Published in final edited form as:

Expert Opin Ther Targets. 2014 October ; 18(10): 1219–1232. doi:10.1517/14728222.2014.943185.

Targeting Hsp90 and its co-chaperones to treat Alzheimer's disease

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Abstract

Introduction—Alzheimer's disease (AD), characterized by the accumulation of hyperphosphorylated tau and beta amyloid (A β), currently lacks effective treatment. Chaperone proteins, such as the heat shock protein (Hsp) 90, form macromolecular complexes with co-chaperones, which can regulate tau metabolism and A β processing. While small molecule inhibitors of Hsp90 have been successful at ameliorating tau and A β burden, their development into drugs to treat disease has been slow due to the off- and on-target effects of this approach as well as challenges with the pharmacology of current scaffolds. Thus, other approaches are being developed to improve these compounds and to target co-chaperones of Hsp90 in an effort to limit these liabilities.

Areas Covered—This article discusses the most current developments in Hsp90 inhibitors including advances in blood-brain barrier permeability, decreased toxicity, and homolog-specific small molecule inhibitors. In addition, we discuss current strategies targeting Hsp90 co-chaperones rather than Hsp90 itself to reduce off-target effects.

Expert Opinion—While Hsp90 inhibitors have proven their efficacy at reducing tau pathology, they have yet to meet with success in the clinic. The development of Hsp90/tau complex specific inhibitors and further development of Hsp90 co-chaperone specific drugs should yield more potent, less toxic therapeutics.

Keywords

Alzheimer's disease; beta amyloid; chaperone; co-chaperone; Hsp90; Hsp90 inhibitors; PPIase; tau; TPR

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Financial and competing interests disclosure

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

1. Introduction

Chaperone proteins represent critical cellular machinery that can fold nascent chain proteins, refold misfolded proteins, or target irreparable proteins for degradation. One of these chaperones, heat shock protein 90 (Hsp90), is ubiquitously and highly expressed and requires adenosine triphosphate (ATP) to help regulate the metabolism of client proteins in aspects of almost every process in the cell^{1, 2}. Hsp90 functions as a homodimer with three main domains including an N-terminal nucleotide binding domain, a middle domain which binds to clients and co-chaperones, and a C-terminal domain which permits ATP-dependent dimerization³. Hsp90 is a highly conserved protein which exists in multiple isoforms in the cell. The two major cytosolic isoforms are the inducible Hsp90 α and the constitutively expressed Hsp90 β . In addition to these cytosolic forms of Hsp90, there are two organelle-bound forms, glucose-regulated protein 94 (GRP94) and tumor necrosis factor receptor associated protein 1 (TRAP1), which reside in the endoplasmic reticulum (ER) and mitochondria, respectively⁴. These multiple forms of Hsp90 have evolved to help maintain homeostasis throughout the cell⁵. Apart from expression patterns, structural differences and sub-cellular localization⁵⁻⁷, the differences between the two cytosolic Hsp90 variants are just emerging. The functions of the two cytosolic isoforms are not completely redundant. Hsp90 β is ubiquitously and highly expressed throughout all cell types⁸, while Hsp90 α is expressed upon a stressor and is differentially expressed depending on tissue type⁹. In addition, the phenotypes between Hsp90 α and Hsp90 β specific knocked-outs are different. Hsp90 β knockout mice do not survive through embryonic development, which was attributed to a lack of induction of trophoblastic differentiation¹⁰. In contrast, mice lacking Hsp90 α are viable and display modest phenotypic differences compared to wild-type littermates, with the exception that males are unable to produce sperm⁹. Recently, Hsp90 α was shown to be significantly elevated in an inflammatory skin disease¹¹ and cells derived from patients with leukemia¹². Other work has shown that Hsp90 α can be used as a biomarker in certain cancer^{13, 14}, suggesting that Hsp90 α may be more intimately involved in disease processes compared to its constitutive counterpart, warranting further studies investigating the differential roles of Hsp90 α and Hsp90 β . But this is the current limit of our understanding about the differences between these two proteins. Therefore, for the purposes of this review, we will not distinguish between α and β when discussing Hsp90.

Hsp90 is able to successfully refold many non-native proteins in the cell, but fails to properly regulate the intrinsically disordered protein tau. Tau is a microtubule associated protein that stabilizes microtubules; however, tau becomes hyperphosphorylated and aggregates in Alzheimer's disease (AD) and other neurological diseases, called tauopathies^{15, 16}, leading to neurodegeneration. Hsp90 actually facilitates the aberrant aggregation and accumulation of tau, and under certain conditions, Hsp90 can even enhance tau toxicity¹⁷. In fact, inhibiting the ATPase domain of Hsp90 facilitates tau degradation, further indicating that Hsp90 is typically working to preserve tau in the brain^{18, 19}. We speculate that chaperones like Hsp90 preserve tau to enhance fast microtubule kinetics, but as tau begins to accumulate in excess or microtubule damage occurs, this ability of chaperones to preserve tau becomes problematic, resulting in tau aggregation and

accumulation. Based on this, efforts to improve Hsp90 inhibitor design for treating AD have been under intense investigation over the past several years to promote tau degradation²⁰.

Chaperones also play an important role in the processing and clearance of A β , another major pathological hallmark of AD. A β peptides are generated by cleavage of the amyloid precursor protein (APP) by beta and gamma secretases. This cleavage is in part regulated by presenilin 1 (PS1), a part of the gamma secretase complex with known mutations linked to AD²¹. A direct link between chaperone regulation and A β was demonstrated when a heat shock element, controlled by heat shock factor 1 (HSF1), was identified in the promoter of the APP gene²². Hsp90 inhibitors are protective against A β -induced toxicity²³, rescuing A β -induced cognitive impairment in mice²⁴, while leaving A β plaque load unchanged²⁵. However, more studies need to be done to parse out the direct effects of Hsp90 inhibition on A β , independent of the elevation of Hsp70 and Hsp27 which occurs with most Hsp90 inhibitors. Regardless, Hsp90 inhibitors ameliorate the pathophysiological effects of tau and A β in many models and further development of Hsp90 targeting drugs will be beneficial in developing treatments for AD, as illustrated in Figure 1.

A third pathology that is common in AD is chronic inflammation²⁶. Hsp90 and other chaperones play an intimate role in regulating many aspects of the inflammatory response, in both pro- and anti-inflammatory pathways²⁷. Hsps are often elevated in areas where inflammation is high, and they are responsible, in part, for controlling expression and signaling of cytokines, along with functioning as antigen presenting molecules^{28,29}. Hsp90, in particular, is involved with the binding of lipopolysaccharide (LPS) to cells and is known to interact with other immune regulating factors including interferon regulatory factor 3, major histocompatibility complex (MHC) maturation, I κ B kinase (IKK), multiple toll-like receptors (TLRs) and T cells, along with natural killer (NK) cells^{30–34}. Since Hsp90 is involved in the activation of pro-inflammatory cytokines, as was elegantly demonstrated with IL-17³⁵ and IL-4³⁶, a number of Hsp90 inhibitors have been shown to be protective against various inflammatory insults^{37–41}. It is also important to note that inflammation has been shown numerous times to be coupled with an increased in Hsp90 α specifically^{11, 36}, possibly making it an important target for AD treatment. Since both tau and amyloid pathologies are affected by inflammatory pathways^{42–44}, controlling inflammation may be one of the ways Hsp90 inhibitors are modulating tau and A β .

Hsp90 functions as a scaffold for a large multi-protein chaperoning complex. In some ways, Hsp90 is just a bridge between a client and other enzymes termed co-chaperones. Recent work defined the nature of the relationship between Hsp90 and its client tau, showing that Hsp90 reduces the need for client specificity. Hsp90 recognizes structural elements of a client⁴⁵ rather than a particular primary sequence, and then allows other co-chaperones with enzymatic activity such as cis/trans peptidyl-prolyl isomerases or protein phosphatases to try and fold or repair the Hsp90-bound client. There are two classes of co-chaperones, those that bind to Hsp90 via a tetratricopeptide (TPR) domain and those that bind to Hsp90 without the need for a TPR domain. There is a growing list of TPR containing co-chaperones, which recognize and bind to MEEVD motifs found in Hsp90, along with EEVD motifs in Hsp70⁴. Some well-known members of this group of proteins include the carboxy-terminus of the heat shock cognate 70-interacting protein (CHIP), Hsp70-interacting protein (HIP), Hsp70-

Hsp90 organizing protein (HOP), protein phosphatase 5 (PP5) and several proteins with peptidyl-prolyl isomerase (PPIase activity): FK506 binding protein 51 kDa (FKBP51), FKBP52, peptidyl-prolyl isomerase D (PPID)/cyclophilin 40 (Cyp40), DNAJC7, Xap2, and Tom70⁴⁶. Hsp90 co-chaperones that bind to Hsp90 without the need for a TPR domain include: cell division control 37 kDa (CDC37), activator of Hsp90 ATPase (Aha) 1, and p23^{47, 48}. These co-chaperones can control Hsp90 ATPase activity as well as impact client metabolism. As such, they represent drug targets within the Hsp90 system that may provide greater specificity than classical Hsp90 inhibitors. These will be discussed in greater detail below.

This review will focus on the known effects of Hsp90 modulation on AD pathology, and in particular, tau. Chemicals that are known to modulate both Hsp90 and its co-chaperones will be discussed. An expanded list of TPR-containing Hsp90 co-chaperones is included in Table 1, while a list of current Hsp90 inhibitors is provided in Table 2. Many co-chaperones have not been well studied in regards to tau and A β pathology, but could provide new directions for future research.

2. Hsp90 Inhibitors: New Developments

Since the two terminal domains of Hsp90 both contain ATPase activity, small molecule inhibitors have been made that target each end and inhibit Hsp90 in different steps of the protein folding cycle⁴⁹. N-terminal Hsp90 ATPase inhibitors activate HSF1 and the expression of other chaperones such as Hsp70 and Hsp27¹⁹. Inhibitors which bind to the C-terminus do not activate HSF1. Hsp90 inhibition through either route is effective at decreasing tau levels. There have been many advances in designing small molecule inhibitors targeting the ATPase activity of Hsp90. This review will discuss the recent advances in Hsp90 ATPase inhibitors, N-terminus and C-terminus specific inhibitors, and isoform specific inhibitors. An extended list of Hsp90 inhibitors can be found in Table 2 and further details are provided in Zhao et al²⁰.

2.1 Hsp90 Inhibitors Scaffolds

2.1.1 N-terminus Inhibitors: Geldanamycin Scaffold—Geldanamycin (GA) was the first small molecule to be identified as an Hsp90 inhibitor⁵⁰. This natural antibiotic was shown to potently and preferentially reduce phospho-tau levels⁵¹, although with a high degree of toxicity²³. Improving upon the potency and safety of GA led to the second generation of Hsp90 inhibitors, including the well-known, less toxic analog 17-(allylamino)-17-demethoxygeldamycin (17-AAG). This class of inhibitors binds to the N-terminus of Hsp90 and was shown to preferentially bind to Hsp90 in a disease state⁵² and effectively clear tau in a mouse model of tauopathies²⁵. This scaffold, while promising, has multiple off-target effects including eliciting a strong heat shock response²⁴.

2.1.2 N-terminus Inhibitors: Purine Scaffold—Another class of N-terminus Hsp90-inhibiting small molecules was built from the purine scaffold. This class of inhibitors includes the blood-brain barrier (BBB) permeable EC scaffold, including EC102, and PU class-derivatives, like PU24FCI¹⁸. The EC scaffold has been previously shown to potently reduce tau levels¹⁹, underscoring the utility of targeting Hsp90 to treat tauopathies. The PU

scaffold derivatives are water soluble and have been shown to be potent Hsp90 inhibitors⁵³, although studies involving their modulation of tau through Hsp90 are still forthcoming.

2.1.3 C-terminus Inhibitors: Novobiocin Scaffold—One important class of Hsp90 inhibitors made from the small molecule antibiotic novobiocin targets the C-terminus ATPase. Improvements on the structure of novobiocin as an Hsp90 inhibitor have led to the generation of KU-32 and A4²³. KU-32 improves mitochondrial bioenergetics and diabetic peripheral neuropathy in an Hsp70-dependent manner⁵⁴.

2.2 Hsp90 Homologue Specific Inhibitors

Inhibitors which are preferentially active toward the inducible, cytosolic Hsp90 α have been identified. Using a computerized docking program, Mahanta et al. described a GA analog which preferentially binds Hsp90 α ^{55, 56}. More recently, Ernst et al. identified compound 31, a BBB permeable inhibitor for Hsp90 α/β , which preferentially inhibits cytosolic Hsp90 over its organelle-bound counterparts⁵⁷. A similar compound, CP9, inhibits Hsp90 α/β and disrupts oncogenesis in mice⁵⁸. Therefore, isoform specific inhibitors, especially those developed against Hsp90 α , may be more disease relevant with fewer side effects given the mild phenotype of Hsp90 α knockout mice⁹. While Hsp90 inhibitors were shown to partially mimic the spermatogenesis defect seen in the Hsp90 α knockout animals⁹, this side effect would be irrelevant for an aged AD population.

GRP94 specific inhibitors were recently developed^{59,60}. One of these inhibitors, now known as BnIm, was designed based on subtle structural differences between the cytosolic Hsp90 isoforms and GRP94, leading to very specific inhibition of only GRP94 ATPase function⁵⁹. BnIm has already been successfully used in follow-up studies, with low toxicity, to facilitate degradation of another aggregation-prone protein, myocilin⁶¹. Additional GRP94-selective inhibitors have been developed based on a different approach. Using a fluorescence polarization (FP) assay as previously described^{62, 63}, it was found that unlike other known Hsp90 inhibitors including GA, 17-AAG, PU-H71, CUDC-305, SNX-2122, NVP-AUY922, and STA-9090, only BIIB021 showed preference for GRP94 over other Hsp90 isoforms. Based on this structure, derivatives were generated that had GRP94 selectivity, including PU-H54, PU-WS13, and PU-H39⁶⁰. This is the first step towards designing specific Hsp90 isoform inhibitors that could reduce the toxic liabilities of a chaperone-based drug approach.

3. Targeting Hsp90 More Specifically through Co-Chaperones

Hsp90 works with a number of co-chaperones with diverse functions to regulate protein metabolism. Most Hsp90 co-chaperones are thought to participate in the refolding of client proteins, but there are examples of co-chaperones that facilitate client degradation, such as the ubiquitin ligase CHIP^{64–66}. Our group and others have shown that these Hsp90 co-chaperone complexes can have dynamic and robust effects on tau metabolism^{17, 67–71}. It is conceivable, as suggested by Zhao and colleagues, that existing Hsp90 inhibitors which target the C-terminus of Hsp90 could interfere with co-chaperone binding, since these sites overlap²⁰. However, this approach to inhibiting Hsp90 interacting proteins may also lead to promiscuity due to structural similarities between co-chaperone binding domains. The sansalvamide A (San A-amide) scaffold has yielded a promising set of compounds to target

Hsp90 co-chaperone interactions. This scaffold prevents the binding of Hsp90 clients and C-terminal co-chaperones proteins⁷². Derivatives which differentially regulate co-chaperone binding to Hsp90 have been developed but require more extensive testing to verify their utility in AD⁷³. Nonetheless, this natural product scaffold may provide a good starting point for the development of novel co-chaperone specific drugs. Using potential drugs from this scaffold or other small molecules which specifically modify Hsp90 co-chaperones could result in increased specificity for modulating tau with fewer side effects. This is well illustrated in a study out of the Picard lab looking at the predicted interactome of Hsp90². While Hsp90 was shown to interact with hundreds of proteins throughout almost every process in the cell, when they isolated the interacting partners of the Hsp90-Aha1 complex, it was only a subset of the original Hsp90 map.

3.1 Hsp90/Aha1

Uniquely, Aha1 is the only known co-chaperone which increases Hsp90 ATPase activity⁷⁴. There is little known about the regulation of tau by the Hsp90/Aha1 complex, but our laboratory previously showed that Aha1 knockdown using siRNA decreases tau levels⁴⁷. Similarly, Aha1 knockdown decreased misfolded cystic fibrosis (CF) transmembrane conductance regulator (CFTR) in a cellular model of CF⁷⁵. Although inhibition of the Aha1/Hsp90 complex seems like a promising strategy to reduce tau levels, there are no known small molecule inhibitors specific to this complex.

3.2 Hsp90/Cdc37

Cdc37 is most well-known for the role it plays in kinase refolding. Our group previously showed that Cdc37 regulates tau stability⁷¹. We found that higher levels of Cdc37 correlated with higher levels of tau, and when Cdc37 was knocked down using siRNA, tau was cleared. Celastrol⁷⁶ and Withaferin A^{77, 78} are small molecule inhibitors which have been shown to inhibit Hsp90-Cdc37 complexes. We found that celastrol treatment leads to modest changes in tau levels; however it can increase the potency of other drugs like methylene blue, an Hsp70 inhibitor⁷⁹. It is also important to note that inhibition of Cdc37 by celastrol leads to a heat shock response⁸⁰. We previously showed TDP-43, a protein that accumulates in amyotrophic lateral sclerosis and some AD cases, is significantly reduced following treatment with celastrol⁸¹. Importantly, celastrol more potently cleared TDP-43 than 17-AAG did, due to TDP43 being a binding partner of Cdc37. This shows proof of concept that targeting Hsp90 co-chaperones can lead to more potent and specific outcomes. Similarly, Withaferin A treatment induces Hsp70 and Hsp27 expression, but was also shown to lessen tau aggregate burden in a mouse model of tauopathies⁸².

3.3 Hsp90/Hop

Hop has three TPR domains, which allow simultaneous binding of a client protein, Hsp90, and Hsp70. This allows transfer of clients between Hsp70 and Hsp90⁸³. Tau levels are increased when Hop is knocked down⁴⁷. Recently, an Hsp90/Hop specific inhibitor, C9, was synthesized⁸⁴. No work has been done yet to see how this regulation of the Hop/Hsp90 complex affects tau.

3.4 Hsp90/CHIP

CHIP is an E3 ubiquitin ligase well characterized for its role in targeting Hsp90 clients, such as tau, for degradation through the proteasome^{19, 85}. In fact, CHIP knockout mice have greater tau accumulation than their wild-type littermates, though this tau was not pathogenic⁸⁶. BCL-Associated anthanogene 2 (BAG-2), has been identified as an intrinsic CHIP inhibitor^{87, 88}. BAG-2 was also shown to regulate tau degradation via a ubiquitin-independent pathway leading to tau accumulation⁸⁹. Thus, drugs which increase CHIP expression or decrease BAG-2 expression should negatively regulate tau levels.

3.5 Hsp90/p23

p23 is a co-chaperone which binds exclusively to ATP-bound Hsp90, but can remain bound even following ATP hydrolysis⁹⁰. p23 binds to Hsp90 simultaneously with other co-chaperones and can extend client-Hsp90 binding by regulating the length of time between ATP hydrolysis⁹¹. Knockdown of p23 decreases tau stability^{19, 47}. The anti-inflammatory compound celastrol has been reported to disrupt p23 function, making this drug a potentially attractive tool to test whether p23 inhibition can alter tau⁹². In addition to celastrol, Gedunin, a natural product with a structure similar to celastrol, was shown just last year to directly inactivate p23 and lead to the degradation of Hsp90 clients⁹³. Gedunin was found to have no effect on the levels of APP or a major cleaving enzyme of APP, beta-secretase 1 (BACE-1). Whether or not gedunin treatment changes tau biology has yet to be elucidated, more studies will need to test the relevance of this inhibitor with tau.

3.6 Hsp90/DNAJC7

DNAJC7 (Tpr2) is an Hsp40 chaperone that is highly expressed in the brain. It has two TPR domains, allowing it to bind both Hsp90 and Hsp70 simultaneously, along with a J domain, which stimulates the ATPase activity of Hsp70^{94, 95}. It was shown that DNAJC7 can pass clients from Hsp90 to Hsp70 in a retrograde fashion⁹⁶. More studies are necessary to understand the regulation of tau by DNAJC7, but this unique co-chaperone provides a novel target for drug development.

3.7 Hsp90 and cis/trans peptidyl-prolyl isomerase complexes

Hsp90 can interact with a host of PPIases; however there is very little known about their role in the Hsp90 folding cycle. They are thought to cooperate with protein phosphatases and regulate protein structure by isomerizing proline residues⁹⁷. But how these specific proteins function with Hsp90 to impact diverse folding modalities remains unknown. PPIase proteins have been linked to tau pathogenesis for quite some time, as Pin1 was shown to alter tau pathogenesis and phosphorylation⁹⁸. Recently, work from our lab and others has shown that other PPIases, such as FKBP51 and FKBP52 can also regulate tau metabolism^{17, 70, 99–101}. But unique from Pin1, these proteins impact tau through their interaction with Hsp90. Below, we detail the properties of the PPIases that interact with Hsp90 and their potential as drug targets from AD.

3.7.1 Hsp90/Protein Phosphatase 5—PP5 is a member of the protein phosphatase family which regulates the phosphorylation of many pathways including MAPK, p53, and

Raf-MEK-ERK^{102–104}. We found that PP5 knockdown decreased tau levels⁴⁷, in spite of the known role PP5 plays in the dephosphorylation of tau¹⁰⁵. PP5 can be self-inhibited by its TPR domain¹⁰⁶. Okadaic acid, a non-specific inhibitor of PP5 which inhibits additional phosphatases, is known to hyperphosphorylate tau in ex-vivo human brain slices¹⁰⁷ and in a neuroblastoma cell line¹⁰⁸. Therefore, drugs designed to increase PP5 activity or decrease total PP5 levels may be beneficial at treating tauopathies.

3.7.2 Hsp90/PPID—PPID, also known as Cyp40, is a cyclophilin with PPIase activity, as well as a TPR domain. PPID is abundant in the brain and has been shown to regulate axonal degeneration¹⁰⁹. The immunosuppressant cyclosporin A (CsA) is a known inhibitor of PPID¹¹⁰, although with many off-target effects is not an ideal drug to use. However, in our previous siRNA screen, we found that PPID knockdown has minimal effects on tau levels.

3.7.3 HSP90/FKBP51—Our group has shown that FKBP51, most well-known for its regulation of glucocorticoid receptors (GR) in concert with Hsp90, has a clear role in tau metabolism^{17, 70}. A rigorous characterization of *FKBP5*–/– mice has shown that there are no adverse effects associated with FKBP51 depletion. In fact, these *FKBP5*–/– mice were protected from depressive-like phenotypes when exposed to stress or in an aged paradigm, making FKBP51 a prime target for therapeutics^{111, 112}. Recent work from our laboratory showed that there is a clear increase in *FKBP5* expression with aging in the human brain, due to demethylation of the *FKBP5* gene, with an additional increase in expression in AD patients. High levels of FKBP51 correlated with increased accumulation of tau oligomers¹⁷. We recently showed increased expression of FKBP51 in AD, but no SNP in *FKBP5* has been linked to this disease¹⁷. Thus FKBP51 could be an ideal drug target for a number of diseases.

FKBP51 is made up of two FKBP-like domains (FK1 and FK2), which have PPIase activity, and a TPR domain. Since FKBP51 has PPIase activity, it is categorized as an immunophilin, which means this domain can directly bind immune suppressive drugs like rapamycin, FK506, and CsA¹¹³. Because this domain is shared between the other FKBP proteins, these drugs promiscuously bind many of the FKBP proteins. Much of the effort directed at designing drugs towards FKBP51 is centered on locating drugs which selectively bind the PPIase pocket, but selectivity is challenging given the similarities with other PPIase containing proteins¹¹⁴. Even if targeting FKBP51 alone were feasible, this approach could also interfere with regulation of other known substrates including GR, Akt, androgen receptors (AR) progesterone receptors (PR), and others. Alternatively, drugs could be made to target the TPR domain of FKBP51, but this domain is also highly homologous to other co-chaperones. Perhaps the high affinity of FKBP51 for Hsp90 could be exploited for such an approach¹¹⁵. Interestingly many of the deleterious effects of FKBP51 in psychiatric diseases have been linked to its regulation of GR that are not dependent on the PPIase activity¹¹⁶. Thus targeting the TPR domain could be the only effective strategy for regulating FKBP51-mediated control of glucocorticoid signaling. However, perhaps rather than focusing on a single protein, a more appropriate strategy would be to target modulators of the FKBP51-Hsp90-substrate complex, but this type of approach would require a complete ternary complex structure, which is currently unavailable.

3.7.4 HSP90/FKBP52—The Hsp90/FKBP52 complex is most well characterized with regards to steroid hormone regulation¹¹⁷. This immunophilin is known to replace FKBP51 in the Hsp90/hormone complex just prior to nuclear translocation. However, quite a bit is also known about the regulation of tau by FKBP52. FKBP52 can directly bind to tau and preferentially binds to hyperphosphorylated, pathogenic tau species¹⁰⁰. Furthermore, this study showed that this interaction had a functional effect on tau by preventing tau from stabilizing microtubules. We found that FKBP52 knockdown preferentially increased total tau, but not phospho-tau⁴⁷. In a more recent study, FKBP52 was found to interact with tau to produce tau oligomers¹⁰¹, similar to the results we demonstrated with FKBP51 and tau¹⁷. There is a known inhibitor of the Hsp90/FKBP52/AR complex, MJC13¹¹⁸, but its characterization has not been extended beyond the application of prostate cancer treatment.

4. Conclusions

Hsp90 is a potent regulator of tau biology and a valid target for decreasing pathological tau. However, this major chaperone is a critical regulator of many cellular processes throughout the body, making it a difficult protein to target without adverse effects. Inhibition of Hsp90 leads to the clearance of many tau species. First generation Hsp90 inhibitors were effective at decreasing tau levels, but had many off-target effects, some of which were toxic. Recent developments in Hsp90 inhibitors have increased specificity for homologues of Hsp90, lowered toxicity, and increased preference for pathological tau. Targeting co-chaperones of Hsp90 may enhance specificity and provide the necessary target engagement of tau to permit therapeutic efficacy. There are few drugs available which specifically regulate these Hsp90 co-chaperone complexes. More studies in this area, along with the discovery and synthesis of new inhibitors, will reveal if targeting Hsp90 through these co-chaperones leads to a novel, more efficacious tau-regulating drug.

5. Expert Opinion

AD has received much attention recently due to increased incidence and an aging population that is living longer than ever before. Directly targeting A β or tau with immunotherapy or small molecule inhibitors has to date proven ineffective, though there are currently several therapeutic candidates that are undergoing pre-clinical work or are in clinical trials¹¹⁹. A number of studies have shown that chaperone modulating chemicals can also regulate tau and amyloid metabolism^{18, 19, 79, 120}. Therapeutic approaches that directly target the Hsp90 family of proteins are clinically relevant for a number of diseases and have shown promise from an efficacy and safety perspective^{18, 52, 79, 121}. In fact, it has been proposed that the increased activity of Hsp90 that is often found in diseased cells can be exploited to titrate dosing and avoid targeting normal cells^{19, 52}. However, it is likely that these compounds will certainly affect other targets in cells besides chaperones, and moreover, even if perfectly selective compounds could be made to only target Hsp90 proteins or even a single isoform, there are a large number of potential clients of Hsp90 that could also be affected by this approach. While this potential for off-target problems seems high, we propose that targeting Hsp90 proteins is no different from targeting any other signaling pathway in the cell, such as mTOR, CdKs, GSK3 β , PI3K, or even the proteasome itself, all of which will affect essential cellular processes^{122–124}. But despite the pleiotropy of these targets, there are successful

examples of targeting each of these proteins in the clinic. This suggests that targeting major pathways in the cell may be tolerable for particular diseases. In the case of Hsp90 inhibitors, the efficacy of this strategy was tested and proven *in vivo* multiple times^{18, 24, 19, 82}, with no signs of overt toxicity. There have been pharmacological issues with some of the Hsp90 inhibitors that are due to their chemical composition rather than their target¹²⁵, and these challenges have dampened enthusiasm for the approach to some extent. But there are new small molecules without these liabilities that have started to emerge, giving hope once again to the field.

Nevertheless, while controlling Hsp90 itself may be challenging and simply too rife with liabilities to pursue in the clinic, the approach of controlling proteostasis warrants much greater attention. Co-chaperones in particular have the potential to inherently confer specificity to a particular disease target through Hsp90. Because co-chaperones modulate significantly fewer processes than the parent chaperone², it becomes easier to avoid undesirable off-target effects. Hsp90 co-chaperones in particular hold promise for targeting A β and tau in a more specific manner than through Hsp90 alone. However, like many other targets, the similar structural and binding profiles of these co-chaperones make it difficult to design ligands specific to an individual protein. For example, the immunophilins have structurally similar ligand binding pockets, which may explain why most compounds developed thus far have lacked selectivity for one isoform over another. Importantly, FKBP51 and FKBP52 do differ at key residues which could be exploited for drug design¹²⁶. Alternatively, recent work has illustrated how manipulating the conformation of a compound can increase its efficiency, even in a difficult binding site such as FKBP51¹²⁷.

Techniques developed recently to facilitate high-throughput screening (HTS) of potential lead compounds should assist pharmacogenesis in upcoming years. FP assays are amenable to an HTS format and can be manipulated considerably to ideally suit the needs of the investigator. FP probes have been developed to validate ligands targeting FKBP51¹²⁸ and Hsp90⁶⁰. Extending this approach to other Hsp90 co-chaperones in a high-throughput setting could reveal which of these targets is most efficient at regulating A β or tau and help direct novel therapeutic approaches.

A wealth of emerging evidence strongly supports using chaperones to target A β and tau. The dynamic nature of Hsp90 and its co-chaperones make ideal drug candidates; all that is needed is the proper specificity and target engagement. Ligands that maintain selectivity for a particular member of the Hsp90 family will not only have utility as lead compounds, but will also be important tools for studying Hsp90 biology. For example, understanding the effects of inhibiting Hsp90 α compared to Hsp90 β in a mouse model of AD would provide vital information prior to the initiation of clinical trials. Nevertheless, Hsp90 and its ever-expanding list of co-chaperones have great potential for success in AD.

Acknowledgments

This work was supported by NIH/NINDS R01 NS073899.

Abbreviation List

17-AAG	17-(allylamino)-17-demethoxygeldamycin
AD	Alzheimer's disease
Aha	activator of Hsp90 ATPase
ATP	adenosine triphosphate
BACE	beta secretase
BAG	BCL-associated anthanogene
BBB	Blood–brain barrier
Aβ	beta amyloid
Cdc37	Cell division control 37 kDa
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator
CHIP	Carboxyl terminus of Hsc70-interacting protein
CsA	cyclosporin A
ER	endoplasmic reticulum
FKBP	FK506 binding protein
FP	fluorescence polarization
GA	geldanamycin
GRP	Glucose-regulated protein
HIP	Hsp70-interacting protein
HIP	Hsp70-Hsp90 organizing protein
Hsc	Heat shock cognate
HSF1	Heat shock factor protein 1
Hsp	Heat shock protein
HTS	high-throughput screening
IKK	I κ B kinase
KU-32	N-(7-((2R,3R,4S,5R)-3,4-dihydroxy-5-methoxy-6,6-dimethyl-tetrahydro-2H-pyran-2-yloxy)-8-methyl-2-oxo-2H-chromen-3-yl)acetamide
LPS	lipopolysaccharide
MB	Methylene blue (phenothiazine methylthioniumchloride)
NK	natural killer
PP5	protein phosphatase 5

PHLPP	pleckstrin homology domain and leucine-rich repeat protein phosphatases
PPI	peptidyl-prolyl isomerase
PR619	2,6-diamino-3,5-dithiocyanopyridine
PS1	presenillin1
San A-amide	sansalvamide A
TLR	Toll-like receptor
TPR	tetratricopeptide
TRAP	tumor necrosis factor receptor associated protein

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Papers of special note have been highlighted as either of interest (*) or of considerable interest (**) to readers.

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Highlights

- Hsp90 inhibitors have been shown to effectively reduce tau levels and decrease A β -induced toxicity and behavioral deficits.
- N-terminal Hsp90 inhibitors elicit a heat shock response, while C-terminal inhibitors do not.
- New developments in Hsp90 inhibitors have yielded more potent, less toxic drugs, including PU24FCI and A4.
- Additional improvements in the bioavailability and toxicity profile of Hsp90 inhibitors are necessary to meet with success in clinical trials.
- Recently, a novel Hsp90 drug screen was developed which uses fluorescence polarization to screen drug libraries for specific inhibitors of Hsp90 α , Hsp90 β , TRAP1, and GRP94.
- Hsp90 co-chaperones provide a large group of targets to identify drugs for targeting specific pathways of Hsp90 interactions.
- Immunophilins (e.g. FKBP51 and FKBP52) directly regulate tau biology, making them attractive therapeutic targets.

Table 1

Mammalian TPR co-chaperones

Name	Full name	Alternative names	Function (other than TPR)
CHIP	carboxy-terminus of the heat shock cognate 70-interacting protein	STUB1	E3 ubiquitin ligase
HIP	Hsp70-interacting protein	ST13	stress inducible by STI domain
HOP	Hsp70-Hsp90 organizing protein	p60, STIP1, STI1	binds Hsp70 and Hsp90 simultaneously
PP5	protein phosphatase 5	PPP5C	Phosphatase, PPIase-like
FKBP51	FK506 binding protein 51 kDa	FKBP5	PPIase
FKBP52	FK506 binding protein 52 kDa	FKBP4	PPIase
FKBP38	FK506 binding protein 38 kDa	FKBP8	PPIase
FKBP36	FK506 binding protein 36 kDa	FKBP6	PPIase
PPID	peptidyl-prolyl isomerase D	Cyp40	Phosphatase, cyclophilin
Tom70	translocase in the outer mitochondrial membrane 70kDa	TOMM70, KIAA0719	Import receptor for mitochondria
Xap2	Aryl Hydrocarbon Receptor Interacting Protein	FKBP16, ARA9, AIP	PPIase
Cns1	cyclophilin seven suppressor	TTC4	involved in cell cycle regulation
UNC-45	UCS domain containing protein	GCUNC-45, SMAP1	contains UCS (Unc-45/Cro1/She4p) domain to bind myosin
DNAJC7	DnaJ (Hsp40) Homolog, Subfamily C	TPR2	stimulate Hsp70 ATPase, E3 ubiquitin ligase
CRN	crooked neck protein	CRNKL1	E3 ubiquitin ligase
WISp39	WNT1- inducible-signaling pathway protein 3	FKBPL	Extracellular matrix signalling protein
Tah1	TPR-contating protein associated with Hsp90	Spaghetti	preferentially binds Hsp90 to Hsp70
NASP	Nuclear Autoantigenic Sperm Protein		Histone 1 binding protein
TPR1	Tetratricopeptide Repeat Domain 1	Ttc1	scaffold for protein-protein interactions
SGT	Small Glutamine-Rich Tetratricopeptide Repeat	Ubp	binds viral-encoded protein U
DYX1C1	Dyslexia Susceptibility 1 Candidate 1	EKN1, DUX1	essential role in dynein assembly
AIPL1	Aryl Hydrocarbon Receptor Interacting Protein-Like 1	LCA4, AIPL2	role in photoreceptors

Name	Full name	Alternative names	Function (other than TPR)
Tom34	Translocase Of Outer Mitochondrial Membrane 34	TOMM34, URCC3	Import receptor for mitochondria

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Table 2

List of Hsp90 Inhibitors

Class/Related patents	Drug	Family	Mechanism	Toxicity	BBB permeability	Clinical trials	Reason of Failure
Hydroquinone							
4,261,989	geldanamycin	natural product	N-terminal Inhibitor	highly toxic	not permeable	no	low solubility and hepatotoxicity
8,466,140	17-AAG	geldanamycin derivative	N-terminal Inhibitor	some toxicity	permeable	yes	low solubility and bioavailability
8,551,964	17-DMAG	17-AAG derivative	N-terminal Inhibitor	some toxicity	permeable	yes	toxicity profile
6,335,157			N-terminal Inhibitor	some toxicity	Not known	yes	high mortality rate
6,946,456	IP1-504	geldanamycin derivative	N-terminal Inhibitor	low toxicity	Not known	no	N/A
7,566,706	KOSNI559	geldanamycin derivative	N-terminal Inhibitor	low toxicity	Not known	yes	anticipated mortality rates of patients
7,465,718							
7,674,795							
7,691,392							
Purine							
7,247,734	PU3	synthetic	N-terminal Inhibitor	some toxicity	Not known	no	N/A
7,999,006	PU-H71	PU3 derivative	N-terminal Inhibitor	low toxicity	poor permeability	yes	Phase I trial in progress
7,138,402	PU24FCI	PU3 derivative	N-terminal Inhibitor	low toxicity	not known	no	N/A
7,148,228	EC102	purine derivative	N-terminal Inhibitor	low toxicity	permeable	no	N/A
7,241,562							
7,241,890	PU-DZ8	purine derivative	N-terminal Inhibitor	low toxicity	permeable	no	N/A
7,138,401							
7,648,976	CUDC-305	purine derivative	N-terminal Inhibitor	some toxicity	permeable	yes	Phase I trial in progress
8,703,942	MPC-3100	synthetic	N-terminal Inhibitor	low toxicity	not permeable	yes	Completed Phase I trial
8,586,605	BIIB021	synthetic	N-terminal Inhibitor	some toxicity	permeable	yes	Completed Phase II trial
8,604,032							
Pyrazole							
	CCT018159	pyrazole derivative	N-terminal Inhibitor	low toxicity	not known	no	N/A

Class/Related patents	Drug	Family	Mechanism	Toxicity	BBB permeability	Clinical trials	Reason of Failure
7,247,734 8,604,032 5,629,337 7,608,611 7,608,635 8,329,899	VER49009 STA-9090 STA-1474	CCT018159 derivative Resorcinol derivative Resorcinol derivative	N-terminal Inhibitor N-terminal Inhibitor N-terminal Inhibitor	some toxicity low toxicity low toxicity	not known not known not known	no yes yes	N/A Phase II trials in progress Recruiting for Phase I trial
Isoxazole							
7,705,027 8,071,766 8,383,619 8,450,310 8,507,480	VER50589 NVP-AUY922/ VER52296	VER49009 derivative VER52296 derivative	N-terminal Inhibitor N-terminal Inhibitor	some toxicity low toxicity	not known permeable	no yes	N/A Phase II trials in progress
Dihydroindazolone							
8,604,032 8,426,396	SNX-2112 SNX5422 Compound 31	synthetic SNX-2112 derivative Octahydro- β -carboline derivative	N-terminal Inhibitor N-terminal Inhibitor N-terminal Inhibitor	low toxicity low toxicity low toxicity	permeable permeable permeable	no yes no	N/A Phase II trial in progress N/A
Radical							
6,670,187 7,115,651 7,674,795	radical cycloproparadical	natural product radical derivative	N-terminal Inhibitor N-terminal Inhibitor	some toxicity some toxicity	poorly permeable Not likely permeable	yes yes	low bioavailability and non-specific cytotoxicity dienone moiety responsible for toxicity and loss of activity
	KF 25706 KF 55823	radical derivative radical derivative	N-terminal Inhibitor N-terminal Inhibitor	low toxicity low toxicity	Not likely permeable Not likely permeable	no no	N/A N/A
Coumarin	Novobiocin	natural product	C-terminal Inhibitor	low toxicity	poorly permeable	yes	poor safety and effectiveness

Class/Related patents	Drug	Family	Mechanism	Toxicity	BBB permeability	Clinical trials	Reason of Failure
6,887,853 7,608,594 7,622,451 7,811,998 7,960,353 8,212,011 8,212,012	chlorobiocin coumermycin A4 4TCNA 4TCCQ KU-32 KU-135 Compound 83 DHN1 DHN2	Novobiocin derivative natural product Novobiocin derivative 3-aminocoumarin derivative 3-aminocoumarin derivative Novobiocin derivative Novobiocin derivative Novobiocin derivative Novobiocin derivative Novobiocin derivative Novobiocin derivative	C-terminal Inhibitor C-terminal Inhibitor C-terminal Inhibitor C-terminal Inhibitor C-terminal Inhibitor C-terminal Inhibitor C-terminal Inhibitor C-terminal Inhibitor C-terminal Inhibitor C-terminal Inhibitor C-terminal Inhibitor	low toxicity low toxicity low toxicity some toxicity some toxicity low toxicity highly toxic some toxicity low toxicity low toxicity low toxicity	Not likely permeable Not likely permeable permeable not known not known permeable likely permeable not known likely permeable likely permeable	no no yes no no no no no no no no	N/A N/A Recruiting for Phase II trial N/A N/A N/A N/A N/A N/A N/A N/A
8,637,494	Withaferin A	natural product	CDC37/Hsp90 Inhibitor	some toxicity	permeable	yes	Recruiting for Phase I trial
7,117,466	Celastrrol	natural product	CDC37/Hsp90 Inhibitor	some toxicity	permeable	no	N/A
7,117,466	gedunin	natural product	p23/Hsp90 Inhibitor	some toxicity	likely permeable	no	N/A
8,685,966	Bnfm	1,2-imidazole derivative	GRP94 inhibitor	low toxicity	not known	no	N/A
8,492,345	Sansalvamide A-amide	natural product	Prevents binding of C-terminal co-chaperones	some toxicity	not known	no	N/A
8,293,718	KW-2478	synthetic	Hsp90 Inhibitor	low toxicity	not known	yes	Recruiting for Phase II trial
8,604,032	AT13387	Dihydroxybenzamide derivative	N-terminal Inhibitor	low toxicity	not permeable	yes	Recruiting for Phase II trial

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Class/Related patents	Drug	Family	Mechanism	Toxicity	BBB permeability	Clinical trials	Reason of Failure
6,572,899	EGCG	natural product	C-terminal Inhibitor	low toxicity	permeable	yes	Completed Phase IV trial; poor bioavailability and potency