Small Molecule Inhibitors to Disrupt Protein-protein Interactions of Heat Shock Protein 90 Chaperone Machinery

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Heat shock protein 90 (Hsp90) is an adenosine triphosphate dependent molecular chaperone in eukaryotic cells that regulates the activation and maintenance of numerous regulatory and signaling proteins including epidermal growth factor receptor, human epidermal growth factor receptor 2, mesenchymal-epithelial transition factor, cyclin-dependent kinase-4, protein kinase B, hypoxia-inducible factor 1α, and matrix metalloproteinase-2. Since many of Hsp90 clients are oncogenic proteins, Hsp90 has become an attractive therapeutic target for treatment of cancer. To discover small molecule inhibitors targeting Hsp90 chaperone machinery, several strategies have been employed, which results in three classes of inhibitors such as N-terminal inhibitors, C-terminal inhibitors, and inhibitors disrupting protein-protein interactions of Hsp90 chaperone machinery. Developing small molecule inhibitors that modulate protein-protein interactions of Hsp90 is a challenging task, although it offers many alternative opportunities for therapeutic intervention. The lack of well-defined binding pocket and starting points for drug design challenges medicinal chemists to discover small molecule inhibitors disrupting protein-protein interactions of Hsp90. The present review will focus on the current studies on small molecule inhibitors disrupting protein-protein interactions of Hsp90 chaperone machinery, provide biological background on the structure, function and mechanism of Hsp90’s protein-protein interactions, and discuss the challenges and promise of its small molecule modulations.

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INTRODUCTION

Over the last two decades, several cancer drugs that target a single genetic abnormality have been discovered as so called ‘targeted cancer drugs’ and eradicated cancer cells in more specific ways without undesired side effects.1 Despite their superiority in the therapeutic efficacy and selectivity, these drugs often encounter the emergence of drug-resistance in cancer chemotherapy and that remains a major obstacle to cure cancers. Most cancers are heterogeneous and driven by multiple genetic abnormalities and hence, it is being recognized that cancers are more likely invincible by a single-targeted drug.2,3 In this regard, Heat shock protein 90 (Hsp90) provides a number of advantages to overcome drug resistance and has become an attractive therapeutic target for treatment of cancer. Hsp90 is an essential molecular chaperone in eukaryotic cells with an important role in activation and maintenance of numerous regulatory and signaling proteins including epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (Her2), mesenchymal-epithelial transition factor (Met), cyclin-dependent kinase-4 (Cdk4), protein kinase B (Akt/PKB), hypoxia-inducible factor 1α (HIF-1α), and matrix metalloproteinase-2 (MMP2).4 Disruption of Hsp90 folding machinery induces client proteins degradation via the ubiquitin-proteasome pathway, which leads to cell death. Hsp90 is constitutively expressed at 2 to 10 fold higher levels in cancer cells than their normal counterparts.5,6 Hsp90 requires a series of cochaperones to form a complex for its function and cell division cycle 37 (Cdc37). Heat shock protein 70 (Hsp70). Heat...
shock protein 40 (Hsp40), Hsp organizing protein (Hop), p23, activator of Hsp90 ATPase (Aha1), and immunophilins belong to the cochaperones (Fig. 1). In cancer cells, the cochaperones assemble into a superchaperone complex with Hsp90 and they assist Hsp90 to carry out its chaperoning job. By contrast, Hsp90 from normal cells does not generally form a superchaperone complex and has lower ATPase activity compared to cancer cells. 9,10 Besides, it is reported that the binding affinity of Hsp90 inhibitor 17-AAG to Hsp90 in cancer cells is approximately 100 times stronger than in normal cells. 9,11 Therefore, Hsp90 present in cancer cells forms a superchaperone complex and displays higher susceptibility to the inhibition by Hsp90 inhibitors than normal cells. Considering that therapeutic selectivity for cancer cells over normal cells is the Holy Grail in the war on cancer, the differential property of Hsp90 in cancer cells over normal cells shows particular promise in cancer chemotherapy.

Three major strategies have been employed to discover Hsp90 inhibitors. Most widely used strategy in academia and the pharmaceutical industry is to target ATP-binding pocket of Hsp90’s N-terminal domain. 12-18 To date, hundreds of N-terminal inhibitors have been discovered. Inhibitors to target adenosine triphosphate (ATP)-binding pocket of N-terminal domain are considered to be the most effective as an anticancer agent. Currently, there are thirteen Hsp90 inhibitors undergoing clinical developments and all Hsp90 inhibitors under clinical evaluation are classified into N-terminal inhibitors. 19 Despite the significant progress of this approach, N-terminal inhibitors induce the pro-survival heat shock response, which is speculated to limit their utility as anticancer therapy. 4,20 Another strategy employed to inhibit Hsp90 function is to block the C-terminal nucleotide-binding domain that exhibits allosteric control over both substrates and ATP-binding site of N-terminal domain. 21-26 Unlike N-terminal inhibitors, C-terminal inhibitors have not advanced to clinical investigations probably due to the absence of the co-crystal structure with their inhibitors. Intriguingly, C-terminal inhibitors are reported not to induce the heat shock response that is believed to be a detrimental consequence of N-terminal inhibitors. 27 Lastly, the strategy to disrupt protein-protein interactions of Hsp90 has been investigated as an alternative to modulate Hsp90 chaperone machinery. A small number of studies on the inhibition of protein-protein interactions have been conducted up to date. 30,32 The present review will focus on the recent developments of the inhibitors disrupting protein-protein interactions of Hsp90 chaperone machinery and provide an overview on their biological insights and therapeutic implications.

### MAIN SUBJECT

#### 1. Inhibitors to disrupt heat shock protein 90-heat shock protein organizing protein interaction

Hsp organizing protein (Hop) is an abundant, highly conserved eukaryotic protein that mediates the association of the molecular cochaperones, Hsp90 and Hsp70. 33,34 The current consensus model for Hsp90 mediated protein folding assumes that a newly synthesized polypeptide is first chaperoned by Hsp70 and then transferred to Hsp90 for the correct folding and maturation (Fig. 2). To do so, Hsp70 and Hsp90 are brought into close proximity via their interactions with Hop. C-terminal peptides of Hsp70 and Hsp90 independently interact with two tetratricopeptide repeat (TPR) domains of Hop, TPR1 and TPR2A respectively, and form a multichaperone complex of Hsp70-Hop-Hsp90. 33,34 If Hsp90 is blocked to interact with TPR2A domain of Hop, a multichaperone complex of Hsp70-Hop-Hsp90 can no longer form. Besides, mounting evidence has demonstrated that the interaction of Hsp90 with Hop via the TPR2A is critically essential for Hsp90 chaperoning function. Therefore, the blockage of Hsp70-Hop-Hsp90 folding relay system will lead to the ubiquitination and proteasomal degradation of Hsp90 client proteins.

To discover inhibitors disrupting Hsp90-Hop interactions, Yi and Regan 31 performed an AlphaScreen technology based
high-throughput in vitro screen to identify six compounds that disrupted the interaction of Hsp90-Hop (Fig. 3). In the AlphaScreen technology, the excitation of the donor bead bound to Hsp90 at 680 nm produces singlet state oxygen species, which then diffuse up to 200 nm and activate fluorophores in the acceptor bead bound to Hop. As a result, protein-protein interaction of Hsp90 with Hop emits light at 520 to 620 nm. To rule out any false positives in the AlphaScreen technology, they executed fluorescence polarization assay and isothermal titration calorimetry (ITC) and confirmed the six compounds. ITC study showed that compound 1 directly bound to TPR2A domain of Hop with dissociation constant (Kd) value of 16 μM and a 1:1 binding stoichiometry. In addition, they demonstrated that compound 1 inhibited Her2-positive human breast cancer cell lines BT474. Interestingly, these compounds did not induce the upregulation of Hsp70, which was speculated to limit the efficacy of Hsp90 inhibitors under certain circumstances. All six compounds that they identified have a 7-azapteridine ring system in common and displayed similar activities, with no substitution effect causing a dramatic change in activity. They speculated that C3 position of 1,2,4-triazine ring that they modified substitution was probably not a key position and that may exert a relatively flat structure-activity relationship (SAR). Up to date, the optimization and SAR studies of the hit compounds have not been reported. Accordingly, further extensive SAR studies along with structural characterization of TPR2A-small molecule complex will provide more detailed information about TPR2A-small molecule interactions and direct how the compounds can be chemically modified for the higher potency and specificity. Inhibitors disrupting Hsp90-Hop interaction could eventually lead to efficacious alternatives to Hsp90 N-terminal inhibitors.

2. Inhibitors to disrupt heat shock protein 90-cell division cycle 37 interaction

Cdc37 was originally identified in mammalian cells as a component of a protein complex involving Hsp90 and the Rous
sarcoma virus protein, pp60^v-src. The cochaperone adaptor Cdc37, also known as p50, pp50, and p50^CDC37 plays an important role in regulating the Hsp90 chaperone cycle by assisting the recruitment of client proteins that include numerous oncogenic kinases. Accumulating evidence suggested that the overexpression of Cdc37 is related to the oncogenic transformation and Cdc37 is required for the folding and the maturation of Hsp90-dependent protein kinases including Her2, Raf-1, Cdk4, and Akt. Consequently, Hsp90-Cdc37 chaperone complex maintains a population of protein kinases in cells, which is implicated in signal transduction, cell proliferation, and survival. The critical role of the Hsp90-Cdc37 chaperone complex makes it an attracting therapeutic target for anti-cancer drug development.

The co-crystal structure of Hsp90 with Cdc37 revealed that the large helical domain in the C-terminal of Cdc37 binds to the N-terminal domain of Hsp90, which is believed to close over bound ATP in ATP-binding pocket of Hsp90’s N-terminal domain (Fig. 4). The core interactions of Hsp90-Cdc37 complex involves a relatively flat hydrophobic patch formed by the hydrophobic residues of Hsp90 and Cdc37. Despite the interacting regions of Hsp90-Cdc37 are located in close proximity to the ATP-binding pocket of Hsp90’s N-terminal domain, most N-terminal inhibitors do not disrupt Hsp90-Cdc37 interactions.

In 2008, Zhang et al. first identified that a natural product, celastrol blocked Cdc37 binding to Hsp90 (Fig. 5). Celastrol is a quinone methide triterpene isolated from *Tripterygium wilfordii* Hook F (the Chinese Thunder of God vine). Using ATP-sepharose binding and co-immunoprecipitation assay, they concluded that celastrol disrupted Hsp90-Cdc37 without blocking ATP binding to Hsp90. The molecular modeling predicted that celastrol binds to the mouth region of ATP-binding pocket in N-terminal domain of Hsp90 and induced a major conformational change in the binding pocket, resulting in a significant decrease of the binding affinity of Cdc37 to Hsp90. The study demonstrated that celastrol disrupted only Hsp90-Cdc37 complex without affecting other Hsp90-cochaperone interactions such as Hsp90-Hop and Hsp90-p23. As a consequence, celastrol degraded Hsp90 client proteins including Akt and Cdk4 in pancreatic cancer cells. In Panc-1 cell xenograft mice, celastrol inhibited 80% of tumor growth, effect of which is slightly better than N-terminal inhibitor, geldanamycin. Interestingly, they observed that celastrol induced heat shock response, leading to the upregulation of Hsp70. The finding may explain that celastrol has multiple mechanisms of action together with Hsp90-Cdc37 disruption.

In 2012, the same research group reported that a natural product, sulforaphane disrupted Hsp90-Cdc37 complex to impair the growth of pancreatic cancer. Sulforaphane is a major compound from broccoli and broccoli sprout, containing an...
isothiocyanate structure on one end (Fig. 5).\textsuperscript{42,43} ATP-sepharose binding assay displayed that sulforaphane inhibited Hsp90 function through an ATP-binding independent manner. Hsp90 co-immunoprecipitation, proteolytic fingerprinting assay, nuclear magnetic resonance spectroscopy, and LC-MS peptide mapping revealed that sulforaphane modified N-terminal domain of Hsp90 and disrupted Hsp90-Cdc37 complex. In this study, sulforaphane promoted the proteasomal degradation of Hsp90 client proteins such as Akt, Cdk4, and mutant p53, resulting in pancreatic cancer cell death.

3. Inhibitors to disrupt heat shock protein 90-human epidermal growth factor receptor 2 interaction

Few studies have been reported to discover small molecule inhibitors specifically inhibiting Hsp90-client interactions. It is probably due to the lack of information on how exactly a single client protein interacts with Hsp90. Nonetheless, Yan et al.\textsuperscript{30} reported that emodin azide methyl anthraquinone derivative (AMAD) blocked Her2 binding to Hsp90 (Fig. 6). Emodin AMAD is an emodin azide methyl anthraquione derivative, obtained from chemical modification of emodin. Emodin is a natural product extracted from nature’s giant knotweed rhizome of traditional Chinese herbs.\textsuperscript{44} The study revealed that AMAD significantly impaired the binding of Her2 to Hsp90, promoted Her2 ubiquitination and destroyed the plasma membrane location of Her2. To investigate the molecular mechanism of emodin AMAD in blocking Hsp90-Her2 complex, they performed \textit{in silico} docking study. The docking study demonstrated that emodin AMAD could bind to both Hsp90 and Her2 nucleotide binding sites. They speculated that emodin AMAD either binds to ATP-binding pocket of Hsp90 or Her2, resulting in structural distortion of chaperone complexes and subsequent degradation of Her2.

CONCLUSION

Hsp90 is responsible for diverse cellular function in normal cells, but also involved in malignant transformation and disease progression. Therefore, Hsp90 has become an active molecular target for the treatment of many diseases, including cancer, neurodegenerative diseases, and pathogenic infections. At present, clinical applications for Hsp90 modulation primarily focus on the treatment of cancer and the progress has been made using Hsp90-targeted therapies. Although N-terminal inhibitors, C-terminal inhibitors, and inhibitors disrupting protein-protein interactions of Hsp90 have been investigated as anticancer agents, only competitive inhibitors that target N-terminal ATP-binding site have entered into clinical developments. Vast amount of studies were conducted to discover N-terminal inhibitors in clinical applications. To date, however, there is no Food and Drug Administration in USA approved Hsp90 inhibitor. Accordingly, alternative strategy might provide an opportunity for the development of Hsp90 inhibitors with clinical applications and inhibiting protein-protein interactions of Hsp90 chaperone machinery may represent a promising strategy to find clinical application in cancer research. In this review, we overviewed the current studies of small molecule inhibitors disrupting protein-protein interactions of Hsp90 chaperone machinery, their biological importance, and therapeutic applications for cancer treatment.

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CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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