found in Hsp90-steroid hormone receptor (SHR) maturation complexes along with the co-chaperone p23 (Faou and Tropschug 2003; Ratajczak et al. 2009, Zgajnar et al. 2019). Interestingly, immunophilins differentially regulate Hsp90 ATPase activity through the formation of distinct immunophilin-Hsp90-SHR complexes that lead to differential receptor function (FKBP51-GR, FKBP52-PR, Cyp40-ER) (Carrello et al. 1999; Davies and Sanchez 2005; Ratajczak et al. 2009).

Posttranslational regulation of immunophilin co-chaperones has recently been reviewed in detail (Daneri-Becerra et al. 2019) and will be contextualized here. FKBP51 binds to the open conformation of Hsp90 and decreases the rate of Hsp90 ATP hydrolysis (Oroz et al. 2018). Previous work has shown that FKBP51 is present in Cdk4-Hsp90-FKBP51 heterocomplexes that inhibit cell proliferation by preventing cyclin D1 binding and inhibiting Cdk4-T172 phosphorylation (Ruiz-Estevez et al. 2018). Interestingly, acetylation of FKBP51 at K28 and K155 promotes activity of the Hsp90 client Akt (Yu et al. 2017), suggesting a context-dependent role for FKBP51 in the regulation of Hsp90 client kinases. Although FKBP51 also appears subject to serine phosphorylation by PINK-1 (Boonying et al. 2019) and PKA (Toneatto et al. 2013), the effects of modification at individual phosphorylation sites have yet to be confirmed.

SUMOylation of FKBP51-K422 by the E3 SUMO ligase PIAS4 is essential for Hsp90 interaction and regulation of GR signaling (Antunica-Noguerol et al. 2016). Impairment of FKBP51 SUMOylation abolishes its interaction with both Hsp90 and GR, leading to recruitment of FKBP52 and nuclear translocation of GR (Antunica-Noguerol et al. 2016). Interestingly, SUMOylation of Hsp90 has been shown to recruit Aha1, thereby inducing closure of the Hsp90 dimer (Mollapour et al. 2014) and suggesting opposing roles for SUMOylation in the FKBP-mediated regulation of Hsp90-dependent GR transcriptional activity.

CK2-mediated phosphorylation of FKBP52-T143 attenuates its interaction with Hsp90 (Miyata et al. 1997), with a concomitantly impaired ability to activate GR (Fig. 11.2g) (Cox et al. 2007). Interestingly, phosphorylation of Hsp90 by CK2 has no impact on its interaction with FKBP52 (Miyata et al. 1997). Other works have shown that Hsp90-K292 and Hsp90-K294 acetylation reduced its interaction with FKBP52 (Scroggins et al. 2007; Prodromou 2016), highlighting the importance of PTM reversibility in the regulation of Hsp90-co-chaperone interactions.

SGTA

Small glutamine-rich TPR-containing protein alpha (SGTA) is an Hsp90 co-chaperone that is critical to cellular signaling in hormone-regulated tissues (Philp et al. 2013). Previous work has demonstrated that SGTA knockdown affects androgen receptor (AR) maturation and prostate cancer proliferation (Buchanan et al. 2007; Trotta et al. 2012, 2013; Paul et al. 2014). SGTA is also known to support the growth of non-small cell lung cancer though the stabilization of the

Hsp90 client PDGFRα (Moritz et al. 2010; Smyth et al. 2012). Phosphorylation of SGTA-S305, potentially by Akt2, augments this effect (Moritz et al. 2010). The impact of this modification on SGTA co-chaperone activity and Hsp90-binding dynamics remains under investigation.

Sgt1

Another TPR-containing Hsp90 co-chaperone, the unrelated suppressor of G_2 allele of SKP1 (Sgt1), is known to be regulated by phosphorylation (Gangula and Maddika 2017). Sgt1 dimerization is integral to kinetochore assembly, and this dimerization is antagonized by CK2 phosphorylation of Sgt1-S361 in *S. cerevisiae* (Bansal et al. 2004, 2009). The lack of Sgt1 dimerization precludes a functional Sgt1/Hsp90 complex which is necessary for kinetochore function and proper chromosome segregation. Interestingly, subsequent work has shown that nuclear translocation of Sgt1 is dependent on its phosphorylation at the same site, potentially by CK2, suggesting a complex regulatory role for CK2 phosphorylation of Sgt1 (Prus and Filipek 2011; Prus et al. 2011). Plk1 is a key mitotic regulatory kinase involved in kinetochore-microtubule attachment (Sumara et al. 2004). Sgt1-S331 (S361 in yeast) phosphorylation by Plk1 promotes kinetochore-microtubule attachment (Liu et al. 2012b), emphasizing a role for differential signaling input in the regulation of Hsp90 co-chaperone function.

C-Terminus of Hsc70-Interacting Protein (CHIP)

CHIP is a TPR-containing co-chaperone that acts as an E3 ubiquitin ligase, targeting unfolded proteins for proteasomal degradation (Edkins 2015). CHIP works in concert with Hsp70 and Hsp90 in the ubiquitination of client substrates such as CFTR, GR, ER, and p53 (Edkins 2015; Quintana-Gallardo et al. 2019). HOP and CHIP compete for binding to the Hsp70 and Hsp90 chaperones, providing a mechanism to control the balance between pro-folding and pro-degradation complexes (Stankiewicz et al. 2010; Muller et al. 2012; Edkins 2015).

Both CK2-mediated phosphorylation (Muller et al. 2012) and acetylation of Hsp90 (residues K69, K100, K294, K327, K478, K546, and K558) decrease Hsp90 binding to CHIP, suggesting these specific modifications represent pro-folding Hsp90 complexes (Scroggins et al. 2007; Yang et al. 2008). Indeed, it seems that CHIP participates in a negative feedback loop where CHIP ubiquitinates and targets HDAC6 for degradation, inducing Hsp90 hyperacetylation and attenuating CHIP-Hsp90 interaction (Rao et al. 2008; Cook et al. 2012). Interestingly, more than ten lysine residues on Hsp90 itself were identified to be targets of CHIP ubiquitination, resulting in proteasomal degradation of Hsp90 (Kundrat and Regan 2010b; Abu-Farha et al. 2011).

As a ubiquitin ligase, CHIP effector function is mediated by posttranslational modification. This suggests that posttranslational cross talk has an exaggerated impact on CHIP function and downstream targeting. In fact, CHIP-S20 phosphorylation by Cdk5 contributes to neuronal cell death via disruption of the interaction between CHIP and truncated apoptosis-inducing factor (tAIF) (Kim et al. 2016, 2018). A recent report shows that this residue can also be phosphorylated by protein kinase G, which enhances CHIP binding to Hsc70 and protein quality control following cardiac ischemic injury (Ranek et al. 2020).

New Co-chaperones

Folliculin-Interacting Proteins 1 and 2 (FNIP1/2)

FNIP1 and FNIP2 are large, multidomain proteins which have recently been identified as Hsp90 co-chaperones (Woodford et al. 2016b). Prior to the discovery that FNIP1/2 are co-chaperones, their functions were thought to be exclusive to mTOR regulation through stabilization of the tumor suppressor folliculin (FLCN) (Baba et al. 2006; Hasumi et al. 2008). Recently, a partial structure of FNIP2 was solved in complex with FLCN, Rag GTPase, and Ragulator complex. The structure shows that FNIP2 has two distinct domains, a longin and a DENN domain, and has several highly unstructured regions (Shen et al. 2019; Lawrence et al. 2019). FNIP1/2 play critical roles in cellular function and in disease, specifically cancer, through their association with FLCN (Hasumi et al. 2008, 2015; Baba et al. 2012). The carboxy-terminal end of FNIP1 interacts with Hsp90-MD and Hsp90-CTD, while FNIP2 interaction is confined to Hsp90-MD (Woodford et al. 2016b). The function of FNIP1/2 as Hsp90 co-chaperones provided an explanation for their protective role toward the tumor suppressor folliculin (FLCN) (Woodford et al. 2016b). FNIP1/2 also contribute to the chaperoning of additional Hsp90 kinase and non-kinase clients. The FNIPs are displaced from Hsp90 by Aha1 allowing the Hsp90/client complex to progress through the chaperone cycle (Woodford et al. 2016b).

FNIP1 co-chaperone activity is uniquely regulated by relay phosphorylation. CK2 phosphorylates FNIP1-S938 in the C-terminal domain leading to sequential phosphorylation of FNIP1-S939, FNIP1-S941, FNIP1-S946, and FNIP1-S948 (Sager et al. 2019). FNIP1 relay phosphorylation provides stepwise deceleration of Hsp90 ATPase activity and client activation. FNIP1 relay phosphorylation is reversed by the co-chaperone PP5. PP5 initially dephosphorylates FNIP1 on S948 residue which is essential for a complete dephosphorylation of all modified serine sites in a relay fashion. Dephosphorylation disrupts FNIP1 binding to Hsp90 and promotes O-GlcNAcylation of FNIP1-S938 (Sager et al. 2019). The addition of GlcNAc precedes ubiquitination of FNIP1-K1119 and subsequent proteasomal degradation (Sager et al. 2019).

FNIP1 is also ubiquitinated upon reductive stress. Under oxidative conditions, three cysteine residues in the middle, unstructured region of FNIP1 are oxidated

leading to FNIP1-mediated mitochondrial shutdown (Manford et al. 2020). Reductive stress reverses the oxidation of FNIP1 cysteine residues, allowing for the E3 ubiquitin ligase adaptor, FEM1B, to recognize FNIP1. FEM1B docks CUL2 onto FNIP1. CUL2, a component of an E3 ubiquitin ligase complex, polyubiquitinates FNIP1 leading to FNIP1 proteasomal degradation. FNIP1 degradation results in hyperactive mitochondrial function and increased ROS production. Fine-tuned oxidation of FNIP1 is therefore a key regulatory mechanism of mitochondrial function (Manford et al. 2020).

FNIP1 and FNIP2 are upregulated in a variety of cancer cell lines including breast, bladder, prostate, lung, colorectal, and renal cancer (Woodford et al. 2016b; Hasumi et al. 2008). FNIP1/2 also bind more to Hsp90 in these cancer cell lines compared to normal kidney (HEK293) cells. Further, FNIP1/2 protein levels were found to be higher in tumor tissue compared to normal from patient samples and upregulation of FNIP1/2 correlated with increased binding of Hsp90 to ganetespib (GB). Taken together, regulation of FNIP1 protein levels, via ubiquitination and degradation, in cancer is critical to sensitivity of Hsp90 to its inhibitors.

Tuberous Sclerosis Complex 1 (Tsc1)

Tsc1 was identified as an Hsp90 co-chaperone as a result of its known role in stabilization of its binding partner Tsc2 (Woodford et al. 2017; Benvenuto et al. 2000; Chong-Kopera et al. 2006). Notably, Tsc1 is also important for the stabilization and activation of many kinase and non-kinase clients. Mechanistically, the C-terminal portion of Tsc1 binds to the Hsp90-MD and decelerates Hsp90 ATPase activity. Additionally, Tsc1 serves as a scaffold to load Tsc2, and potentially other clients, onto the Hsp90 chaperone machinery. Interestingly, Tsc1 also decelerates Hsp70 ATPase activity; however, the impact of this on clients has not yet been determined (Natarajan et al. 2020). It is noteworthy that phosphorylation of Tsc1-T417 is required for interaction with Hsp70 (Inoue et al. 2010).

The Tsc1/Tsc2 complex has a well-established function inhibiting the mTOR pathway via Rheb inhibition. The observation that Tsc1 is phosphorylated during nocodazole-induced G2-M arrest led to the finding that Tsc1 is phosphorylated by cyclin-dependent kinase Cdk1 at T310, S332, and T1047. Mutation of these residues to nonphosphorylatable alanine causes increased suppression of p70S6K activity, suggesting that phosphorylation of these residues downregulates Tsc1 activity (Astrinidis et al. 2003). Interestingly, Tsc1-T310 required for Tsc1 binding to Plk1 (Astrinidis et al. 2006). Plk1 phosphorylates Tsc1-S467 and Tsc1-S578 leading to Tsc1 ubiquitination and proteasomal degradation. Cells expressing the phosphomimetic Tsc1-S467E/S578E show increased mTOR activity and hypersensitivity to mTOR inhibition by rapamycin (Li et al. 2018b). Notably, Plk1 is an Hsp90 client, and its stability is dependent on the co-chaperone Sgt1, whereas Plk1 expression is negatively correlated with Tsc1 expression (Simizu and Osada 2000; Martins et al. 2009; Astrinidis et al. 2006). Intriguingly, co-deletion of FNIP1 and

TSC1 promotes synergistic hyperactivation of mTOR and drives polycystic kidney disease (PKD) development (Centini et al. 2018). Together, the evidence shows complex regulation of mTOR activity and subsequent mitosis by posttranslational regulation of Tsc1.

Another mechanism of mTOR regulation through Tsc1 is the result of IKK-β-mediated phosphorylation of Tsc1-S487 and Tsc1-S511. IKKβ phosphorylation of Tsc1 suppresses Tsc1 activity, resulting in subsequent mTOR activation. Notably, the mechanism of Tsc1 suppression is similar to Plk1-mediated Tsc1 suppression. Mutation of Tsc1-S487 and Tsc1-S511 to phosphomimetic aspartic acid caused increased ubiquitination and a shorter half-life of Tsc1 (Lee et al. 2007). Tsc1-S511 was found to be hyperphosphorylated in breast cancer tissues with high IKKβ expression and promoted inflammation-mediated tumor angiogenesis. IKKβ phosphorylation of Tsc1-S511 also plays a role in insulin resistance (Lee et al. 2008). The diminished activity of Tsc1-pS511 results in hyperactivity of mTOR and downstream inhibitory phosphorylation of insulin receptor substrate 1 (IRS1) and decreased insulin response.

Although no studies have investigated the impact of posttranslational regulation of Tsc1 on its co-chaperone function, Tsc1 loss disrupts Hsp90 posttranslational regulation. Mutation and loss of function of Tsc1 leads to hypoacetylation of Hsp90-K407 and Hsp90-K419. Hypoacetylated Hsp90 has a higher ATPase activity and decreased affinity for Hsp90 inhibitors (Woodford et al. 2019). Hsp90 acetylation and drug binding can be restored by treating Tsc1 null cells with the HDAC inhibitor ACY-241, demonstrating a potential therapeutic utility in cancer.

TIMP-2

The tissue inhibitor of metalloproteinase-2 (TIMP-2) is a small extracellular protein responsible for regulating the activity of matrix metalloproteinases (MMPs) (Bourboulia and Stetler-Stevenson 2010; Brew and Nagase 2010, Olson et al. 1997). Together with its ability to impact angiogenesis, TIMP-2 is an established regulator of the tumor microenvironment (Kim et al. 2012; Remillard et al. 2014).

Recent work has demonstrated that TIMP-2 functions as a co-chaperone of extracellular Hsp90 (eHsp90) (Baker-Williams et al. 2019). Mechanistically, the NTD of TIMP-2 interacted with the MD of Hsp90, antagonizing eAha1 binding to eHsp90. The eHsp90-TIMP-2 chaperone complex was shown to mediate the inhibitory role of TIMP-2 toward its established extracellular target, the eHsp90 client MMP2 (Baker-Williams et al. 2019). In agreement, the presence of TIMP-2 sensitizes Hsp90 binding to its inhibitor, ganetespib (Baker-Williams et al. 2019), underscoring the importance of understanding TIMP-2-mediated regulation of eHsp90 chaperone function.

Previous work has also shown that extracellular phosphorylation of TIMP-2-Y90 by c-Src regulates its inhibitory activity toward MMP2 (Fig. 11.2h) (Sanchez-Pozo et al. 2018). This finding highlights the potential effect of co-chaperone

posttranslational modification on eHsp90 client activity; however, the impact of TIMP-2-Y90 phosphorylation on the direct regulation of eHsp90 chaperone activity remains unexplored.

Concluding Remarks

The Hsp90 chaperone machinery is a complex network of tightly regulated proteins which concertedly maintain a multitude of cellular pathways. Posttranslational modification of co-chaperones and Hsp90 itself provides regulation by controlling chaperone complex assembly, ATPase activity, and client activation to meet cellular requirements.

Hsp90 inhibitors have shown great promise for clinical utility. In fact, Taiho Pharmaceutical's Hsp90 inhibitor TAS-116 recently met its primary endpoint of prolonged progression-free survival in the phase III clinical trial CHAPTER-GIST-301 (Taiho 2021). However, reports of toxicity and induction of the heat shock response, as seen with other Hsp90 inhibitors, have led to the evolution of alternative approaches (Neckers and Workman 2012; Biamonte et al. 2010; Bagatell et al. 2000). Recent efforts toward inhibiting specific chaperone complexes have yielded encouraging results. In attempts to overcome the reported toxicity of Hsp90 inhibitors, Cdc37 and Hsp90/Cdc37 complex inhibitors have been developed (Wang et al. 2019; Zhang et al. 2008). The rationale for targeting Cdc37 was reviewed in detail and highlighted the importance of future investigation (Li et al. 2018a). Aha1-Hsp90 inhibitors have also been shown to selectively inhibit Aha1/Hsp90 complexes and hinder client activation (Stiegler et al. 2017). Another study showed that specific inhibition of Aha1/Hsp90 complexes was able to overcome the negative impact of Aha1 on maturation of mutant CFTR (Ihrig and Obermann 2017). Current developments toward chaperone complex inhibitors have been recently reviewed in detail (Gestwicki and Shao 2019; Serwetnyk and Blagg 2020). It is noteworthy that direct disruption of Hsp90 interaction with clients such as CDK4 by client-mimicking peptides has also been shown to be a promising strategy for selective Hsp90 kinase inhibition (Paladino et al. 2020). Treatment of clear cell renal cell carcinoma cell lines with CDK4 mimicking peptide caused dissociation of CDK4 from Hsp90 and apoptosis.

Several enzymes demonstrate the ability to modify a number of chaperone and co-chaperone proteins (Fig. 11.3). Hsp90-T90 phosphorylation by protein kinase A (PKA) has been shown to increase association with p23, PP5, and CHIP while decreasing interaction with HOP and Cdc37 (Mollapour et al. 2011b) and while also directly phosphorylating Cdc37 and FKBP51, suggesting bidirectional phosphorylation directs the formation of specific Hsp90 chaperone complexes (Wang et al. 2012). It is likely that other PTMs have a comparable effect on several co-chaperones simultaneously.

Similarly, it is common for a single enzyme to modify several co-chaperones with varied effects. The Hsp90 client Plk1 phosphorylates Sgt1, which had a positive

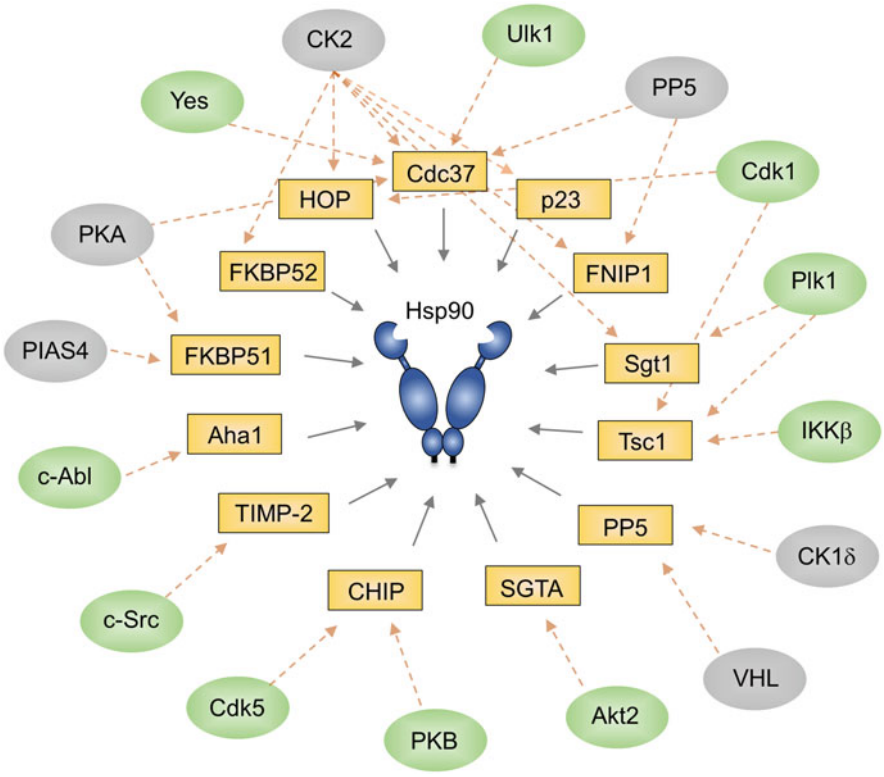

Fig. 11.3 A wide range of enzymes modify co-chaperones. Enzymes known to modify Hsp90 co-chaperones and alter their activity are represented in circles. Enzymes that are known Hsp90 clients are colored green. Co-chaperones discussed in this review are in yellow boxes

impact on Sgt1 function, whereas phosphorylation of Tsc1 by Plk1 led to its ubiquitination and degradation. CK2 phosphorylates several co-chaperones with greatly differing effects. Interestingly, phosphorylation of both HOP and Sgt1 by CK2 led to their translocation to the nucleus, while CK2 phosphorylation of Cdc37, p23, and FNIP1 enhances their binding to Hsp90 and is critical to their co-chaperone function. Notably, the opposite effect is observed when FKBP52 is phosphorylated by CK2. Phosphorylation is a frequent signal for co-chaperone/Hsp90 complex formation. It follows that PP5 may serve as common regulator of co-chaperone release from Hsp90, as PP5-mediated dephosphorylation of both Cdc37 and FNIP1 triggers their dissociation from Hsp90.

Most studies to date have focused on the impact of a singular PTM on a co-chaperone, leaving a gap in understanding of how the modification impacts the chaperone system as a whole. Stetz et al. thoroughly investigated a cross talk of structurally conserved Hsp90 PTMs and propose a model in which PTMs are allosterically coupled in an effector-sensor residue pair (Stetz et al. 2018). Further investigation into the commonality of this phenomenon or PTM cross-talk patterns

could yield insight into the impact of PTMs on overall changes in client chaperoning and subsequent cellular functions. A detailed characterization of the PTM makeup of specific Hsp90-co-chaperone-client complexes will provide the ability to identify unique druggable targets and a fundamental understanding of the chaperone code.

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