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REVIEW

HSP90: Chaperone-me-not

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Abstract With increasing understanding of the molecular basis of carcinogenesis, its progression and metastasis, the cancer therapy has shifted from empirical approaches to targeting specific molecules that regulate the complex network of signalling pathways for cell survival and proliferation. These include key players in malignant transformation like protein kinases, transcription factors, steroid hormone receptors, cell cycle regulators, signal transduction proteins and regulators of apoptosis. Almost all these proteins depend upon the molecular chaperone Hsp90 for their proper folding, stability and function and thus are a part of the Hsp90 clientele. Dependence of these proteins on Hsp90 makes this chaperone an appealing target for cancer therapeutics. Inhibition of Hsp90 can affect multiple oncogenic pathways simultaneously. Moreover Hsp90 inhibitors selectively kill cancer cells compared to normal cells and cancer cells have greater dependence on Hsp90 for the maintenance of intracellular protein homeostasis. All this has led to a rapid pace discovery of Hsp90 clients as well as chemical inhibitors of Hsp90. The role of hsp90 in cancer, tumor selectivity of Hsp90 inhibitors and the current status of Hsp90 inhibitors are discussed in the present review.

Keywords Hsp90 · Cancer hallmarks · Hsp90 inhibitors · Tumor selectivity · Client proteins · Inhibitor classes

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Introduction

Cells progress to malignancy due to repeated mutations and the retention of these mutations over generations [1]. Such genetic plasticity makes it difficult to target the cancer cells due to the ever-changing domains of molecular targets. Most of the solid tumour cells are genetically so diverse, that a single molecular target therapy is completely ineffective [2]. Also in the presence of complex interactive network of signalling pathways, disruption of only one or two targets may not abrogate the malignant phenotype [3]. A simultaneous attack on many signalling pathways and molecular players of these pathways is more likely to jeopardize the survival of cancer cells than a single target. However multiple molecular targets used in combination, make therapy more complicated and prolong trials. Instead, pharmacological manipulation of a single molecule, involved in a multitude of signalling pathways leading to simultaneous adverse effects on oncogenic proteins, would prove promising. Hsp90 (Heat shock protein 90), a member of the chaperone family of proteins is one such molecule.

Due to its protective role Hsp90 is overexpressed in stressful environment of cancerous cells. Hsp90 also permits accumulation of mutations, acting as a buffer of genetic variation. It is needless to say how such a molecule can prove to be an asset for genetically unstable cells to survive in the presence of high mutation rates.

Structure

Hsp90 is a molecular chaperone that plays a role in the folding and assembly of other proteins. It binds to substrate proteins at a 'client' site and encourages folding into the proper conformation and prevents aggregation. The binding and release of polypeptides is accompanied by hydrolysis of ATP to ADP and Pi. The structure and function of HSP90 has been elucidated and described in considerable detail with the aid of biochemical evaluation and X-ray crystallography. Hsp90

protein is a flexible homodimer. Each monomer has three subunits, the N terminal domain (N), the middle domain (M) and the C terminal domain (C) [4, 5].

In eukaryotes, the N-terminal and middle domains are connected by a charged linker. Thus the M domain is the client-binding site. The C-terminal is the dimerization domain and the C domains of the two monomers interact with each other to form the dimer. The eukaryotic C-terminal is implicated in binding to co-chaperones, which support Hsp90 in folding and also in the activation of its substrate proteins. The ATP hydrolysis site is located at the junction of the N and M domains. The chaperone cycle requires ATP binding and hydrolysis. The N terminal domains are free in the absence of the nucleotide and associate with each other on ATP binding leading to transition from open V shaped conformation to closed ring shaped structure [5, 6]. Thus Hsp90 opens and closes to capture and release the substrate proteins (Fig. 1) [7, 8]. Hydrolysis of ATP leads to opening of the clamp by the separation of the N terminals. The chaperone cycle requires the coordination of other co-chaperones like the Hsp70/Hsp40, Cdc37, Hop [4], p23 and Aha1 [5].

It has been pointed out that, the N-terminal domain ATP-binding site of hsp90 is very unique in its shape compared to the other ATP-binding proteins [4]. This fact to some extent explains the high molecular selectivity of most HSP90 inhibitors [9]. Hence the ATPase activity of Hsp90 has been targeted previously to inhibit the chaperone. Most inhibitors of Hsp90 share the property of displacing ATP from its binding domain. Interference of Hsp90 activity, targets the client proteins to degradation by the proteosome machinery. The central role of HSP90 in chaperoning cancer was first identified in 1994. With increased understanding of ATP dependence of Hsp90 chaperoning and the structural characterization of the binding site in 1997, almost 200 different Hsp90 client proteins have been identified. Similarly vast data is accumulating about Hsp90 inhibitors, many of which have entered the Phase I or II clinical trials in cancer.

The present review gives a comprehensible account of the role of HSp90 in cancer, dependency of tumor cells on Hsp90 chaperoning and the potential of this chaperone as a molecular target in cancer therapy. The current status of Hsp90 inhibitors is discussed on the basis of several informative reviews published in the past few years. We try to identify gaps in the current understanding and highlight areas for future research.

Hsp90 in Cancer

The goal of any therapeutic strategy is to produce impact on the target tumor cells with limited detrimental effect on normal cell functioning [10]. A greater understanding of the hallmark capabilities and the multiple biochemical pathways involved in regulating these [11, 12], has lead to the recognition of Hsp90 as an important target in cancer therapy.

Aetiology of cancer is associated with a plethora of signal transduction and other pathways [7]. Most of the regulatory proteins of the intracellular circuitry are as diverse as the pathways themselves. However, there is one common factor that places most of these in the same boat. That is their dependence on the molecular chaperone Hsp90 for their stability and folding. Which means that manipulation of Hsp90 function may produce an impact on more than one pathway simultaneously. This possibility is what makes Hsp90 a promising and attractive target for cancer therapy.

Hsp90 is one of the most abundant cell chaperone proteins. It accounts for 1–2 % of the total protein in unstressed cells

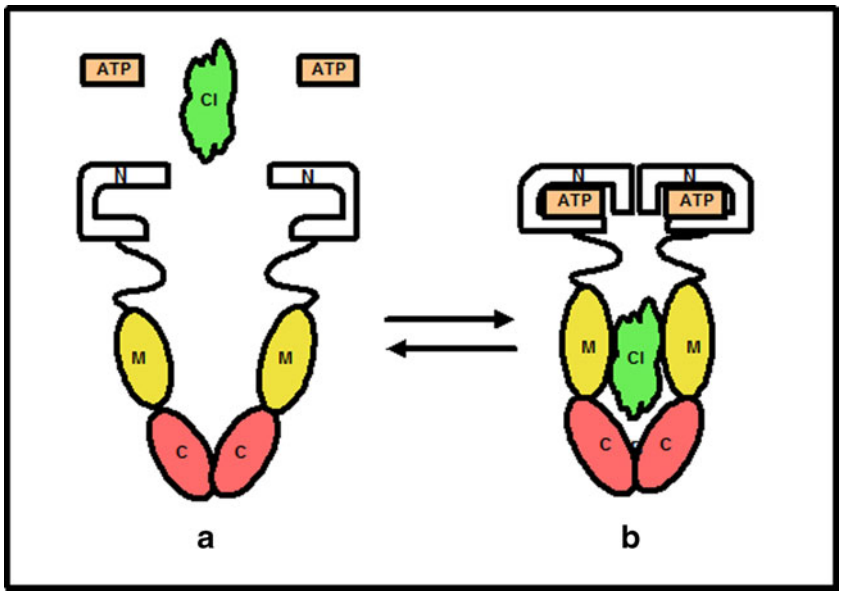

Fig. 1 Diagrammatic representation of Hsp90 chaperone cycle. **N**- N terminal Domain, **M**-Middle Domain, **C**- C terminal Domain, **Cl**- Client. The chaperone acts like a clamp that opens and closes in response to ATP hydrolysis and binding. **a** Open conformation, **b** closed conformation

and increases to 4–6 % of cellular proteins under stress [3]. In face of the multitude stresses, expression of Hsp90 in cancerous cells is two to three fold higher than normal cells and is co-related to poor prognosis [9, 13–16]. Hsp90 characteristically chaperones a number of mutated or chimeric kinases that are key mediators of the disease.

Role of Client Proteins

Malignant transformation involves the over-expression and/or the mutation of several modulators of signal transduction pathways. A considerable number of these modulators are Hsp90 clients. Almost 50 proteins known to play important roles in the control of cell cycle and growth, including receptor protein kinases and transcription factors have been identified as *bona fide* clients of HSP90 [1, 15–18]. The Hsp90 clients can be grouped in three main classes namely the protein kinases, transcription factors and a miscellaneous group of structurally unrelated clients [3, 7]. For an updated record of Hsp90 clients refer to the website http://www.picard.ch/downloads/Hsp90interactors.pdf. Hsp90 is indispensible for the maintenance of its client proteins in an active conformation and thereby is believed to chaperone cells to immortality and malignancy [4, 19–21]. It stablizes both wild and mutant forms of its client proteins like transcription factors, steroid hormone receptors, protein kinases, regulators of apaoptosis and pro-angiogenic proteins [11, 16, 22–25].

Thus HSP90 has a crucial role to play in almost all hallmark traits of oncogenesis, such as self-sufficiency in growth signals, evasion of death signals and angiogenesis [2, 26, 27]. It also allows tumor cells to tolerate otherwise lethal mutations by stabilizing mutant proteins [4, 16]. This explains the dependence of cancer cells on Hsp90 and their sensitivity to pharmacologic Hsp90 inhibition. Thus Hsp90 provides a broader target for anti-cancer therapies than a single, oncogenically activated signaling pathway. Hsp90 inhibitors, by interacting specifically with single molecular target (Hsp90) can cause the inactivation, destabilization and eventual degradation of multiple oncogenic Hsp90 client proteins simultaneously [13, 28]. The emergence of Hsp90 as a promising target for cancer therapy is therefore not surprising and evaluation of Hsp90 inhibitors is the current focus of drug discovery [18, 24, 25, 29].

Tumor Selectivity of Hsp90

A major flaw in the rationale of most of the conventional cancer therapies is the lack of selectivity for tumor cells versus normal cells. There is a need to identify the differences between normal and transformed cells at the molecular level to recognize cancer-specific molecular targets. This will help the designing of drugs that are more specific, efficient and less toxic [30]. While we continue to validate Hsp90 as a target in cancer therapy, another interesting feature of this chaperone has surfaced in the past few years. This is the tumor selectivity of Hsp90 inhibitors. It has been demonstrated that Hsp90 inhibitors selectively kill tumor cells at doses that are not toxic to normal cells. Several inhibitor classes have shown selective binding to Hsp90 in tumor cells. Also, cancer cells have proven to be significantly more sensitive to Hsp90 inhibition than the non-transformed cells [1, 3, 4, 31, 32]. Studies on binding affinity of the inhibitors to Hsp90 from normal and tumor cells report higher response for Hsp90 from tumor cells. Binding affinity for ATP is also higher in Hsp90 from tumor cells [32]. Also although Hsp90 is abundantly expressed in cancer as well as normal cells, certain Hsp90 inhibitors show significantly higher accumulation in tumor cells [33–35]. Scientists have often questioned the specificity of Hsp90 as a molecular cancer target since Hsp90 is an abundant protein that is present in all cells. The molecular basis for tumor selectivity is not clear, but a number of hypotheses are reported in explanation.

Tumor cells over-express Hsp90 clients and hence greater amount of Hsp90 is engaged in active chaperoning and exists as multi-chaperone complex. Hsp90 from tumor cells also has higher ATPase activity as compared to that of normal cells. It is suggested that all soluble hsp90 from tumor cells exists as multi-chaperone complexes whereas; hsp90 from normal cells is in uncomplexed inactive form Fig. 2. The functionally distinct molecular form of Hsp90 in cancer cells probably accounts for greater affinity of inhibitors. [32]. Maroney et al. 2006 [36], demonstrated that the amount of Hsp90 complexed to co-chaperones is higher in tumor cells than in normal ones.

Another observed finding is the enhanced Hsp90 affinity for mutated or functionally deregulated client proteins. Several examples of this behavior have been documented. For example, *v*-src (oncogene) exhibits unusually stable interaction with Hsp90 [35], while the non-oncogenic *c-src* requires only limited assistance from the Hsp90 machinery for its maturation and cellular function.

Also selective sensitivity of transformed cells for Hsp90 inhibitors may be partly due to selective accumulation of these drugs in cancer cells [35]. This in turn is accounted for by the fact that these compounds have higher binding affinity for tumor derived Hsp90 compared to Hsp90 derived from non-transformed cells [21, 32, 35, 37]. Tumor-specific accumulation has been observed for a number of Hsp90 inhibitors, such as 17-AAG (17-allylamino-17-demethoxygeldanamycin), 17-DMAG, IPI-504, radicicol derivatives and purine-scaffold inhibitors [35].

Several groups are currently examining altered states of post-translational modification of Hsp90 in tumor vs. normal cells as a possible contributing factor to this phenomenon [21].

It can be summarized that, under normal conditions, Hsp90 exists in a ‘latent state’ and interacts with client proteins in a

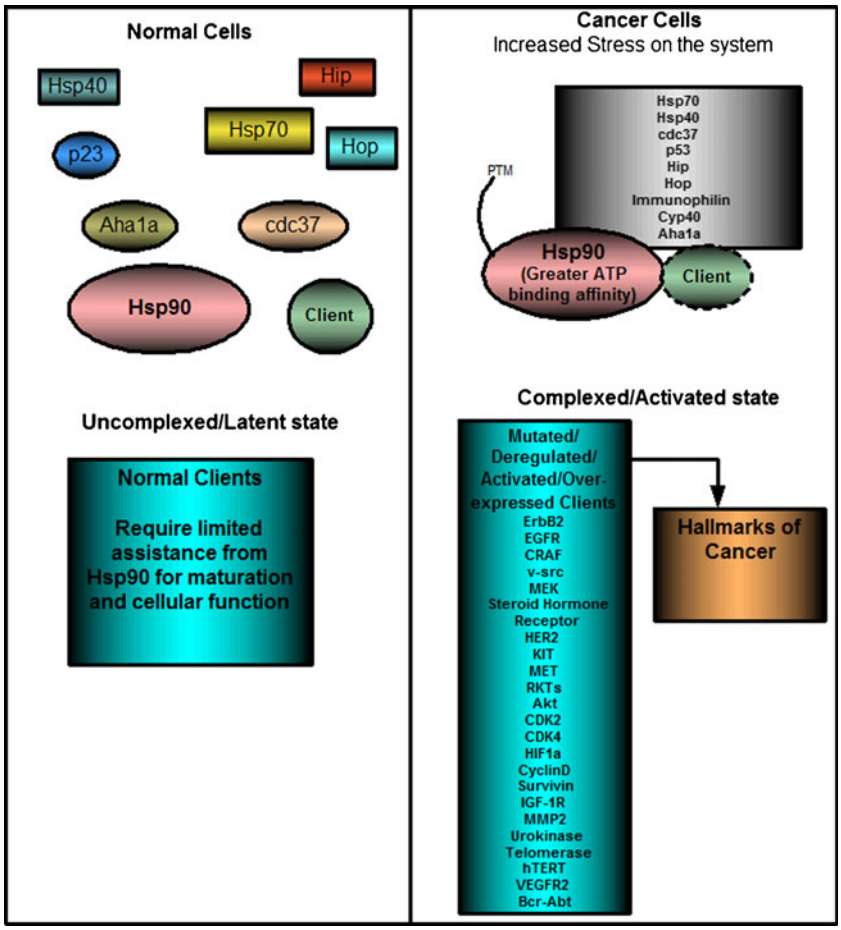

Fig. 2 Altered structure and functions of Hsp90 in tumor/ cancer cells is the molecular basis of the tumor selectivity of Hsp90 inhibitors. *PTM* Post-translational modification

dynamic, low-affinity manner regulated by low-affinity binding and release of ATP and ADP. On the other hand, in cancerous state, Hsp90 exists in an, 'activated state' containing co-chaperone complexes. The shift in equilibrium from the latent to the activated state may be governed by the degree of transformation or amount of 'stress' on the system in the form of abundance of mutated and deregulated proteins, hypoxia, low-nutrient environment etc.

Irrespective of the underlying mechanism, the apparent increased affinity of Hsp90 in tumor cells for inhibitors as compared to Hsp90 in normal cells makes this chaperone a particularly attractive target for cancer therapy [13].

Current Status of Hsp90 Inhibitors

The ability of Hsp90 inhibitors to diminish the levels of multiple protein targets in parallel is therapeutically more attractive and potentially more efficient than highly selective single target drugs [4, 28]. After the demonstration of potent anti-cancer effects of geldanamycin (GA) through Hsp90 inhibition, great deal of efforts have been devoted to this area and a diversity of Hsp90 inhibitors have either been identified or synthesized [4, 9].

Current Hsp90 inhibitors are categorized into several classes based on distinct modes of inhibition, including i) blockade of ATP binding, ii) disruption of cochaperone/Hsp90 interactions, iii) antagonism of client/Hsp90 associations and iv) interference with post-translational modifications of Hsp90 [4]. Tables 1 and 2 give a summary of the different classes of Hsp90 inhibitors. These range from ATPase activity blocking inhibitors that impart non-selective anti-cancerous activity as shown in Table 1, to inhibitors that impair Hsp90-specific client protein interactions that offer ultimate selectivity Table 2. Chemically Hsp90 inhibitors are categorized into three groups 1) Benzoquinone ansamycins and its derivatives 2) Radicicol and its derivatives and 3) Small synthetic inhibitors.

Accordingly, Geldanamycin is a benzoquinone microbial product classified as ansamycin antibiotic [4, 9, 28, 38]. Other analogues of geldanamycin namely, 17-allylamino-17-demethoxygeldanamycin (17-AAG, tanespimycin), 17-DMAG (17-Dimethylaminoethylamino-17-demethoxygeldanamycin) (alvespimycin) and another water-soluble hydroquinone hydrochloride analogue of 17-AAG, IPI-504 exhibit improved pharmacodynamic properties compared to geldanamycin and IPI-504 is in Phase I/II clinical trials [4]. Radicicol is yet another natural product inhibitor which is a 14-member macrocyclic antibiotic isolated from fungus *Monocillium nordinii* and

Table 1 Inhibitors of Hsp90 that bind to the N-terminal ATP binding site

Sr. no.	Class	Types	Examples	Name	Source	Combination Therapy	Phase
1.	Ansamycin macrolactames (natural compounds and derivatives)	Quinone derivatives	Geldanamycin				Trials terminated
			17-AAG	KOS953 (Tanespimycin) CNF1010 (Tanespimycin)	Kosan-BMS Conforma-Biogen	Bortezomib	II/III
						Paclitaxel	
						Sorafenib	
			17-DMAG	(KOS1022) Alvespimycin	Kosan-BMS	Paclitaxel Trastuzumab	I
		Hydroquinone derivatives	IPI504	Retaspimycin	Infinity	Trastuzumab	III
			IPI493		Infinity		I
		Quinone methide triterpene	Celastrol	Tripterine			
2.	Purines (synthetic small molecules)	Purine analogues	PU3				
			PUH58				
			PU24F-Cl				
			BIIB021		Conforma-Biogen	Aromasin	II
			CNF2024		Biogen Idec		
			PUH71				I
3.	Pyrazole and Isoxazole derivatives (synthetic small molecules)	Pyrazole analogues	CCT018159				
			G3129				
			G3130				
			VER49009		Vernalis		
			STA-9090	Ganetespib	Synta		II
			STA-1474		Synta		I/II
		Isozaxole analogues	VER50589				
			VER52296/NVP-AUY922		Vernalis-Novartis	Bortezomib plus dexamethasone	II
4.	Dihydroindazolone derivatives		SNX2112				I
			SNX5422 mesylate		Pfizer Inc		
5.	Radicicol derivatives (natural compounds and derivatives)	Oxime derivatives	KF55823 KF58333 KF25706				None
			KW2478		Kyowa Hakko	Bortezomib	I/II
			AT13387		Astex Pharma		I
6.	Other synthetic small molecules		MPC-3100		Myriad Genetics		I
			XL 888		Exelixis		I
			Debio 0932		Debiopharm		
			(Nano-particle albumin bound 17-AAG) ABI 010		Abraxis Bioscience		I
			HSP990		Novartis		I

Table 2 Hsp90 inhibitors binding to sites other than the N terminal ATP binding site

Sr No	Class	Type	Examples	Name	Binding site	Phase
1.	Coumarin derivatives	Novobiocin (Albamycin, Cathomycin)			C terminal	Pre-clinical trails
		Novobiocin analogues	KU135, KU174			
			A4			
			DHN2			
			4TDHCNA			
			4TCNA			
			4TCCQ			
2.	Others		Cisplatin		C terminal	Completed trials
			EGCG	Epigallocatechin-3-Gallate	C terminal	Pre-clinical/Clinical
			Taxol and derivatives	Paclitaxel	C terminal	Pre-clinicla/Clinical
				Sansalvamide A	N middle domain	
		Prenylated Isoflavone	Derrubone		Not known	Lead compound
			Gedunin			
			Pochonin A and D			

Monosporium bonorden [4, 39]. The oxime derivatives of radicicol, unlike radicicol itself, have potent antitumor activity in vivo by disrupting client-Hsp90 interaction, are more stable and exhibit less severe hepatotoxicity [4, 7, 28].

Novobiocin [15], a coumarin antibiotic isolated from *Streptomyces* species (Table 2) led to the discovery of a new ATP binding site in the C terminal of Hsp90 for which it is specific [4]. Recent examples of new natural product scaffolds being discovered and tested are, isoflavone derrubone from the Indian tree *Derris robusta* [37] and a green tea polyphenol catechin, epigallocatechin 3-gallate (EGCG) [4].

Most of the natural product inhibitors designated as 'first generation' Hsp90 inhibitors suffer certain pharmacologic drawbacks and toxicity-associated adverse [4, 9, 40–55] events. These and formulation issues are dose limiting [38]. Dose limiting toxicity for some of the inhibitors includes, diarrhea, fatigue and the ocular effects of darkening of vision, night blindness, syncope, dizziness and ocular toxicity, constitutional, gastric and hepatic (transaminitis) effects [9]. Another problem that limits further application of this category of drugs is resistance developed due to induction of stress response in the form of Hsp70 expression that interferes with the efficacy of Hsp90 inhibition [4]. Nonetheless, these natural product non-specific inhibitors have acted as pathfinder molecules, helping us to understand the biology of Hsp90 as well as the consequences of Hsp90 inhibition [4, 9, 15, 40, 41, 46–50, 53, 56–58].

These were also essential in biological validation of Hsp90 as a drug target (33) as well as establishing the technical druggability of N terminal domain of Hsp90.

All synthetic or semisynthetic inhibitors are designated as 'Second Generation' Hsp90 inhibitors. The first series of synthetic small molecule HSP90 inhibitors was based on a purine-scaffold and were conceived by structure-based modeling [4]. PU3 [4, 59] and more potent PU24FCl were the first prototype small molecule inhibitors developed in this series, but several derivatives have followed suite. BIIB021/CNF2024 are oral purine scaffold compounds in phase II trials after being well tolerated in phase I [4, 60, 61].

The Second series is the resorcylic pyrazole/isoxazole series, members of which share the anchoring resorcinol warhead used by radicicol [9]. This series includes, CCT018159, the diarylpyrazole-scaffold HSP90 inhibitor, resorcinylic pyrazole amide VER-49009 [62], resorcinylic isoxazole amide VER-50589, pyrazole amide CCT0129397 and isoxazole CCT0130024 and an optimized analogue NVP-AUY922 has just entered clinical trials [4].

All synthetic small molecule inhibitors including 6,7-dihydro-indazol-4-one scaffold SNX-2112, SNX-5422 and STA-9090 seem to have several advantages over the first generation Hsp90 inhibitors. These have better pharmacological profiles with favorable water solubility, lower toxic-

ity, oral bioavailability, metabolic stability and insensitivity to multi-drug resistance. Yet eye-disorders and ocular toxicity led to the discontinution of a few [63–65]. Besides, the intrinsic non-selectivity of ATP-binding Hsp90 inhibitors for Hsp90 clientele limits their further application.

Recently Hsp90 inhibitors that bind to sites other than the N terminal ATP binding site of Hsp90 have been discovered. These inhibitors do not target ATPase activity of the chaperone but impair its association with co-chaperones and client proteins. These inhibitors are potentially more specific. These include Novobiocin, natural product Macrocycle Sansalvamide A that binds between the N-terminal and middle domains [9], CTPR390+ that impairs Hsp90-Hsp70 interaction and Celastrol [4] that causes disruption of CDc37-Hsp90 association. However, these drugs have modest selectivity for tumor cells over normal non-tumor ones. The feasibility of targeting other co-chaperones like Hop, Aha etc. still needs to be addressed.

Inhibition of client/Hsp90 interactions offers the ultimate selectivity, however, details of client protein- chaperone interactions are still un-clear [7]. Hence, targeting these associations still remains a challenge. Moreover, the structure of the C-terminal of Hsp90 and its nucleotide-binding site is still not very clear. Continued efforts on the Hsp90 C-terminal are required to fully understand the mechanism of action of C-terminal inhibitors.

Inhibitors used in combination with molecularly targeted drugs exhibit promising clinical responses. For example, tanespimycin (17AAG) in combination with the multikinase inhibitor sorafenib demonstrated clinical effect in 75 % and 67 % of renal cancer and melanoma patients, respectively. Similarly, administration of 17-AAG in combination with HER-2 targeting monoclonal antibody, trastuzumab clearly demonstrated promising anti-tumor activity and acceptable toxicity for patients with HER-2 positive breast cancer [4, 9, 66].

Research in the last 10 years has led to the entry of almost 20 compounds in clinical trails and several others are in pre-clinical development [9]. For a comprehensive analysis of, the phase I/II clinical trial outcomes of Hsp90 inhibitors, refer to Jon Travers et al. 2012 [9] and Neckers L and Workman P 2012 [67].

Future Directions

Today significant progress has been made in illustrating and validating the potential of Hsp90 inhibition in cancer therapy. Despite good activity and clinical progression current Hsp90 inhibitors like 17-AAG have several potential limitations. Therefore, development of new synthetic HSP90 inhibitors, with improved pharmacologic profiles and with diverse chemical scaffolds is underway. Also, improved clinical trials involving stratified patient groups for current investigational drugs, has become a greater task. Selecting patients who have the molecular defect that the inhibitor is designed to target is important. This ensures that the efficacy of the drugs is being tested on the 'right' patients. Several features of Hsp90 inhibition that make its employment more promising and attractive have emerged and these need a closer look. Besides this several aspects of the process of carcinogenesis itself are still unclear. Better understanding of these will prove instrumental in deciding Hsp90 inhibition strategies with improved pharmaco-dynamic efficacy.

Molecular Understanding of Hsp90 Structure

The basis for antagonizing client-Hsp90 interactions is the structural and biochemical understanding of these associations. Even today very little is known about the association of Hsp90 with its clients. Better understanding of the molecular structure of Hsp90 gives an opportunity for molecular modelling studies using bio-informatics tools to screen Hsp90 inhibitors with greater potential.

Biomarkers

With more Hsp90 inhibitors entering clinical trials, identification of effective and convenient pharmaco-dynamic markers for Hsp90 inhibition is becoming increasingly important. The clinical trials of 17-AAG used depletion of client proteins like CRAF and induction of Hsp70 as biomarkers in peripheral blood mononuclear cells (PBMCs) and tumor biopsies of treated patients.

It has been pointed out that the most appropriate pharmacodynamic marker to test for Hsp90 inhibition in oncoprotein addicted cancer type is the depletion of the client oncoprotein itself and not the expression of Hsp70. This is because Hsp70 expression is an overly sensitive molecular response to Hsp90 inhibition and may not be followed by actual clinical benefit. Therefore refining pharmacologic approach by focusing on key clients will give a clearer picture of relation between Hsp90 inhibition and clinical benefit [33]. In recent years two serum biomarkers have been identified, namely the IGFBP-2 and HER-2 extracellular domain. The expression of these serum proteins is closely related to Hsp90 regulation. Besides, these can be readily detected and quantified in the sera of the patients [68, 69].

With the use of techniques like gene expression microarrays and protein profiling, potential biomarkers of Hsp90 inhibition can be identified. These biomarkers will help predict sensitivity so as to allow identification of cancer types that are most likely to benefit from Hsp90 inhibition. Employing minimally invasive functional imaging techniques like PET scans and magnetic resonance spectroscopy can improve detection of biomarkers [15].

For example, one novel approach currently being assessed is the use of radiolabeled antibody fragments to measure, non-

invasively, changes in HER2 expression by PET imaging [69, 70]. Recent studies have also led to the emergence of yet another protein that promises to be a highly sensitive biomarker for Hsp90 inhibition. This is the prostate-specific antigen (PSA). The studies have clearly demonstrated decrease in PSA in BT-474 and LNCaP cell lines following inhibitor treatment. This reduction is superior to the decrease in IGFPB-2, making PSA a more promising biomarker [71]. PSA is an androgen receptor (AR)-dependent protein and AR is an Hsp90 client. Hence inhibition of Hsp90 leads to degradation of AR by the proteosome and hence reduced expression and levels of PSA. PSA also acts as a marker for Hsp90 inhibitor efficacy.

Combination Therapy

It is hypothesized that the Hsp90 inhibitors used in clinically relevant doses can enhance cancer cell sensitivity to radiation. Several research groups have reported such findings for 17AAG [21, 28]. Since such effect is observed for 17-AAG it can be expected that other Hsp90 inhibitors may also exhibit similar activity. Further studies on the effect of other inhibitors on response of cancer cells to radiation are wanting.

The dose limiting toxicity of Hsp90 inhibitors will be an important issue especially when the inhibitor is used alone as a single agent. Combining Hsp90 inhibitors with other therapeutic agents, especially those that directly block the function of a given Hsp90 oncoprotein client, should enhance efficacy and lower toxicity [67]. Data obtained from several preclinical and clinical trials have shown promising results of combining Hsp90 inhibitors with other chemotherapeutic agents. For example, combining inhibition of Hsp90 and proteosome inhibitors. Hsp90 inhibitor +/−bortezomib has clinical activity and reduced peripheral neuropathy in patients with relapsed/refractory multiple myeloma [66].

Pre-clinical Studies

It is crucial to determine the best manner in which to use the available inhibitors in order to achieve the greatest benefit from inhibiting the target in a disease specific manner [4]. It will be beneficial if pre-clinical modelling and patient stratification preceeds clinical studies. Rigorous pre-clinical evaluation strategies can facilitate the clinical hypotheses to go forward [9].

Since most mutant proteins in cancer cells use Hsp90 to compensate for their structural instability, Hsp90 activation could itself become an independent prognostic tool. Genetic profiling of tumors and Hsp90 usage in cancer cells could be a valuable diagnostic tool to select patients in clinical trials.

Targeting cancers that are addicted to clients of Hsp90 like HER2, ALK, EGFR, BRAF etc. will show greater pharmacologic success [67]. Diagnostic assays to measure Hsp90 usage could be done using binding assays to Hsp90 extracted from tumor lysates using a routine blood sample for leukemias or fine-needle aspirate for other tumors. If it is possible to develop antibodies against the activated form of Hsp90, then simple immunoassays can be employed to measure Hsp90 usage. Adeela K et al have reasoned that since Hsp90 exists as a multichaperone complex in its activated form, antibodies can be raised not against an epitope of Hsp90 but against one formed by close juxtaposition of Hsp90 and a co-chaperone protein. Developing such will be a challenging task and it remains to be seen if such an antibody can be developed [72].

Resistance

Another possibility that follows the use of Hsp90 in cancer therapy is the acquisition of resistance both intrinsic as well as extrinsic by the cancer cells. For example, inherent resistance of cells to 17-AAG (a quinone moiety containing Hsp90 inhibitor) due to low expression of the enzyme NQO1. Counter expression of Hsp70 and Hsp27 in response to Hsp90 inhibition may also impart resistance. Mutation in Hsp90 of cancer cells or overexpression of certain co-chaperones may also impart Hsp90 inhibitor resistance [73]. Effective designing of second-generation Hsp90 inhibitors will benefit greatly from a much more detailed understanding of the factors that contribute to resistance. Refer, Piper PW and Millson SH, 2011 [61] for information on the routes of resistance. Appropriate drug redesign can help overcome the problem of resistance.

Summary

It can be summarized that current research has led to the recognition of new aspects in the complex process of carcinogenesis. The promise of hsp90 as a cancer target continues to hold true in the light of the broadening knowledge of transformation pathways. Employment of new strategies based on the findings of previous clinical trials might provide a broader and more effective anti-cancer therapy. There is much more to learn about the, emerging hallmarks of cancer, identification of appropriate biomarkers for various cancer types, overcoming possible resistance to Hsp90 inhibitors etc.

The significance of combination therapy cannot be understated and recognition of cancer types that can benefit from a particular drug recipe will help in offering more individualistic treatment. Modulating a driver oncoprotein using a combination of a drug that inhibits its biochemical function (e.g. kinase activity) together with its overall depletion at the protein level via HSP90 inhibition could be especially damaging for the cancer cell, particularly if proteotoxic stress is also induced. Such an approach can lead to breakdown of robustly evolved oncogenic system [9]. Studies on structure of Hsp90 and improved understanding of Hsp90/client/co-chaperone associations will

assist designing second-generation inhibitors with improved pharmacologic profiles.

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