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Heat shock protein 90 as a new drug target in cancer research

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ABSTRACT

Current anticancer drug development strategies involve identifying novel molecular targets which are crucial for tumourigenesis. The molecular chaperone heat shock protein (HSP) 90 is of interest as an anticancer drug target because of its importance in maintaining the conformation, stability and function of key oncogenic client proteins involved in signal transduction pathways leading to proliferation, cell cycle progression and apoptosis, as well as other features of the malignant phenotype such as invasion, angiogenesis and metastasis. HSP90 inhibitors geldanamycin, geldanamycin derivatives and radicicol exert their antitumour effect by inhibiting the intrinsic ATPase activity of HSP90, resulting inactivation, destabilization and degradation of HSP90 client proteins. HSP90 is new therapeutic target attack on all of the hallmark traits of cancer such as self – sufficiency in growth signals, evading apoptosis, sustained angiogenesis, limitless replictive potential, tissue invasion, insensitivity to anti-growth signals. Clinical activity has been seen with 17-AAG in breast and prostate cancer. Hsp90 inhibitors have entered Phase II and III clinical trials, and have shown therapeutic activity in several types of cancer.

Introduction

Cancer is a group of diseases in which cells are aggressive (grow and divide without respect to normal limits), invasive (invade and destroy adjacent tissues), sometimes metastatic (spread to other locations in the body) and normally resistant to apoptosis (programmed cell death).Cancer may affect people at all ages, even fetuses, but risk for the more common varieties tends to increase with age [1]. Cancer causes about 13% of all deaths [2]. 7.6 million People died from cancer in the world during 2007 [3].

In human genome if activation of oncogenes and inactivation of tumor suppressor genes, resulting excess growth and division, loss of normal function, increase surviving of in diverse tissue environment and metastasis. Many cancers can be treated and cured according to their type, location and stage. Combination of surgery, chemotherapy and radiotherapy is normally used for treatment of cancer. Every time new drug develops for treatment of various cancers for their more selectivity for cancer cell and less damage to normal cells.

Overview of Hsp90

Cancer metastasis is the result of complex processes, including alteration of cell adhesion/motility in the microenvironment and neoangiogenesis that are necessary to support cancer growth in tissues distant from the primary tumor. The molecular chaperone heat-shock protein 90 (Hsp90), also termed the 'cancer chaperone', plays a crucial role in maintaining the stability and activity of numerous signaling proteins involved in these processes.Hsp90 inhibitors cause the eventual inactivation, destabilization, and degradation of numerous chaperone-dependent client proteins, and these drugs have shown promising antitumor activity in preclinical model systems.

Heat Shock proteins (HSP) is a family of proteins found in all cells. In normal conditions where they act as chaperones making sure that the cell's proteins are in the right shape and in the right place at the right time by folding and unfolding (modulate conformation and function) of proteins eg.steroid receptor, help shuttle proteins from one compartment to another inside the cell, transport old proteins to "garbage disposals" and inside the cell of cell signalling molecules, transcription factors, cytoskeleton. HSP expressed in response to cold, heat, oxygen deprivation and other environmental stresses and stabilize protein in abnormal configuration.

Hsp90 (heat shock protein 90) is a molecular chaperone and is one of the most abundant proteins expressed in cells [4]. The protein is named "HSP" for obvious reasons whereas the "90" comes from the fact that it weighs roughly 90 kiloDaltons. Hsp90 is an abundant eukaryotic protein because they makes up about ~2% of cytosolic protein content.Hsp90 production increased by cellular stresses. Hsp90 exists in some bacteria but not archaea [5].

Isoforms of Hsp90

Hsp90 is highly conserved and expressed in a variety of different organisms from bacteria to mammals including the prokaryotic analogue htpG (high temperature protein G) with 40% sequence identity and 55% similarity to the human protein [5]. Yeast Hsp90 is 60% identical to human Hsp90α. In mammalian cells, there are two or more genes encoding cytosolic Hsp90 homologues [5] with the human Hsp90α showing 85% sequence identity to Hsp90β [6]. The α- and the β-forms are thought to be the result of a gene duplication event that occurred millions of years ago [5] The five functional human genes encoding Hsp90 protein isoforms are listed below [6].

There are 12 human pseudogenes (non-functional genes) that encode additional Hsp90 isoforms which are not expressed as proteins. Recently, a membrane associated variant of cytosolic Hsp90, lacking an ATP-binding site, has been identified and was named Hsp90N [7]. This HSP90α-Δ-N transcript is a chimera, with the first 105 bp of the coding sequence derived from the CD47 gene on chromosome 3q13.2, and the remaining coding sequence derived from HSP90AA1

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[6]. It is possibly a cloning artifact or a product of chromosomal rearrangement occurring in a single cell line [8].

Table 1: Isoforms of Hsp90

Family	Subcellular location	Subfamily	Gene	Protein
HSP90 A	Cytosolic	HSP90AA (inducible)	HSP90AA 1	Hsp90-α_1
			HSP90AA 2	Hsp90-α_2
		HSP90AB (constitutively expressed)	HSP90AB1	Hsp90-β
HSP90B	endoplasmic reticulum	-	HSP90B1	GRP94
TRAP	Mitochondrial	-	TRAP1	TNF Receptor-Associated Protein

Structure of Hsp90

Dimer structure

HSP90 resides primarily in the cytoplasm, where it exists predominantly as a homodimer (Figure 1). Each homodimer is made up of monomers. The monomer of the Hsp90 consists of a four structural domain [9-11].

1. A highly conserved N-terminal (NTD) domain of ~25 kDa.
2. A "charged linker" region, that connects the N-terminus with the middle domain.
3. A middle domain (MD) of ~40 kDa.
4. A C-terminal domain (CTD) of ~12 kDa.

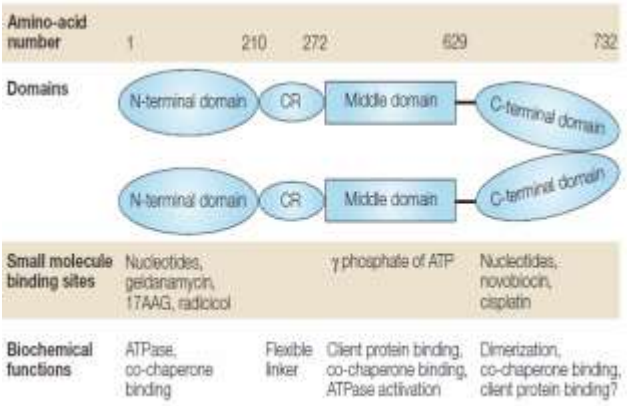

Figure 1: Dimer Structure of Hsp90. the numbering 1-732 indicates the approximate positions in the amino acid sequence of the human proteins that define its functional domains. 'CR' refers to a charged region which serves as a flexible linker between the N-terminal and middle domains. The locations where various small molecules bind HSP90 (heat-shock protein of 90 kDa) and modulate its function are indicated. The biochemical functions of each domain are also shown. 17AAG: 17-allylaminogeldanamycin; GA: geldanamycin.

Hsp90 function

Normal cells

In unstressed cells, Hsp90 plays a number of important roles, which include assisting in folding, intracellular transport, maintenance, and degradation of proteins as well as facilitating cell signaling.Hsp90 interacts with, regulates the conformation of, and the activity of, a large variety of cell signalling molecules, transcription factors, cytoskeleton, etc (Table 2).

Table 2: Physiological targets of Hsp90

Substrates	Notes
Heat-shock factor (HSF)	Hsp90 down regulates activity in conjunction with Hsp70 system
Other transcription factors	Receptors (steroid, glucocorticoid), hypoxia-inducible factor-1 (HIF-1), etc.
Kinases	Tyrosine kinases (v-src, lck, insulin receptor, etc.) serine-thronine kinases (eIF-2 kinase, v-raf, c-raf, etc.),protein kinase CK-II (casein kinase-II)
Cytoskeleton	Actin, Tubulin (protection during heat stress)

Hsp90 never functions in isolation in eukaryotes; it always appears to be associated with a variety of cofactors (Table 3)

Table 3: Hsp90-associated proteins

Cofactor	Notes
Hsp70	Hsp90 activity dependent on Hsp70 system (incl. Hsp40)
HOP	HOP, Heat-shock Organizing Protein, brings Hsp70 and Hsp90 together via TPR interaction domains
p23	Modulates ATPase activity of Hsp90
HIP	Co-chaperone of Hsp70
PPIases	Cyclophilin-40, FKBP51, FKBP52
Others	Kinase-targeting co-chaperone Cdc37/p50

Protein folding

Hsp90 not only assists the folding of proteins, but can also modulate the conformation/function of proteins (Figure 3). Hsp90 is known to associate with the non-native structures of many proteins which have lead to the proposal that Hsp90 is involved in protein folding in general [12]. Hsp90 has been shown to suppress the aggregation of a wide range of "client" or "substrate" proteins and hence acts as a general protective chaperone [13-15]. However Hsp90 is somewhat more selective than other chaperones [16].

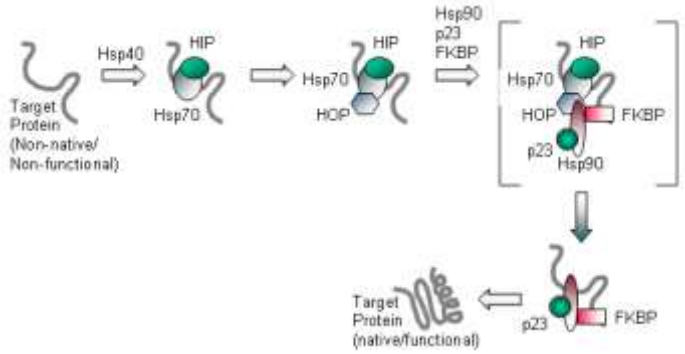

Figure 3: Schematic representation of protein Folding and modulate the conformation.HIP: Hsp70-interacting protein; HOP: Hsp70/Hsp90-organizing protein; FKBP: FK506 binding protein.

Protein Degradation

Proteolysis: Biochemical degradation of protein through hydrolysis of peptide bonds.

Lysosomal: Steps= Uptake (Autophagy) into lysosome: Secretory vesicles; Cytoplasm; Organelles, and enzymatic degradation. Non-lysosomal: Steps= tagging of protein to be degraded (by ubiquitnation).Recognition of proteolytic system: exposure of peptide sequence or distinction of unfolded protein segments Example: Proteasome.

Eukaryotic proteins which are no longer needed or are misfolded or otherwise damaged are usually marked for destruction by the Ubiquitin-mediated degradation pathway (Figure 4). These ubiquitinated proteins are recognized and degraded by the 26S proteasome [17-18]. Hence the 26S proteasome is an integral part of the cell's mechanism to degrade

proteins. Furthermore a constant supply of functional Hsp90 needed to maintain the tertiary structure of the proteasome [19].

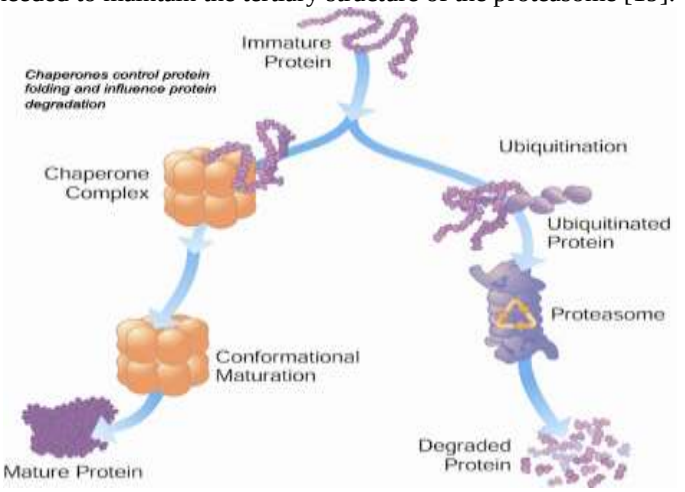

Figure 4: Hsp90 Multichaperone pathway and Ubiquitin-mediated degradation pathway

Role in cancer

Hsp90 Client proteins and cancer

In cancer cells overexpression of hsp90 muti-chaperone complex (Hsp90complex with its client protein) having high ATPase activity and binding affinity to ligand resulting increase regulation, function and stability of many key signal proteins (like HER-2/ErbB2, Akt, Raf-t, Bcr-Abl and mutated p53) that help cancer cells to escape the inherent toxicity of their own environment, to evade chemotherapy, and to protect themselves from the results of their own genetic instability (uncontrolled cell growth and survival.). Small list of Hsp90 client proteins [20] included mechanism of action and potential target cancer are described in below (Table 4).

Hsp90 plays multiple roles in cancer metastasis

HSP90 exerts its chaperone function to ensure the correct conformation, activity, intracellular localization and proteolytic turnover of a range of proteins that are involved in cell growth, differentiation and survival [21-22] Of particular importance is that HSP90 is essential for the stability and the function of many oncogenic client proteins, which contribute hub (Figure 5) to the hallmark traits of cancer (Figure 6).

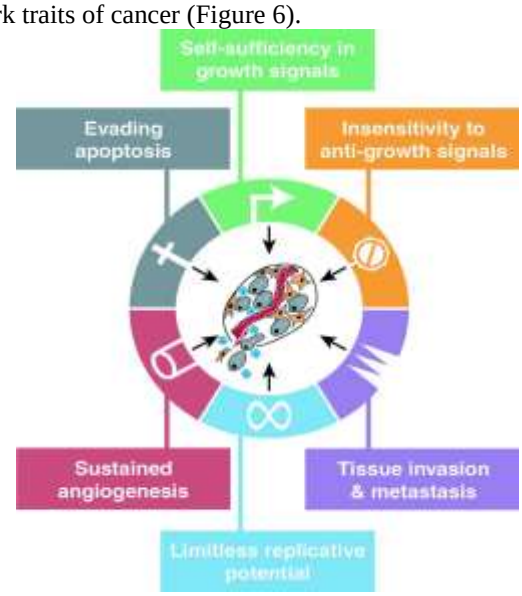

Figure 5: Hsp90 as a hub of cancer

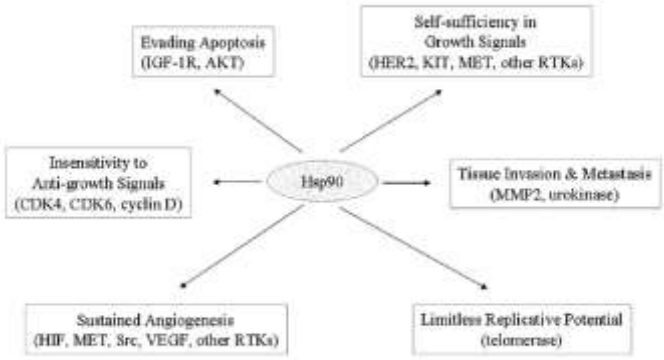

Figure 6: Hsp90 and the six hallmarks of a cancer cell. Multiple Hsp90 client proteins mediate acquisition and maintenance of the six properties necessary for transformation of a normal cell into a cancer cell: (a) ability to evade apoptosis, (b) ability to be self sufficient for growth, (c) ability to invade surrounding tissue and to metastasize to distant sites, (d) ability to undergo limitless replication, (e) ability to promote neoangiogenesis, and (f) ability to not respond to antigrowth signals. IGF-1R: Insulin like growth factor-1 receptor; RTKs: Receptor tyrosin kinase; MMP2: matrix metalloproteinases2; HIF: Hypoxia-inducible factor; VEGF: vascular endothelial growth factor.

Hsp90 and inhibitors play multiple roles in cancer metastasis are described in below (Table 5). Cancer cells must rely on a number of signaling proteins that regulate these events [23-24].

Hsp90 Inhibitors and Client proteins

Hsp90 association is important for maintaining the stability and function of numerous proteins referred to as client proteins [58]. Hsp90 clients are frequently mutated or activated in cancer cells, and include the oncogenic tyrosine kinase v-Src, the mutated oncogene Bcr/Abl, the receptor tyrosine kinases ErbB2 and c-MET, and the serine/threonine kinase Raf-1 [58]. Client-protein interaction is regulated by the adenine nucleotide binding status of Hsp90. Nucleotide exchange and ATP hydrolysis that occur in an amino-terminal binding pocket in Hsp90 direct the mechanistic binding, chaperoning and release of client protein in what is referred to as the chaperone cycle [59,60]. Hsp90 bound to ATP has higher affinity for client proteins than its ADP-binding counterpart. Although Hsp90 itself has weak ATPase activity, the ATPase activity is both positively and negatively regulated by cochaperones, such as p23 [60]. Disruption of the association of client proteins with Hsp90 is generally followed by their ubiquitination and subsequent degradation in proteasomes [61]. Hsp90 inhibitors block cancer cell proliferation in vitro and cancer growth in vivo. These drugs inhibit Hsp90 function by competing with ATP binding, thereby freezing the chaperone cycle, which in turn decreases the affinity of Hsp90 for client proteins and leads to proteasome-mediated client protein degradation (Figure 7).

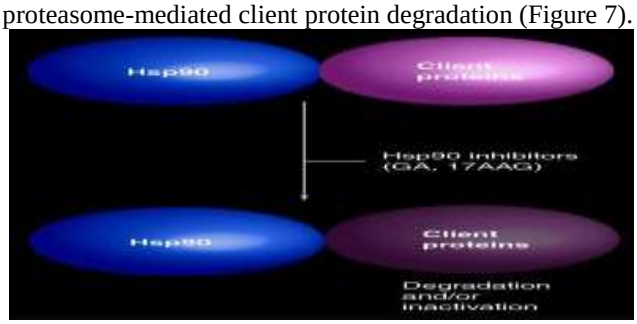

Figure 7: Hsp90 inhibitors induce client protein degradation and /or inactivation

Hsp90 inhibitors and their application

Geldanamycin

Geldanamycin (GA) is a natural product produced by *Streptomyces hygroscopicus*. GA binds with high affinity into the ATP binding pocket of Hsp90. Geldanamycin (GA), a benzoquinone ansamycin (BA) antibiotic, interferes with the action of the heat shock protein 90 (Hsp90) leading to the degradation of Hsp90 client proteins. Since many of these client proteins are oncogenic proteins, GA inhibits the proliferation of cancer cells and shows anticancer activity in experimental animals. Binding of GA to Hsp90 causes the destabilization and degradation of its client proteins [62].

Mechanism of action

(1). Free radical formation

The fact that a quinone ring is part of the GA molecule led to the initiation of detailed studies on the generation of intracellular free radicals through redox cycling [63]. it was shown that the treatment of eukaryotic cells with GA indeed causes the formation of free radicals. However, this could only be observed at high concentrations of GA, eg, 100 μM.

(2). Inhibition of tyrosine kinases

GA inhibited the tyrosine kinase activity of v-src, it was classified as a tyrosine kinase inhibitor [64]. the assumption was that BAs directly act on sulfhydryl groups of v-src. Yet it was known that the tyrosine kinase activity of v-src could only be influenced by herbimycin A an analog of GA in vivo but not in vitro [65]. This suggested that the target molecule of GA is either downstream of v-src or an interacting protein.

(3). Binding of GA to and interference with the function of members of the Hsp90 family of proteins

All these findings could not explain the multitude of effects of BAs. Finally, through chemical cross-linking of GA to Sepharose beads, it was possible to precipitate the target molecule of GA. Further analysis identified it as Hsp90 [66]. The discovery that GA binds to Hsp90 was a decisive breakthrough in understanding the mechanism of action of GA and structurally or functionally related substances. HSP90 interacts with proteins that contribute to all six hallmarks of cancer (for a complete list of HSP90 binding partners) (Figure 8).

Hsp90 stabilizes the active conformations and mutant tyrosine kinase receptors (red-purple), cytosolic serine-threonine and tyrosine kinases (green), transcription factors (blue), structural proteins and other enzymes (gray). Note: no specific sequence within the middle domain as a binding site is implied by the cartoon.

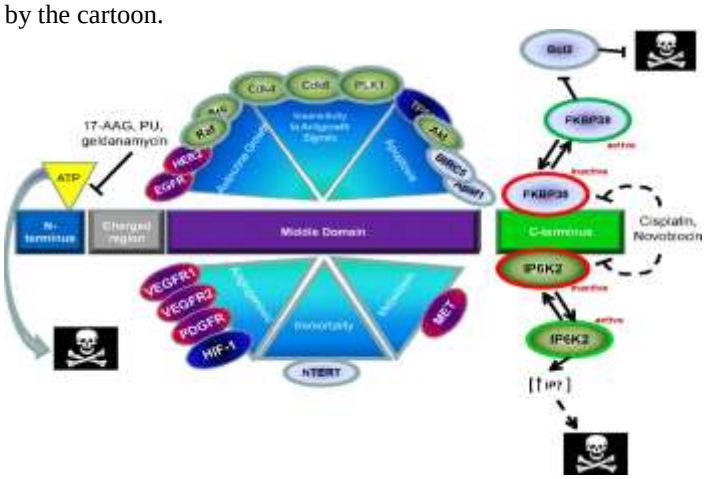

Figure 8: Schematic representation of Hsp90 binding to client proteins, how these proteins affect six hallmarks of cancer, and drug target sites within Hsp90. EGFR: epidermal growth factor receptor; AKT: Serine/ threonine protein kinase etc.

Client binding occurs through the middle domain of Hsp90 (purple rectangle), which leads to Hsp90 dimerization, cochaperone binding (HSP70, HIP, HOP, cdc37), and ATP binding and hydrolysis. Many of these interactions are inhibited by small molecules that compete for the N-terminal.ATP binding pocket such as the benzoquinone ansamycins (geldanamycin, 17-AAG), radicicols, their derivatives, and purine analogues (PU).Thus, many signal transduction pathways require Hsp90 to perpetuate growth promoting signals, and attenuation of these signals by inhibition of Hsp90 ATPase activity leads indirectly to cell death. On the other hand, proteins that bind to the C terminus (green rectangle) of Hsp90, such as FKBP38 and IP6K2 (proapoptatic protein kinase), are maintained in a constitutively inactive state by the interaction (red border). Interestingly, Hsp90β can be found in direct physical association with the MDR protein that is only presented in chemotherapy-resistant but not in wildtype cells [67].

Other substances interacting with Hsp90

Recent research revealed that the most effective drugs for the treatment of epithelial tumors, novobiocin and cisplatin, both bind to Hsp90 [68]. Drugs such as cisplatin and novobiocin (which interact with the C terminus of HSP90 at high concentrations) appear to disrupt these interactions, leading to the release and activation of the now cytosolic c-terminal binding partners (green border) and subsequent apoptosis, either through inhibition of Bcl2 (FKBP38) or increased cytosolic concentrations of IP7 (IP6K2). Dashed arrows indicate interactions that have yet to be demonstrated biochemically (Figure 8).

Geldanamycin analogues

17-AAG

17-Allylamino-17-demethoxygeldanamycin (17-AAG) is an analogue chemically derived from GA. 17-AAG is a less toxic and more stable analogue of geldanamycin (GA) [69]. Even though 17-AAG binding to Hsp90 is weaker than GA, 17-AAG displays similar antitumor effects as GA and a better toxicity profile.17-AAG is currently in phase I clinical trial in several centers worldwide.

17-DMAG

17-(Dimethylaminoethylamino)-17-demethoxygeldanamycin (17-DMAG, NSC 707545) is the first water-soluble analogue of 17-AAG. This Hsp90 inhibitor shows promise in preclinical models.17-DMAG has excellent bioavailability, is widely distributed to tissues, and is quantitatively metabolized much less than is 17-AAG [70].

17-AEP-GA

17-[2-(Pyrrolidin-1-yl) ethyl] aminno-17-demethoxygeldanamycin (17-AEP-GA) is a new geldanamycin (GA) analogue with an alkylamino group in place of the methoxy moiety at C17. 17-AEP-GA is less cytotoxic than GA and remains biologically active.17-AEP-GA was shown to induce similar tumor cell growth inhibition than 17-AAG and, unlike 17-AAG which is soluble in DMSO, to be water soluble [71].

17-DMAP-GA

17-(Dimethylaminopropylamino)-17-demethoxygeldanamycin (17-DMAP-GA) belongs to a new set of geldanamycin analogues that have been synthesized based on binding affinity to Hsp90 and water solubility. 17-DMAP-GA was shown to greatly inhibit the growth of cancer cells [71].

Table 4: A selected list of Hsp90 client proteins, mechanism of action of these proteins and potential target tumours that Hsp90 inhibitors could be applied to

Class of Protein	Client protein or interacting protein of Hsp90	Mechanism of action	Potential target cancer
Receptor tyrosine kinase	EGFR mutant	Activation of downstream prosurvival pathways, such as PI3-AKT and MAPK	NSCLC and glioblastoma
	ErbB2/HER-2		Breast cancer
	KIT		GIST
Signalling molecules or kinases	AKT/PKB	Activation of prosurvival proteins and suppression of proapoptotic proteins	Various cancers
	B-Raf mutant	Constitutively activates ERK signaling	Melanoma
	MET	Involved in cellular proliferation, migration, invasion and morphogenesis	Gastric, lung
	CDK4	Phosphorylates and inactives Rb, allowing cell cycle to proceed	Tumors with CDK4 over expression
	Death domain kinase RIP	Allows activation of NF-κB and its antiapoptotic signals	
Transcriptional factors	HIF-1α	Promoting angiogenesis	Renal cancer
	ERα-receptors	Regulating genes involved in cellular proliferation	Breast cancer
	P53 mutant	Transcription of genes involved in cell cycle arrest or apoptosis	Mutated in ~50% of cancer
Chimeric fusion-proteins	BCR-ABL	Activates numerous signal transduction pathways in leukaemogenesis	CML
	NPM-ALK	Induces cell transformation and proliferation	Anaplastic lymphoma
Others	Telomerase	Prevents telomere shortening	Various cancers
	Apaf-1	Crucial for apoptosome formation	
	Bcl-2	Regulates mitochondrial apoptotic pathway	Follicular lymphoma/small cell lung cancer
	MMP2	Facilitates invasion through cell adhesion, matrix digestion and cell migration	Overexpressed in various cancers

Table 5: Hsp90 and inhibitor role in cancer metastasis

Cancer Metastasis Processes	Hsp90 client proteins	Role of Hsp90	Role of Hsp90 inhibitor	References
Cell Adhesion	Focal-adhesion kinase (FAK, substrate of the v-Src oncogene)	Interacts with integrin cytoplasmic tails, and stimulates the recruitment of other adaptor proteins, such as $p130^{Cas}$, paxillin and talin, to form complexes known as focal adhesions and rearrangement of the actin cytoskeleton, cell migration and cell invasion	Stimulates the proteasome-mediated degradation of FAK, reduces tyrosine phosphorylation of FAK(The function of FAK is regulated by phosphorylation, particularly tyrosine phosphorylatio)	[25]
	Integrin-linked kinase (ILK). ILK is a serine/threonine protein kinase	An interacting partner of the β1-, β2- and β3-integrin cytoplasmic tails. ILK also complexes with PINCH1 and PINCH2, paxillin, α-parvin and β-parvin, and these associations are reported to be important for cell polarization and adhesion.	_	[26]

Cell Motility	Receptor tyrosine kinases (RTKs), erythroblastosis oncogene B (ErbB), ErbB2 (also called HER2/neu) EGF receptor (EGFR; also called ErbB1)	ErbB2 amplification is often observed in breast and ovarian cancers. RTKs are activated and complex with intracellular signaling molecules, stimulating cell motility via classical signal transduction pathways.	Interact with ErbB2 through the ErbB2 kinase domain induces the dissociation of hsp90 complex, after which rapid proteasome-mediated degradation of ErbB2.	[27-31]
	Hepatocyte growth factor (HGF)	Activating a tyrosine kinase signaling cascade after binding to the proto-oncogenic RTK cellular-MET (c-MET and work as a potent mitogen, motogen and morphogen	Destabilizes both mature and immature MET in breast and lung cancer cells. Disrupts HGF-induced association of MET with FAK and inhibits subsequent rearrangement of the actin cytoskeleton and cell motility. Furthermore, inhibit HGF-induced cell scattering and MET-dependent transformation	[32-34]
	The type 1 insulin-like growth factor receptor (IGF-1R) is an RTK	Induce IGF-1R expression	Reduce IGF-1R expression at the transcriptional level and induce IGF-1R protein degradation. Inhibit IGF-1-induced phosphorylation of IGF-1R and IGF-1R-induced cell migration in pancreatic cancer cells	[35-37]
	c-Src	Transiently activated by multiple growth factors or ECM proteins and RTK-mediated signaling pathways related to cell motility	Disruption of the c-Src-Hsp90 interaction by GA ultimately induces Src downregulation	[38]
	The serine/threonine kinase phosphoinositide 3-kinase (PI3K)	Activation of the serine/threonine kinase Akt through 3-phosphoinositide-dependent protein kinase (PDK) -1 and -2	Reduces phosphorylation of Akt and induces its ubiquitination and subsequent proteasomal degradation without any effect on the PI3K protein level	[39-41]
	rho GTPases Rho, Rac and Cdc42	Act as a molecular switch for the RTK-mediated rearrangement of the actin cytoskeleton, When coupled with RTKs, many signaling proteins such as c-Src and PI3K can modulate Rho GTPase	–	[42-45]

	Cdc42	Associate with activated Cdc42-associated kinase 1 and 2 (Ack1 and Ack2), members of the FAK family of nonreceptor tyrosine kinases. Ack1 induces phosphorylation of $p130^{Cas}$ and recruits the adaptor protein Crk, stimulating subsequent rearrangement of the actin cytoskeleton	Reduces the kinase activity of Ack1 without any effect on total Ack1 protein level, and suppresses Ack1-induced tumorigenesis an in animal model, inhibiting Ack2 kinase activity	[46-48]
	Matrix remodeling through matrix-degrading enzymes, such as plasminogen activators and matrix	Regulates the transcription of a variety of pro-angiogenic genes including VEGF	Attenuate HGF-induced urokinase (uPa) activity, and subsequent activation of MMP-2 and MMP-9. Inhibit chemical hypoxia-induced activation of uPA, MMP-2 and MMP-9.17-AAG reduces both phosphorylation of FAK and NF-κB activation, leading to inhibition of MMP-9 activity and reduction of subsequent cell invasion into the ECM	[34,32,49,50]
Neoangiogenesis	Hypoxia-inducible factor (HIF)-1	A transcriptional activator that regulates the transcription of a variety of pro-angiogenic genes including VEGF. The VEGF receptor (VEGFR) are key mediators of angiogenesis and are specifically expressed in endothelial cells	Induces ubiqutination and proteasome-mediated degradation of PDGFRα in cancer cells but not in normal cells	[51,52]
Metastasis	Cell surface HSP90, MMP2	Stimulated by environmental stresses and growth factors and affected by post-translational modification, such as phosphorylation and acetylation. Stimulate cell migration through the cell surface receptor CD91, independent of its ATP-binding and ATPase activity Binds to the extracellular domain of ErbB2. Heregulin-induced ErbB2 phosphorylation, downstream kinase signaling, result rearrangement of the actin cytoskeleton and subsequent cell migration	Cell-impermeable small-molecule Hsp90 inhibitor decreases MMP2 activity and cell invasion	[53-57]

Its binding affinity to Hsp90 was not significantly affected while its water solubility was highly improved compared to 17-AAG.

Advantages, Limitations and Future aspects

Advantages

- Preclinical trials emphasize the important role of Hsp90 inhibitors in clinical applications.
- Combination therapies, applying low doses of these drugs together with convention chemotherapeutic agents, seem to be an effective way to target various cancers.
- For example, in the case of Bcr/Abl-expressing leukemia's, a low dose GA is sufficient to sensitize these cells to apoptosis.
- Among the hallmarks of cancer, up regulation of growth signals and evasion of apoptosis are the most important.
- As most growth regulatory signals depend on Hsp90 for their function stability, Hsp90 is an ideal molecule to intervene in complex oncogenic pathways.
- Hence, most drugs are targeting Hsp90, which is more beneficial than the selective oncogene pathway inhibitors.

Limitations and future aspects

- 17-AAG contains poor solubility.
- These include relatively weak target potency, reduced activity in the presence of P-glycoprotein[72] and low bioavailability and metabolism by polymorphic cytochrome P450 CYP3A4 [73].
- But now Recent studies have confirmed that this increase in potency is indeed due to the metabolism of 17-AAG to the more active hydroquinone form.
- The analogue 17-DMAG is more water soluble than 17-AAG, but exhibits equal or greater activity.
- The hydroquinone form of 17-AAG, IPI-504 is very water soluble and has now entered clinical evaluation.
- The 3,4diaryl pyrazole CCT018159 (diaryl pyrazole resorcinol series) is able to inhibit human HSP90ß with a similar potency to 17-AAG and with a very high degree of selectivity towards HSP90 compared with topoisomerase II, HSP72 and a representative panel of kinases.
- The cellular sensitivity to CCT018159 is not affected by P-glycoprotein and inhibits a range of different cancer cell lines in vitro at micromolar concentrations, which caused degradation of client proteins and reduce tumour cell invasion and exhibit antiangiogenic activity.
- The diaryl pyrazole resorcinol series of novel HSP90 inhibitors have similar cellular properties to 17-AAG, but have several possible advantages (e.g. aqueous solubility, independence from NQO1 and P-glycoprotein), which may provide the basis for the future development of clinically superior HSP90 modulators.

Summary and Conclusion

The word ''cancer'' can be regarded as a gross term for a vast number of many different disease conditions with distinct characteristics and therapeutic requirements. Though the general features of cancer include unrestrained cell proliferation, a great variety of mutations as well as deregulation of numerous genes can cause this. Among the hallmarks of cancer,[74] up-regulation of growth signals and evasion of apoptosis are the most important.HSP90 is an exciting new therapeutic target, inhibition of which delivers a combinatorial attack on multiple oncogenic targets and pathways and on all of the hallmark traits of malignancy. The development of HSP90 inhibitors has moved forward rapidly alongside our growing understanding of the role of the chaperone in normal and malignant cells. The first and second generations of HSP90 inhibitors act by blocking its intrinsic ATPase activity. Following on from the natural product-based agents, exemplified by 17-AAG and related analogues that have entered clinical trials, a variety of HSP90-inhibitory chemo-types are now under development. It is also possible that new classes of inhibitor could be developed which act upon the co-chaperones of HSP90. Clinical activity has been seen with 17-AAG in melanoma, breast and prostate cancer. Although strength of HSP90 inhibitors is their combinatorial action in depleting multiple client proteins, this can, at the same time, obscure the precise mechanism of action that may predominate in a particular cancer. Hsp90 inhibitors have entered Phase II and III clinical trials, and have shown therapeutic activity in several types of cancer. Many novel small-molecule Hsp90 inhibitors are currently being assessed in vitro, in animal models and in clinical trials. It is probable that further investigation will identify Hsp90 inhibitors with improved therapeutic activity and better pharmacologic properties.

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