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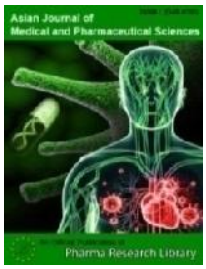

R E V I E W A R T I C L E

A Review on Cancer Therapy by Targeting HSP90

Shaik Salma Sultana*, P. Sukumari

Jagan's College of Pharmacy, Jangala kandriga, SPSR Nellore, Andhra Pradesh, India

A B S T R A C T

Chaperones (stress proteins) are essential proteins to help the formation and maintenance of the proper conformation of other proteins and to promote cell survival after a large variety of environmental stresses. Therefore, normal chaperone function is a key factor for endogenous stress adaptation of several tissues. Development of membrane-interacting drugs to modify specific membrane domains, thereby modulating heat shock response, may be of considerable therapeutic benefit as well. In this review, we give an overview of the therapeutic approaches and list some of the key questions of drug development in this novel and promising therapeutic approach.

Keywords: Chaperone coinducers, chaperones, Hsp90, Hsp70, heat shock proteins, geldanamycin; stress proteins

A R T I C L E I N F O

A U T H O R D E T A I L S
Shaik Salma Sultana
Jagan's College of Pharmacy,
Jangala kandriga, SPSR Nellore,
Andhra Pradesh, India
MS-ID: AJMPS3585

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C O N T E N T S

1. Introduction

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body.[1] Not all tumors are cancerous; benign tumors do not spread to other parts of the body.[2] Possible signs and symptoms include a lump, abnormal bleeding, prolonged cough, unexplained weight loss and a change in bowel movements.[3] While these symptoms may indicate cancer, they may have other causes. Over 100 types of cancers affect humans. There is an urgent need for new treatments for cancer, especially because resistance to traditional cytotoxic and cytostatic drugs is now widespread. Better knowledge of the oncogenic process and cancer cell biology has revealed many potential new targets. One of these is the so-called chaperone machinery. This system of several proteins acting in concert exists in all mammalian cells and serves both to facilitate the correct folding of newly synthesized proteins and to refold proteins that have been denatured due to stress; hence some of the main components are named heat shock proteins. Many of the so-called "client" proteins of this chaperone system are involved in the oncogenic transformation or the stabilization of the cancer cell phenotype.

Among the actors in the chaperonema chinery, the protein hsp90 plays a central role and interference with its function can be considered as a "cluster bomb" touching many aspects of cancer cell function at the same time. Over the last two decades, many small molecules able to inhibit hsp90 have been synthesized with a view to cancer treatment, but only a few have entered clinical trials and none have been commercialized. There are two explanations for this. Firstly, most of hsp90 inhibitors are poorly water-soluble; secondly, because hsp90 is a ubiquitous protein, effects are also seen in normal cells. Nanomedicine could give a second chance to hsp90 inhibitors by improving their apparent solubility and reducing their side effects by delivering them more specifically to cancer cells [4].

2. Classification of Cancer

Tissue of Origin Benign Malignant

1. Tumours of one parenchymal cell type

A. Epithelial tumours:

1. Squamous epithelium- squamous cell carcinoma
2. Transitional epithelium-Transitional cell carcinoma
3. Glandular epithelium -Adenoma carcinoma
4. Basal cell layer skin-Basal cell carcinoma
5. Neuroectoderm- Naevus Melanoma
6. Hepatocytes -Liver cell adenoma
7. Placenta -Choriocarcinoma

B. Non-epithelial (Mesenchymol) tumours:

1. Adipose tissue-Lipoma
2. Adult fibrous tissue-Fibroma
3. Embryonic fibrous tissue-Myxoma
4. Cartilage-Chondroma
5. Bone-Osteoma
6. Synovium- Synovial sarcoma
7. Smooth muscle- Leiomyosarcoma
9. Mesothelium- Mesothelioma
10. Blood vessels-Haemangioma
11. Lymph vessels-Lymphangioma
12. Glomus-Glomus tumour
13. Meninges- Meningioma
14. Haematopoietic cells-Leukaemias
15. Lymphoid tissue-Malignant lymphomas
16. Nerve sheath- Neurogenic sarcoma
17. Nerve cells-Neuroblastoma

2. Mixed tumours:

Salivary glands -pleomorphic adenoma

3. Tumours of more than one germ cell layer: Totipotent cells in gonads or Mature teratoma, immature teratomain embryonal rests

3. Causes of Cancer

The majority of cancers, some 90–95% of cases, are due to environmental factors. The remaining 5–10% are due to inherited genetics.[5]. Common environmental factors that contribute to cancer death include tobacco (25–30%), diet and obesity (30–35%), infections (15–20%), radiation (both ionizing and non-ionizing, up to 10%), stress, lack of physical activity and pollution. Some hormones play a role in the development of cancer by promoting cell proliferation. [6] Insulin-like growth factors and their binding proteins play a key role in cancer cell proliferation, differentiation and apoptosis, suggesting possible involvement in carcinogenesis. [7]Hormones are important agents in sex-related cancers, such as cancer of the breast, endometrium, prostate, ovary and testis and also of thyroid cancer and bone cancer. For example, the daughters of women who have breast cancer have significantly higher levels of estrogen and progesterone than the daughters of women without breast cancer. Osteosarcoma may be promoted by growth hormones.

4. Signs and Symptoms

Local symptoms may occur due to the mass of the tumor or its ulceration. For example, mass effects from lung cancer can block the bronchus resulting in cough or pneumonia; esophageal cancer can cause narrowing of the esophagus, making it difficult or painful to swallow; and colorectal cancer may lead to narrowing or blockages in the bowel, affecting bowel habits.

Some cancers can cause a buildup of fluid within the chest or abdomen. General symptoms occur due to effects that are not related to direct or metastatic spread. These may include: unintentional weight loss, fever, excessive fatigue and changes to the skin. Hodgkin disease, leukemias, cancers of the liver or kidney can cause a persistent fever. Cancer can spread from its original site by local spread, lymphatic spread to regional lymph nodes or by hematogenous spread via the blood to distant sites, known as metastasis. When cancer spreads by a hematogenous route, it usually spreads all over the body. However, cancer 'seeds' grow in certain selected site only ('soil') as

hypothesized in the *soil and seed hypothesis* of cancer metastasis.

5. Pathophysiology

Cancers are caused by a series of mutations. Each mutation alters the behavior of the cell some somewhat.

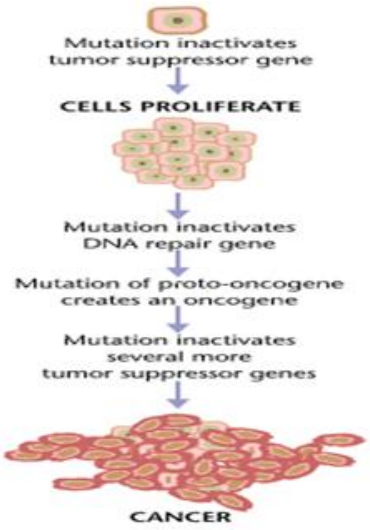

Figure 1

6. Diagnosis

Most cancers are initially recognized either because of the appearance of signs or symptoms or through screening. Neither of these leads to a definitive diagnosis, which requires the examination of a tissue sample by a pathologist. People with suspected cancer are investigated with medical tests. These commonly include blood tests, X-rays, CT scans and endoscopy.

7. Prevention

Cancer prevention is defined as active measures to decrease cancer risk.[8] The vast majority of cancer cases are due to environmental risk factors. Thus, cancer is generally preventable.[9] Between 70% and 90% of common cancers are due to environmental factors and therefore potentially preventable.[10]Greater than 30% of cancer deaths could be prevented by avoiding risk factors including: tobacco, excess weight/obesity, insufficient, diet, physical inactivity, alcohol, sexually transmitted infections and air pollution. Not all environmental causes are controllable, such as naturally occurring background radiation and cancers caused through hereditary genetic disorders and thus are not preventable via personal behavior. The primary dietary factors that increase risk are obesity and alcohol consumption. Diets low in fruits and vegetables and high in red meat have been implicated but reviews and meta-analyses do not come to a consistent conclusion. [11] NSAIDs reduce the risk of colorectal cancer; however, due to cardiovascular and gastrointestinal side effects, they cause overall harm when used for prevention. Aspirin has been found to reduce the risk of death from cancer by about 7%. inhibitors may decrease the rate of polyp formation in people with familial adenomatous polyposis; however, it is associated with the same adverse effects as NSAID, Daily use of tamoxifen or raloxifene reduce the risk of breast cancer in high-risk women. Vaccines have been developed that prevent infection by some carcinogenic viruses. Human papillomavirus vaccine (Gardasil and Cervarix) decrease the risk of developing cervical cancer. The hepatitis B vaccine prevents infection with hepatitis B virus and thus decreases the risk of liver cancer. The administration of human papillomavirus and hepatitis B vaccinations is recommended when resources allow.[12]

Surgery is the primary method of treatment for most isolated, solid cancers and may play a role in palliation and prolongation of survival. It is typically an important part of definitive diagnosis and staging of tumors, as biopsies are usually required. In localized cancer, surgery typically attempts to remove the entire mass along with,the lymph nodes in the area. For some types of cancer this is sufficient to eliminate the cancer. Radiation therapy involves the use of ionizing radiation in an attempt to either cure or improve symptoms. It works by damaging the DNA of cancerous tissue, killing it. To spare normal tissues (such as skin or organs, which radiation must pass through to treat the tumor), shaped radiation beams are aimed from multiple exposure angles to intersect at the tumor, providing a much larger dose there than in the surrounding, healthy tissue. The radiation can be either from internalsource or external sources. The radiation is most commonly low energy x-rays for treating skin cancers, while higher energy x-rays are used for cancers within the body.

8. Prognosis

Survival rates vary by cancer type and by the stage at which it is diagnosed, ranging from majority survival to complete mortality five years after diagnosis. Once a cancer has metastasized, prognosis normally becomes much worse. About half of patients receiving treatment for invasive cancer die from that cancer or its treatment.

9. HSP90

Structure of hsp90:

Based on the crystal structures of yeast Hsp90 and Grp94 (Hsp90 isoform in mammalian endoplasmic reticulum Hsp90 exists as a homodimer, each monomer consisting of three highly conserved domains: an N-terminal ATP-binding domain (25 kDa), a middle domain (35 kDa) and a C-terminal dimerization domain (12 kDa). The major role of the middle domain is to discriminate various types of client proteins to adjust the molecular chaperone for proper substrate activation. The C-terminal dimerization domain strengthens the weak association between the two N-terminal domains of the Hsp90. The C-terminal domain of eukaryotic Hsp90 has a conserved pentapeptide (MEEVD) implicated in binding to the tetratricopeptide repeat (TPR) domain of cochaperones, such as Hop (Hsp organizing protein) and Sti1 (stress-inducible protein 1, yeast homologue of Hop)

The “open” state of the Hsp90 dimer, with its two N-

termini separated, can capture client proteins .ATP binding triggers the closure of the ATP pocket "lid" and brings the N-termini close to each other, resulting in the formation of a compacted, ring-shaped Hsp 90 dimer. These conformational alterations lead to a "closed" state to "clamp" client proteins inside. The ATPase activity of Hsp90 itself drives the chaperone cycle

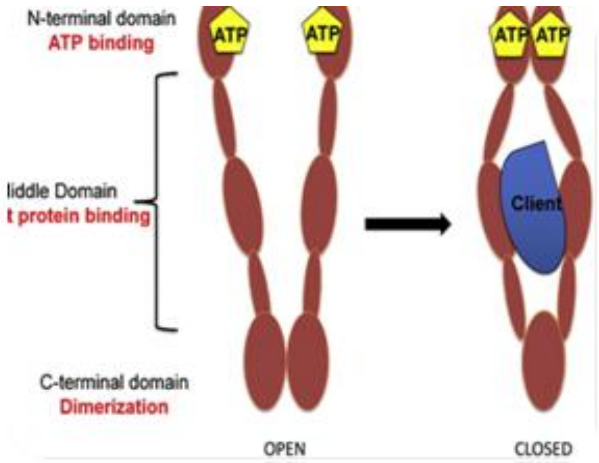

Figure 2

Function of hsp90:

- Hsp90 is a ubiquitous, well conserved and abundant dimeric protein that facilitates the repair and proper refolding of proteins that have undergone stress (such as pH changes, temperature variations, hypoxia or cytokine release resulting from tissue injury) that could modify their structure and thereby their function.
- Hsp90 has a very important role in cell homeostasis and cytoprotection in various stress situations.
- It is involved in the folding of more than 300 proteins this function depends on its dimeric structure.
- The hsp90 monomer consists of three main domains: a N-terminal domain, the binding site for ATP the hydrolysis of which, mediated by hsp90 and its co-chaperones is the driving force for hsp90 function; a middle domain (M) where client proteins and co-chaperones bind; and a C-terminal domain, another binding site for co-chaperones, responsible for hsp90 dimerization .
- Substrate binding to the middle domain induces conformational changes of hsp90 via interactionwith co-chaperones and ATP hydrolysis leading to a "closed" conformation. In this state, hsp90 can exert its activity .

Mechanism:

The Hsp90 multi-chaperone system has been extensively studied in the maturation of steroid receptor on yeast Hsp90. The chaperone cycle begins with a newly synthesized or misfolded steroid receptor binding to Hsp70/Hsp40 complex, associated with the "open" state Hsp90 via the bridging cochaperone Hop that interacts simultaneously with Hsp90 and Hsp70. Hop not only binds to the C-terminal MEEVD motif of Hsp90, but also connect with the N-terminal region of Hsp90, preventing the Hsp90 N-terminal domain association. Hop therefore inhibits the ATPase activity and promotes client transfer from Hsp70 to Hsp90. Upon ATP binding to Hsp90, Hop is replaced by p23 and immunophilins, converting the intermediate chaperone complex into the mature complex. Another cochaperone, Aha1 (activatior of Hsp90 ATPase), associates with the middle domain of Hsp90, facilitating conformational adjustments to favor ATP binding. Both Aha1 and immunophilins stimulate the ATPase activity of Hsp90 Upon ATP hydrolysis, the correctly-folded client protein is released from Hsp90. Recent studies have shown that the mechanistic basis of the Hsp90 chaperone cycle are conserved in yeast and human, although a slower turnover rate was observed with human Hsp90.

HSP90 Target in Cancer Therapy:

The feasibility of targeting Hsp90 for cancer therapy is well supported:

1. First, Hsp90 is involved in the maturation and stabilization of a wide range of *oncogenic* client proteins crucial for oncogenesis and malignant progression, making cancer cells particularly dependent on proper Hsp90 function The harsh environmental conditions found in tumors such as hypoxia, low pH, and bad nutritional status may tend to destabilize proteins, making them even more dependent on Hsp90 activity. The extraordinary reliance of tumor cells on Hsp90 is consistent with a report that Hsp90 comprises as much as 4–6% of total proteins in tumor cells in contrast with the 1–2% in normal cells.

2. Another explanation for tumor selectivity of Hsp90 inhibitors comes from the observation that in cancer cells Hsp90 predominantly exists as *multi*-chaperone complex with unusually high affinity for ATP and drug, whereas in normal cells most Hsp90 is present in an uncomplexed or latent state. Hsp90 derived from tumor cells has an approximately 100-fold higher binding affinity for 17-AAG than does Hsp90 isolated from normal cells .Finally, tumor-specific accumulation has been observed for a number of Hsp90 inhibitors, such as 17-AAG, 17-DMAG, IPI-504, radicicol derivatives and purine-scaffold inhibitors .Thus, this selectivity may not be due to the structural or physicochemical properties of a specific class of compounds, but rather to properties of Hsp90 itself. Although the mechanisms underlying the tumor selectivity of Hsp90 inhibitors are not fully understood, Hsp90 has become validated as a potential target in cancer therapy. Furthermore, preclinical and clinical evaluation of a plethora of Hsp90 inhibitors have already shown promising results as a single agent and/or in combination with chemotherapy.

Among hsp90 client proteins, several are involved in the so-called "hallmarks of cancer", first proposed by Hanahan and Weinberg and therefore contribute to tumorigenic cell stabilization. These proteins have a major role in cancer, and include protein kinases, telomerases and steroid hormone receptors as well as transcription factors . Therefore, proliferation, apoptosis and metabolism pathways are all concerned by the chaperoning of hsp90.

The accumulation of mutated and dysregulated onco-proteins has been associated withmodification of hsp90 properties. Some controversy remains but a number of observations can be cited. although the hsp90 protein can be induced during the transformation process to allow the cell to survive in the microenvironment, it appears to be only 2–3 fold overexpressed compared with non-tumour cells. Finally, as client onco-proteins display stable association with hsp90, the latter ismore activated in tumour cells hence a higher affinity for inhibitors. These singular properties make the targeting of hsp90 a highly promising approach to cancer therapy since the inhibition of a single target can result in multiple effects on oncogenesis-associated phenomena. When the function of hsp90 is inhibited, the client protein cannot be properly stabilized and will be targeted to the proteasome by the ubiquitin-ligase system. As a result, oncogenic proteins involved in the cancer hallmarks are degraded, leading to the possibility of various anticancer effects such as cell growth arrest and apoptosis induction. Importantly, several studies have demonstrated a preferential interaction between the N-terminal inhibitor 17-allylaminogeldanamycin (17-AAG) and hsp90 in cancer cells compared with healthy cells, leading to a selective anti-cancer effect. Hsp90 can be targeted by different families of inhibitors, which act mainly on the N-terminal or the C-terminal domain. The N-terminal domain containing the ATP binding site has been shown to be the binding site of the antitumour antibiotics Geldanamycin (GA) and radicicol. Although GA showed anti-cancer activity on a large panel of cell lines, its low solubility, poor bioavailability and severe hepatotoxicity prevented it from reaching clinical trials. To overcome these difficulties, new derivatives were synthesized with chemical modifications on position 17; among those, 17-AAG, the first to enter clinical trials, showed very interesting results while another derivative, 17-(2dimethylaminoethyl)amino-17 demethoxy geldanamycin (17-DMAG), showed better solubility and potency and also reached clinical trials . Purine scaffolds have also been specifically designed to target the ATP-binding site and have shown a similar hsp90 binding affinity to 17-AAG .N-terminal inhibitors maintain hsp90 in the "open" conformation, thereby preventing the folding of client proteins that are then degraded by the ubiquitin-proteasome system. However, a disadvantage of N-terminal inhibition of hsp90 is that it triggers HSF-1 release and thereby activates a HSR (induction of hsp70 and 90), leading to resistance to treatment and counteracting the inhibition effect. Therefore, it appears very interesting to target the Cterminal domain since its inhibition does not release HSF-1 and efforts have thus been made to synthesize active compounds that can bind to this domain. A knownDNA gyrase inhibitor used as an antibiotic, novobiocin (nvb), showed an ability to bind to the C-terminal domain ofhsp90, leading to the degradation of several client proteins. It has been hypothesized that the site of interaction of nvb on the C-terminal domain might be a second ATP binding site, but this has not been proved.In fact, nvb has a low affinity for hsp90 (~700 μM in SKBR3cells); therefore, many coumarin derivatives were synthesized with the aim of improving binding to the C-terminal domain . Starting from the chemical structure of nvb, Audisio et al. have synthesized quinolinone derivatives. Among these molecules, 6BrCaQ has shown very interesting activity against a number of cancer cell lines in the micromolar range as well as potent inhibition of hsp90 as revealed by client protein degradation. Other inhibitors have been designed to target this domain, for example the cyclic peptide analogues (SM122, SM145 and SM253) that block the binding of TPR co-chaperones. Recently, the N terminal inhibitor X66 has also been shown to avoid activating the HSR which calls into question the systematic HSR after treatment with N-terminal inhibitors.

Relationship between HSP90, CDC37 and Protein Kinase Clients: Protein kinases are the largest class of Hsp90 clients, with a similar chaperone cycle. Cdc37 was originally discovered in yeast as an essential cell cycle protein, and later it was proved to be a kinase-specific cochaperone of Hsp90. The Hsp70/Hsp40 complex first prepares a newly synthesized or misfolded protein kinase for interaction with the N-terminal domain of Cdc37, followed by recruitment of Hsp90 to the complex with the help of Hop. The C-terminal side chain of Cdc37 associates with the "lid" of Hsp90, which closes the N-terminal ATP binding pocket . This holds Hsp90 in an "open" conformation in the intermediate complex, allowing later client loading. Although the release of Cdc37 C-terminus from Hsp90 N-terminal clamp is required for the transition of the "open" to the "closed", mature conformation, Cdc37 could stay in the complex by interacting with the client protein. Other cochaperones, such as p23 and Aha1, may be required as well. More details of kinase maturation in the complex remain to be understood.

Hsp90 inhibitors targeting the ATP Binding Site

Natural compounds and their derivatives

Benzoquinone ansamycins, represented by geldanamycin were the first class of natural Hsp90 inhibitors to be discovered and substantially studied. Geldanamycin, a natural occurring antibiotic, was originally isolated from Streptomyces hygroscopicus as early as 1970s. Structural and biochemical studies demonstrated that GA is a competitive inhibitor of ATP binding to Hsp90. Binding of GA in the N-terminal ATP pocket restrains Hsp90 in its ADP-bound conformation and prevents the subsequent "clamping" of Hsp90 around a client protein, resulting in ubiquitination and proteasomal degradation of the client. This N-terminal ATP pocket has distinctive characteristics in comparison with most other nucleotide-binding proteins, which explains the selectivity of GA.Chemical structures of the natural product Hsp90 inhibitors. Although GA exhibited potent anti-cancer activities in preclinical in vivo studies, it was determined to have little clinical potential mostly due to the high hepatotoxicity observed in animal models. As a result, this has encouraged the search for GA derivatives that maintain similar anti-cancer activities but with better toxicological properties. 17-AAG (17-allylamino-17-desmethoxygeldanamycin; tanespimycin, KOS-953), and more recently 17-DMAG (17-dimethylaminoethylamino-17-demethoxygeldanamycin) and IPI-504 (17-allylamino-17-demethoxygeldanamycin hydroquinone hydrochloride) were therefore synthesized

for further evaluation [13].A considerable amount of understanding has been obtained from the clinical experience of 17-AAG . 17-AAG entered Phase I trials in 1999 [14]. and several intravenous formulations have completed Phase I testing [15]. Early signs of therapeutic activity have been seen in melanoma, breast cancer, prostate cancer, and multiple myeloma [16]. Phase II clinical trials for 17-AAG are currently ongoing.[17] 17-AAG was recently administered in combination with trastuzumab in patients whose disease progressed following trastuzumab treatment; this trial has demonstrated promising anti-tumor activity and acceptable toxicity. [18] However, several drawbacks of 17-AAG, including low water-solubility, instability in solution, and low oral bioavailability. may become the obstacle to further clinical application.In addition to 17-AAG, other GA derivatives have been developed for clinical use as well. Currently 17-DMAG, a more water-soluble analogue of 17-AAG, has entered Phase I and Phase II clinical testing, and displayed higher oral bioavailability, lower toxicity, and increased stability compared with 17-AAG. Another water soluble hydroquinone hydrochloride analogue of 17-AAG is IPI-504. IPI-504 is in Phase I and Phase II clinical trials to evaluate its potential for treating cancer that has become resistant to therapy with tyrosine kinase inhibitors, such as Philadelphia chromosome-positive chronic myelogenous leukemia (CML) [19].New natural product scaffolds are being discovered and tested. A recent example is the isoflavone derrubone from the Indian tree Derris robusta . A green tea polyphenol catechin, epigallocatechin 3-gallate (EGCG) , was shown to inhibit the transcriptional activity of aryl hydrocarbon receptor (AhR) through a mechanism involving direct binding of EGCG to the C-terminus of Hsp90. It remains unclear whether EGCG could inhibit Hsp90 function through this direct binding. These findings may provide new natural product scaffolds to facilitate the development of novel Hsp9inhibitors.

Potential resistance to ansamycins:

Thus, encouraging clinical responses have confirmed the potential of targeting Hsp90. However, binding of these ansamycin drugs not only prevents ATP binding but also induces a stress response through the release, activation, nuclear localization and trimerization of heat shock factor-1 (HSF-1), a transcription factor that binds heat shock elements (HSE) to increase the mRNA and protein levels of Hsp70. This stress-responsive up-regulation of Hsp70 is believed to reduce the Hsp90-targeted drug efficacy by inhibiting apoptosis signaling. Furthermore, these ansamycins are P-glycoprotein (Pgp) substrates. Interestingly, a very recent study suggested that HSF-1-mediated stress induction, such as accumulation of Hsp70, may play a more important role in resistance to 17-AAG than drug efflux by Pgp, since depletion of Hsp70, but not P-gp inhibition increased the sensitivity of 17-AAG resistant cells to 17-AAG. These findings encourage the investigation of combining Hsp90 inhibitors, particularly of the ansamycin class, with abrogation of Hsp70 induction to enhance the clinical efficacy of Hsp90-targeted therapy.

Synthetic small molecules and peptide derivatives

The search for natural Hsp90 inhibitors has been accompanied by a search for synthetic small molecule inhibitors that potentially possess more specific targeting and better pharmacological profiles. The availability of crystal structures of the Hsp90 N-domain and the development of structure-based design and high-throughput screening (HTS) assays have prompted successful exploitation of a range of new synthetic scaffolds that have Hsp90 inhibitory capacity which mimics the unique shape adopted by natural nucleotide ligand inside of the N-terminal pocket of Hsp90. The favorable interaction between PU3 and the ATP pocket of Hsp90 was visualized in crystal structure, and consistently the similar biological effects of PU3 with geldanamycin were observed. PU3 thus became a starting point for the expansion and the improvement of clinically applicable purine-scaffold Hsp90 inhibitors, where efforts aredirected toward either positionC8or C9 of purine. These synthetic derivatives could be roughly categorized to 8-benzyl, 8-phenylsylfanyl, 8-(7′-substituted benzothiazolothio, and 9-benzyl purine derivatives. Major improvements of purine-scaffold Hsp90 inhibitors include insensitivity to multi-drug resistance, favorable water solubility, oral bioavailability, and metabolic stability. For instance, PU-H71 and PU-DZ8 are currently under advanced preclinical investigation. CNF-2024, an orally available 9-benzyl purine derivative with low nanomolar potency, has entered Phase I clinical trials, tested in chronic lymphocytic leukemia, advanced solid tumors, lymphomas, and more recently in advanced breast cancer.Chemical structures of synthetic small molecule Hsp90 inhibitors. The molecular scaffolds are depicted in blue. Pyrazole is another important class of synthetic small molecules that has been identified A molecule of 3, 4-diaryl pyrazole (CCT018159) binds deeply into the N-terminal ATP pocket of Hsp90, as shown in crystallographic studies. Similar with GA, a decrease in Hsp90 client protein levels (Raf-1 and Cdk4) and an increase in Hsp70 expression were observed in CCT018159-treated cancer cells. Structure-based design generated more potent pyrazole amide CCT0129397 and isoxazole CCT0130024; and an optimized analogue NVP-AUY922 has just entered clinical trials. Finally, a novel family of short peptide derivatives composed of the three core amino acids, Phe-D-Trp-Leu, at the center of the molecule was recently reported to inhibit ATP binding to Hsp90 . They killed various cancer cell lines, but not normal cells *in vitro* and did not show toxicity *in vivo*. A few of these peptide derivatives were reported to cause a marked inhibition of multidrug resistance in human *MDR1* gene-transfected mouse lymphoma cells**.**

HSP90 inhibitors targeting co-chaperone/hsp90 interactions: Hsp90 requires a series of co-chaperones to assemble a super-chaperone complex for its function. These co-chaperones bind and leave the complex at various stages to regulate the chaperoning process. Arresting the chaperone cycle at these stages by targeting different cochaperone/Hsp90 interactions is likely to achieve similar consequences with the direct inhibition of Hsp90. Blockade of ATP binding seems to be the most direct and the simplest way to manipulate Hsp90, but its intrinsic non-

selectivity for the Hsp90 clientele may limit its further application. Therefore, a potentially more specific approach is to develop drugs that block the interaction between Hsp90 and cochaperones. Availability of crystal structures of co-chaperone/Hsp90 interactions plays a critical role in this process.

Targeting the cdc37/Hsp90 interaction

Cdc37 has a specialized and indispensable role in the maturation of a kinase sub-population of clients, including receptor tyrosine kinases such as epidermal growth factor receptor (EGFR), non-receptor tyrosine kinases Src and lymphocyte-specific protein tyrosine kinase (Lck), and intracellular serine/threonine kinases such as Raf-1and Cdk4. Cdc37 acts as an adaptor, loading these kinases onto the Hsp90 complex, thereby facilitating their maturation; Depletion of Cdc37 using RNAi in human colon cancer cells diminished the association of kinase clients with Hsp90 and decreased the levels of these clients including Her-2, Raf-1, Cdk4, and Akt, leading to reduced cell proliferation. Another noteworthy observation that silencing of Cdc37 did not induce Hsp70 up-regulation suggests that targeting Cdc37 might be advantageous compared with some Hsp90 inhibitors that induce Hsp70 accumulation. The inhibition of Cdc37/Hsp90 interaction would probably represent an approach that may offer greater specificity and an improved side-effect profile Indeed, our group has recently reported that celastrol, a quinone methide triterpene from Tripterygium wilfordii Hook F, exhibits anti-pancreatic cancer activity both in vitro and in-vivo through the disruption of Cdc37/Hsp90 association and the subsequent degradation of Hsp90 client proteins .Celastrol was identified to block Cdc37 interaction with Hsp90 by molecular modeling, taking advantage of the available crystal structure in which Cdc37 associates with the N-terminal domain of Hsp90 by inserting its C-terminal side chain into the mouth of the ATP binding pocket.

(A) Chemical structure of celastrol. (B) Molecular docking of celastrol with Hsp90-Cdc37 complex. Ribbon view of the Hsp90-Cdc37-celastrol binding pocket.

Targeting the Hsp70/Hsp90 interaction

Assembly of Hsp70/Hsp90 complex is achieved by associations of their C-terminal tails with two independent TPR domains of Hop, TPR1 and TPR2A, respectively. These interactions, especially the one between TPR2A of Hop and the C-terminal MEEVD motif of Hsp90, are essential for function of this multi-molecular complex. Recently, a designed TPR module, CTPR390+, which binds to the Hsp90 C-terminus with higher affinity and more specificity than TPR2A, was shown to be capable of competing with endogenous TPR2A for Hsp90 binding and thereby to prevent the formation of Hsp70/Hsp90 complex, triggering Her-2 degradation with consequent inhibition of breast cancer cell proliferation. Furthermore, a high-throughput Alpha Screen assay was developed to identify novel small molecules that hinder the Hsp90/TPR2A interaction and the identified compounds were shown to inhibit the growth of breast cancer cells, which was correlated with a reduced level of Her-2. it is evident that these novel compounds will have modest selectivity for tumor versus non-tumor cells.

Targeting the Hop/Hsp90 interaction

In addition, recent work revealed that whilst the primary binding site of Hop is the C-terminal MEEVD peptide of Hsp90, binding also occurs at additional sites in the C-terminal and middle domains of Hsp90. The feasibility of targeting Hop/Hsp90 interaction still needs to be addressed.

Targeting the Aha1/Hsp90 interaction

According to crystallogical studies, the N-terminal domain of Aha1 interacts with the middle segment of Hsp90 and triggers the ATPase activity of Hsp90. Similar to Cdc37, Aha1 knockdown was recently reported to decrease the activation status of Hsp90 clients and a synergistic interaction was observed between Aha1 depletion and 17-AAG in inhibition of cancer cell growth, which indicates that modulation of Aha1 might be another potential therapeutic strategy.

Hsp90 inhibitors targeting client/Hsp90 associations

Inhibition of client/Hsp90 interactions offers the ultimate selectivity, but little is known about the molecular basis for these interactions. The key to targeting the client/Hsp90 interaction is the ability to study the structure and biochemistry of the molecular complexes. An Hsp90/Cdc37/Cdk4 complex has been purified and its three dimensional structure has been determined by electron microscopy, providing the first structural view of the interaction between a client protein and Hsp90. Shepherdin, a peptidomimetic, was designed to specifically block the interaction between Hsp90 and the anti-apoptotic client survivin. However, shepherdin can interact with the ATP pocket of Hsp90 as well, and affects a range of Hsp90 clients in addition to survivin . Although shepherdin did exhibit anti-leukemia activity in animal models, its apparent interaction with the ATP pocket of Hsp90 and the effect on a range of Hsp90 clients suggests that it may have a different mode of action.

Post-Translational Modifications of HSP90: Post-translational modifications, such as hyperphorphorylation, S-nitrosylation and reversible hyperacetylation, have been thought to be involved in regulating chaperone function of Hsp90 through affecting co-chaperone association and/or ATP binding. The post-translational modifications of Hsp90 may open up a wide range of opportunities to indirectly interfere with its activity

HSP90 hyperacetylation: Several studies have correlated histone deacetylases (HDACs) with Hsp90 chaperone function. HDACs, in concert with acetyltransferases (HATs), control reversible acetylation of lysine residues on Hsp90, which regulates Hsp90 activity. Lysine K294 in the middle domain of Hsp90 has been recently identified to be an important acetylation site. A very recent work identified p300 as one of the HATs involved in acetylating Hsp90. Hyperacetylation of Hsp90 has been reported after treatment with a variety of pan-HDAC inhibitors, such as LAQ824 and LBH589. HDAC6, among the 18 HDAC family members identified so far, is unique in that it deacetylates *non*-histone proteins, such as Hsp90 and α-tubulin. Specific knockdown of HDAC6 using RNAi enhanced the degree of Hsp90 acetylation and hyperacetylation of Hsp90 by either pan-HDAC inhibitors or HDAC6 knockdown was associated with a reduced

binding of ATP and/or co-chaperones to Hsp90, thus promoting the degradation of Hsp90 client proteins. Also, HDAC1 may contribute to deacetylation of Hsp90. MS-275, a novel synthetic selective HDAC1 (and not HDAC6) inhibitor induces apoptosis of acute myeloid leukemia (AML) cells expressing mutant fms-like tyrosine kinase 3 (FLT3). MS-275, by inducing hyperacetylation of Hsp90 in these leukemia cells, inhibits the direct interaction between Hsp90 and FLT3, by its proteasome-dependent degradation. Overall, the hyperacetylation of Hsp90 was shown to inhibit binding of ATP, co-chaperone p23 and client proteins to Hsp90, directing the latter to polyubiquitylation and proteasomal degradation. Therefore, *inhibition* of *either* one of the two HDAC family members would probably be associated with hyperacetylation of Hsp90 and consequent impairment of Hsp90 chaperoning functions. In addition, siRNA-mediated depletion of HDAC6 improved the affinity of 17-AAG for Hsp90 through enhancing the degree of Hsp90 acetylation.

HSP90 thiol oxidation

A new type of inhibitor, tubocapsenolide A (TA) was recently isolated from Tubocapsicum anomalum and shown to have potent anti-cancer activity in various human cancer cell line. It was suggested that TA rapidly and selectively induced thiol oxidation of Hsp90 and Hsp70 respectively, thereby inhibiting the chaperone activity of the Hsp90/Hsp70 complex; indeed the TA-induced effects could be prevented by N-acetylcysteine, a thiol anti-oxidant. The TA activity was at least in part attributed to proteasomal degradation of Hsp90 clients such as Cdk4, cyclin D1, Raf-1, Akt and mutant p53. This finding may open up another window for the targeting of Hsp90.

HSP90 phosphorylation

It is known that phosphorylation negatively regulates Hsp90 chaperoning function and disruption of a phosphatase responsible for Hsp90 phosphorylation resulted in inhibition of Hsp90 function. However, the role of site-specific phosphorylation in modulating Hsp90 function has not yet been clarified.

Cell Surface HSP90 and Tumor Metastasis

Over the past years, most attention has been given to the study of the *intra*cellular Hsp90. However, a pool of Hsp90α has been described to be loosely attached to the cell membrane and facing the *extra*cellular space, which was associated with tumor cell invasion. A range of conditions, such as serum starvation, hypoxia, high concentration of glucose, as well as oxidative stress have been shown to trigger the *extra*cellular localization of Hsp90α. It remains unknown how Hsp90α reaches the cell membrane and/or the extracellular environment. One possible explanation is that Hsp90α might be secreted via an exosome pathway, as it was reported to be present in exosomes. Although Hsp70, p23, Cdc37 and Hip are present in the extracellular space of tumors as well, their function there has not been explicated. In addition, whether ATP binding and hydrolysis are required for an extracellular chaperone function of Hsp90α remains to be determined. Cell surface Hsp90α was described to serve as a molecular chaperone of matrix metalloprotease 2 (MMP-2), an extracellular enzyme essential for cell invasion, to assist the maturation of this enzyme. This surface interaction is necessary for Her-2 activation and heregulin-induced heterodimerization with ErbB-3, which in turn mediates signal transduction pathways vsia MAPK and PI3K-Akt, leading to cytoskeletal actin re-arrangement essential for cell motility. Inhibition of cell surface Hsp90α with antibodies or cell-impermeable inhibitors could block cell motility and invasion *in vitro* and metastasis *in vivo*. Although DMAG-N-oxide lacks the growth inhibitory ability of cell-permeable Hsp90 inhibitors, it displays significant anti-invasion activity *in vitro* and anti-metastasis activity *in vivo*. Moreover, it was described that the selective blockade of cell surface Hsp90α with a monoclonal antibody, mAb 4C5, could lead to specific disruption of the extracellular Hsp90α/Her-2 interaction, in turn preventing Her-2/ErbB-3 heterodimerization, actin reorganization, and consequent cell invasion. Interestingly, disruption of the extracellular Hsp90α/Her-2 interaction did not lead to a reduced level of Her-2 protein on the cellular membrane; instead, it significantly reduced the phosphorylation of Her-2, suggesting that this extracellular interaction is different from the previously characterized intracellular interaction. These findings suggest that blockade of cell surface Hsp90α may have clinical benefit in limiting cancer cell invasion and metastasis. Recently K69 was identified as one of the acetylated lysine residues on cell surface Hsp90α hyper acetylated by pan-HDAC inhibitor LBH589; and hyperacetylation actually stimulates not only the extracellular localization of Hsp90α, but also promotes its association with MMP-2, resulting in an increased tumor cell invasion. This study further demonstrated that exposure of breast cancer cells to a novel antibody against the acetyl-K69 Hsp90α could markedly inhibit *in vitro* invasion compared with the inhibitory effect of an anti-Hsp90α antibody that non-specifically recognizes both acetylated and unacetylated Hsp90α.Taken together, these intriguing findings raise the possibility that cell surface Hsp90α plays an important role in modulating cancer cell invasion and metastasis that is *in*dependent of the intracellular Hsp90 function, providing a novel extracellular drug target for metastatic cancer therapy.[20]

10. Conclusion

The most attractive advantage of targeting Hsp90 is the combined impact on many oncogenic pathways involved in multiple steps of carcinogenesis and cancer progression, as Hsp90 inhibition eventually leads to the ubiquitin-proteasome degradation of a large population of oncogenic client proteins. In summary, the targeting of Hsp90 for anti-cancer therapeutics has a potentially bright future. Further progress in the development of Hsp90 inhibitors and a deeper understanding of the Hsp90 characteristics further strengthen its promise in cancer therapy. Heat shock protein[Hsp90] is conserved and constitutively expressed molecular chaperone and it has been show to stabilize oncoproteins and facilitate cancer development.Hsp90 is an interesting target for cancer therapy because it is involved in the folding and stabilizing of numerous proteins, include many that contribute to the development of cancer.

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